Food Chemistry 138 (2013) 116–121

Contents lists available at SciVerse ScienceDirect

Food Chemistry

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

Analytical Methods

Development of a rapid and simple voltammetric method to determine total antioxidative capacity of edible oils

Rubin Gulaboski [a,b,*], Valentin Mirčeski [a,b,c], Saša Mitrev [a,b,*]

[a] Department of Chemistry, Faculty of Agriculture, University "Goce Delčev" – Štip, Macedonia
[b] Department of Plant and Environment Protection, Faculty of Agriculture, University "Goce Delčev" – Štip, Macedonia
[c] Department of Chemistry, Faculty of Natural Sciences and Mathematics, "Ss Cyril and Methodius" University, Skopje, Macedonia

ARTICLE INFO

Article history:
Received 21 January 2012
Received in revised form 19 September 2012
Accepted 15 October 2012
Available online 8 November 2012

Keywords:
Reactive oxygen species
Antioxidants
Edible oils
Voltammetry
ABTS
Electron paramagnetic resonance

ABSTRACT

In this work we report on a new, rapid and simple voltammetric method to determine the total antioxidant capacity (TAC) of the edible oils. The method explores the ABTS radical (2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid)) assay as a redox probe and it relays on measuring catalytic voltammetric currents. The electrocatalysis comprises redox regeneration of the electrochemically created $ABTS^{\cdot+}$ radical either by Trolox (6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid) or by antioxidants present in studied oils. The detection limit of the method is determined to be 0.5 mg/L of Trolox equivalent, being a slightly lower than the corresponding UV–VIS spectrophotometric method. Applying the proposed voltammetric method the total antioxidant capacity of three types of commercially available cold-pressed edible oils are determined, and the results are found to be in a very good agreement with those obtained by UV–VIS spectrophotometry. The reported voltammetric method is cheap, rapid and simple, and it can be used as a sustainable alternative to the UV–VIS methods for the determination of total antioxidant capacitance of oils and other liquid lipophilic nutrients. Potent antioxidant capacity of studied oils was also confirmed by electron paramagnetic resonance spectroscopy of superoxide anion produced by macrophages.

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1. Introduction

It is widely recognised that the curbing of many diseases (Aruoma, 1998; Belitz & Grosch, 1999; Bogeski, Kappl, Kummerow, Gulaboski & Hoth, 2011; Cadenas & Davies, 2000; Droge, 2002; Halliwell & Gutteridge, 2002; Stanner, Hughes, Kelly, & Buttriss, 2004) is directly linked to consumption of bioactive compounds such as vitamins A, D, K and E, lipoic acid, and many polyphenolic derivatives as hydroquinones and flavonoids (Beardsell, Francis, & Ridley, 2002; Manach, Scalbert, Morand, & Jimenez, 2004; Novak, Seruga, & Komorsky-Lovric, 2009, 2010; Visioli, Bogani, Grande, & Galli, 2004; Yanishlieva-Maslarova & Heinonen, 2001; Yu, Zhou, & Parry, 2005). Cold-pressed edible vegetable oils are rich in a variety of lipophilic vitamins and polyphenols (Beardsell et al., 2002; Belitz & Grosch, 1999; Manach et al., 2004; Visioli et al., 2004; Yanishlieva-Maslarova & Heinonen, 2001; Yu et al., 2005). Total antioxidant capacity (TAC) of vegetable oils is most commonly assessed using the Trolox-ABTS assay (Re, Pellegrini, Proteggente, Pannala, & Yang, 1999) because of its simplicity, but UV–VIS spectrophotometry, based on the same assay (Apak, Guclu, Demirata, Ozyurek, & Celik, 2007; Gunstone & Harwood, 2007; Re et al., 1999; Visioli et al., 2004; Yu et al., 2005), is also used. The Trolox Equivalent Antioxidant Capacity (TEAC) is often used for vegetables, fruits and other food products, coupled with UV–VIS spectrophotometry (Gunstone & Harwood, 2007). Its capacity to measure activity of lipophilic as well as hydrophilic antioxidants makes it an assay of choice for routine determination of the TAC (Gunstone & Harwood, 2007). In this work we describe a new electrochemical method that allows an easy and accurate determination of the total antioxidant capacity of oils. The proposed method relies on the so-called electrocatalytic mechanism in voltammetry, which comprises a chemical regeneration of ABTS radical in a reaction with Trolox or with the antioxidants that are present in the oils. The method is fast and simple, and it can be seen as a viable alternative for the UV–VIS methodology for the total antioxidant capacity determination.

