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Chemical composition and antioxidant activity of XAD-7 extracts from lingonberry (*Vaccinium vitis-idaea* L.)

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Introduction

Vaccinium vitis-idaea is most commonly known as 'lingonberry' or 'cowberry' belongs to the family Ericaceae-edible fruit. The collection of wild berries is very popular in northern, central and eastern Europe, notably in Nordic countries, the Baltic states, central and northern Europe. The berries are quite tart, so they are often cooked and sweetened before eating in the form of lingonberry jam, compote, juice, smoothie or syrup. The raw fruits are also frequently simply mashed with sugar, which preserves most of their nutrients and taste. Their consumption is strongly related to normalization of blood sugar levels, health hearth and eyes, kidney protection and lowering the risk of some types of cancers.

The present study characterized and identified the most effective extract, only consisting of anthocyanins, copigments or a mixture of both, obtained from a lingonberry juice concentrate. An extract was generated by using a XAD-7 column followed by fractionation into anthocyanins and determination of antioxidant activity by cyclic voltammetry [1].

a

b

Fig. 1 Lingonberry (*Vacciniumvitis-idaea* L.) fruits (a) and flowers (b)

Fig. 2. Lingonberry jam and juice (*Vacciniumvitis-idaea* L.)

Materials and Methods

Lingonberry juice concentrate (JC; Vaccinium vitis-idaea L.) was obtained from Symrise AG (Holzminden, Germany). According to Kostka et al. (2020), carbohydrates, organic acids and minerals were removed from lingonberry juice, while phenolic compounds were enriched. A detailed scheme and protocol for the XAD-7 fruit extract preparation and fractionation by membrane chromatography is given in Kostka et al. (2020) [3].

The characterization and identification of polyphenols was performed by coupling an HPLC system (1100/1200 series, Agilent, Waldbronn, Germany) to a high capacity spherical trap Ultra Ion Trap mass spectrometer (Bruker Daltonics, Bremen, Germany) using an electrospray ionization source. Detailed information about the method is written in Ostberg-Potthoff et al. (2019) [3].

Cyclic and square wave voltammetry were used for the determination of the an tioxidant capacity of the three lingonberry samples. All electrochemical measurements were performed using potentiostat/galvanostat Palm Sens Instrumentation (PalmSens 3, Houten, The Netherlands), controlled by PCT race version 3.0.6 software (Houten, The Netherlands) [4]. Briefly, the antioxidant capacity of the lingonberry XAD-7 extract and its fractions were determined by measuring of 1 mg/mL samples diluted in phosphate buffer using a glassy carbon working electrode (3 mm-φ). An Ag/AgCl (3 mol/L KCl) (i.e., silver/silver chloride electrode, with 0.1 M phosphate buffer, pH = 7.3) served as reference electrode, while platinum wire was used as a counter electrode. Before each measurement, the working electrode was cleaned by polishing with aluminum oxide powder for proximately 1 min, followed by rinsing with water, acetone and air-drying. The conditions were as follows: E1 = 0 V, v = 10 mV/s and Estep = 1 mV. All experiments were performed at room temperature.

Results and Discussion

The main phenolic compounds were identified and quantified by HPLC-PDA and HPLC–PDA–ESI–MS/MS, showing similar results as those described earlier [15,31,34,40,41]. The anthocyanin content of the XAD-7 extract was significantly higher than in lingonberry juice and fruits (2.44 g/100 g vs. up to 65 mg/100 g and 53 mg/100 g; [1-3]). Consequently, the phenolic compounds including anthocyanins were concentrated in the XAD-7 extract. Cyanidin-3-O-galactoside (68%) was the most abundant compound in lingonberries, followed by cyanidin-3-O-arabinoside (12%) and cyanidin-3-O-glucoside (6%). The composition of anthocyanins agreed with the literature, which documented a relative occurrence of 79–92% for cyanidin-3-O-galactoside, 11–13% for cyanidin-3-O-arabinoside and 5–10% for cyanidin-3-O-glucoside [2]. Another important group are the flavonols, such as quercetin-3-O-galactoside [1-3].

Table 1. Compound identification of the anthocyanin XAD-7 extract by HPLC-ESI-MS/MS

[M+H]+	Anthocyanins
287	Cyanidin-3-O-galactoside
287	Cyanidin-3-O-glucoside
287	Cyanidin-3-O-arabinoside

Electron Transfer Reactions Analyzed by Cyclic Voltammetry

The lingonberry XAD-7 extract and its fractions (CF and AF) were analyzed for antioxidant capacity by cyclic voltammetry. The results from the cyclic and square wave voltammetry are presented in Figure 3. Compared to the solvent control, lingonberry samples showed a current increase and had the ability to exchange electrons with the electrode. While the shape from cyclic and square wave voltammetry appeared similar for XAD-7 extract and its fractions, lingonberry samples differ in current intensity. In cyclic voltammograms, CF and XAD-7 extract showed the highest intensities. The results of AF were lower, nevertheless, antioxidant activity was detected. In square wave voltammograms, the ability to transfer electrons was as follows: XAD-7 extract > AF > CF. Phosphate buffer as solvent control had no effects. To sum up, lingonberry extracts showed electron transfer reactions with the highest potential for the XAD-7 extract [1,4].