2. Materials and methods

All chemicals were products of Sigma (Germany) having 99% or higher purity and were used as received. All solutions were prepared by dissolution in ethanol. The oils used were obtained by cold-pressing procedure applied to sunflower, pumpkin and oil-turnip seeds, and were products from "Agrofila" company from

* Corresponding authors.
E-mail addresses: rubin.gulaboski@ugd.edu.mk (R. Gulaboski), sasa.mitrev@ugd.edu.mk (S. Mitrev).

0308-8146/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodchem.2012.10.050

Stip, Macedonia. All cold-pressed oils are commercially available. As a test-sample for purposes of comparison, we used refined sunflower oil.

Cyclic voltammetry (CV) and square-wave voltammetry (SWV) were performed with an AUTOLAB potentiostat model PGSTAT 128 N (Eco Chemie, the Netherlands) in a conventional three-electrode set-up. The reference electrode was an Ag/AgCl (3 mol/L KCl), Platinum wire was used as a counter electrode, while the ultra-trace glassy carbon (Metrohm, 3 mm in diameter) was exploited as a working electrode. The working electrode was cleaned by polishing with aluminium powder for 60 s, followed by rinsing with ethanol, and drying on air. The UV–VIS spectra were recorded with *Ultrospec* spectrophotometer model *2110 pro* over the region from 450 to 900 nm.

The TEAC assay was prepared according to the method reported elsewhere (Re et al., 1999) for both spectroscopic and voltammetric experiments, in order to make the two methods directly comparable. A stable radical cation of $ABTS^{\cdot+}$ was created by mixing solutions of a 7 mmol/L of ABTS salt (2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid)) diammonium salt) and 2.45 mmol/L of potassium persulfate in a volume ratio of 1:1 in ethanol. The reaction mixture was kept in dark for 8 h at 20 °C. The mixture could be used up to 52 h after preparation. The working $ABTS^{\cdot+}$ radical solution was diluted with an appropriate amount of ethanol to get suitable voltammetric responses (or absorbance of the signal at 734 nm in UV–VIS experiments). KCl at concentration of 0.05 mol/L was added to the ethanol solution to serve as a supporting electrolyte in voltammetric experiments. Microlitre amount of the cold-pressed oils were added to the $ABTS^{\cdot+}$ working solution, and the reaction mixture was left to react for 2–3 min at room temperature. Subsequently, we recorded several consecutive voltammograms in order to assess the stability of the system and the reproducibility of obtained results. The reaction between $ABTS^{\cdot+}$ and Trolox (6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid) was used as a reference system. As a control, we ran UV–VIS measurements in parallel to the voltammetric experiments. For each point presented, we recorded at least 10 consecutive voltammograms, and the average value was taken. The standard deviation of the measured currents was 0.125 μA. For the square-wave voltammetric experiments, a potential increment of 1 mV, frequency of 10 Hz, and square-wave amplitude of 50 mV were used.

The electron paramagnetic resonance (EPR) experiments were performed with the Bruker spectrometer (ESP300e) equipped with a TMH cavity, in which the flat cell was placed immediately after filling with the reaction solution. The reaction system consisted of 100 μL of stimulated monocyte-derived macrophage (300,000 units/mL) cell culture solutions in which a spin trapper 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) was added. The DMPO is a specific spin-trapper that is sensitive to the superoxide anions that are being created by the macrophages. The modulation amplitudes varied between 10 mG and 0.2 G. The microwave power was 0.63 mW. The EPR spectra were recorded with scan times of 50 s.

All experiments were performed at room temperature.