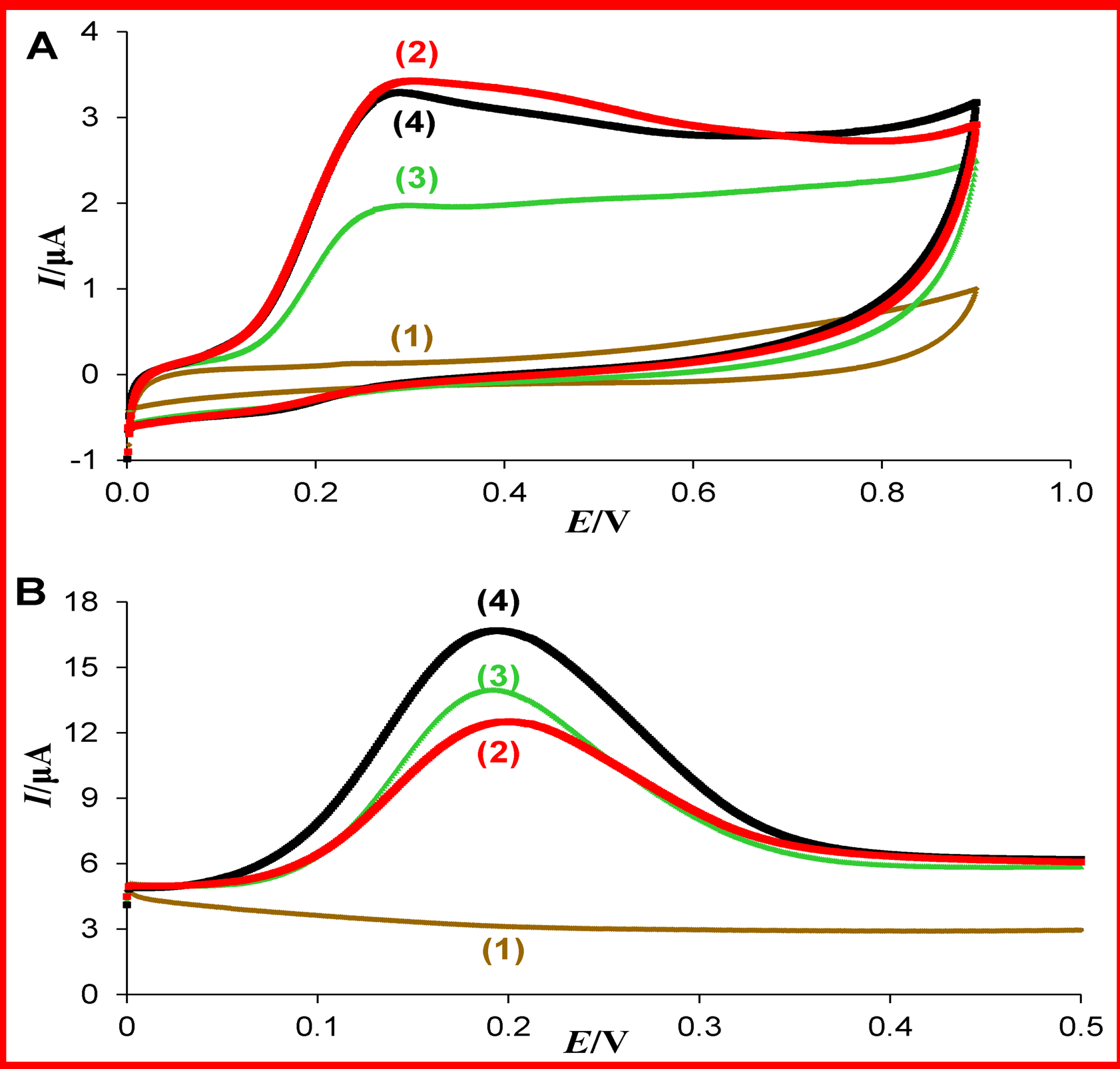

Figure 3. Determination of antioxidant capacity by cyclic voltammetry of lingonberry samples. (A) Cyclic voltammograms of lingonberry extracts at concentration of 1 mg/mL in 0.1 M phosphate buffer (pH = 7.3) recorded at potential scan rate of 10 mV/s at glassy carbon electrode. (B) Square wave voltammograms. For both panels: blank (1); copigment fraction (2); anthocyanin fraction (3); and XAD-7 (4). The parameters of the potential modulation for square wave voltammograms are: starting potential E1 = 0.0 V, square wave frequency f = 10 Hz, the height of the potential pulses Esw = 50 mV and the step potential ΔE = 1 mV.

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