3. Results and discussion

The TEAC assay commonly refers to the ability of a given compound to scavenge the $ABTS^{\cdot+}$ radicals (Re et al., 1999). The main goal of the present work was to develop a fast and simple electrochemical method for assessing the TAC of edible cold-pressed oils. Although the basic voltammetric features of ABTS are discussed elsewhere (Buettner, 1993; Geletii, Balavoine, Efimov, & Kulikova, 2002; Stenken & Neta, 1982), to date no voltammetric method has been developed for determination of the TAC in edible oils by using the ABTS assay.

3.1. Basic electrochemistry of ABTS

Fig. 1A shows the cyclic voltammogram of ethanol ABTS solution containing 0.05 mol/L KCl as a supporting electrolyte. Under such conditions, ABTS features two quasireversible electrode processes with mid-peak potentials of +0.230 V and +0.520 V, respectively. These are referred to the oxidation of ABTS to $ABTS^{\cdot+}$ (the peak "1" at +0.230 V), and to the oxidation of $ABTS^{\cdot+}$ to $ABTS^{2+}$ (the second peak "2" at +0.520 V) (Buettner, 1993; Geletii et al., 2002; Stenken & Neta, 1982). The redox reactions associated with the two voltammetric signals in Fig. 1A can be described by following reaction scheme:

$$ABTS \leftrightarrows ABTS^{\cdot+} + e^- \leftrightarrows ABTS^{2+} + e^- \quad (I)$$

Note that after certain period of time (after 8–12 h from the preparation), the less positive potential peak diminishes significantly; the effect is attributed to the chemical conversion of ABTS to $ABTS^{\cdot+}$ radical. While at lower scan rates one clearly observes two distinct processes, at higher scan rates the first redox process (1–1′) is not so obviously pronounced in cyclic voltammetry. The reason for this can be attributed to the slower kinetics of the first redox process (Stenken & Neta, 1982). After period of 12 h, successive potential scanning gives a stable cyclic voltammogram, which is a good indicator for the stability of the system (see Fig. 1B). The linear dependence of the peak current vs. the square-root of the applied scan rate reveals the mass transfer of ABTS to the working electrode occurs primarily via diffusion (see Fig. 1C and D). The ratio between the cathodic and anodic peak currents of the second voltammetric process (peak pair 2–2′) measured by all scan rates is relatively constant, ranging between 0.97 and 1.02. Moreover, the peak-to-peak separation between the anodic and cathodic peaks of the voltammetric signal 2–2′ reads 60 mV (at 10 mV/s), and it only slightly changes by increasing the scan rates. These results imply that the second voltammetric process is attributed with a high degree of electrochemical reversibility. This is a very good indicator for exploring this process for analytical applications. All results described in this section are consistent with the voltammetric features of ABTS described elsewhere (Buettner, 1993; Geletii et al., 2002; Stenken & Neta, 1982).

3.2. Electrochemistry of ABTS in the presence of trolox

Addition of Trolox to the electrochemical cell containing ABTS caused changes in the features of the voltammograms. Concentrations of Trolox higher than 0.01 mmol/L led to a successive increase of the oxidation peak currents of the second voltammetric signal that was followed by a decrease in the backward (reduction) component (see Fig. 2A). The voltammogram in Fig. 2A featuring the lowest oxidative current component was recorded in the absence of Trolox. When Trolox is added in the cell, it easily undergoes chemical reaction with the $ABTS^{2+}$, which is the product of the $ABTS^{\cdot+}$ electrode oxidation. Such homogeneous redox reaction causes regeneration of the electrochemical reactant ($ABTS^{\cdot+}$) in the course of the voltammetric experiment, enabling $ABTS^{\cdot+}$ to be again re-oxidised at the electrode surface. This phenomenon is portrayed by the increase of the oxidation current component, and concomitant decrease of the backward (reduction) current component. As the concentration of Trolox increases, the oxidative voltammetric currents increase further, since more $ABTS^{\cdot+}$ gets regenerated during the voltammetric experiment. These features are typical for the so-called electrocatalytic regenerative EC' (or ECat) redox mechanism that are well known in the literature (Gosser, 1994; Gulaboski, 2008; Mirceski, Komorsky-Lovric, & Lovric, 2007; O'Dea, Osteryoung, & Osteryoung, 1981; Osteryoung & Osteryoung, 1985). In the present case, this EC' reaction mechanism can be described with the following scheme:

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