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Voltammetric determination of β-carotene in raw vegetables and berries in Triton X100 media

Guzel Ziyatdinova*, Endzhe Ziganshina, Herman Budnikov

Department of Analytical Chemistry, Kazan (Volga Region) Federal University, Kremlyevskaya, 18, Kazan 420008, Russian Federation

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ABSTRACT

Electrochemical behavior of β-carotene in polar organic media and solubilized systems has been investigated. β-Carotene is irreversibly oxidized at 500 and 920 mV on glassy carbon electrode in 0.1 M $LiClO_4$ ethanol containing 10% of CH_2Cl_2. Effect of surfactants (cationic, nonionic and anionic) on voltammetric characteristics of β-carotene oxidation has been evaluated. High concentrations of surfactants facilitate the electrooxidation of β-carotene independently of surfactant type. The increase of oxidation current by 7–11% has been obtained in the presence of nonionic surfactant. The best results have been observed in 10 mM Triton X100 media. The peak current showed a linear dependence with the β-carotene concentration over the range 10–380 μM. The calculated detection limit was 2.5 μM and the quantification limit was 8.3 μM. Liquid extraction of β-carotene with dichloromethane from raw vegetables and berries has been developed. Quantitative determination of β-carotene in real samples using cyclic voltammetry in Triton X100 media combined with preliminary extraction has been carried out. The results obtained are in good agreement with data of nutrient database for standard references.

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1. Introduction

β-Carotene is one of the most important carotenoids and widely occurs in plants, algae and few fungi [1]. Being precursor of vitamin A, β-carotene is considered as its major source in the human diet [2]. β-Carotene contains 11 carbon–carbon double bonds in conjugation (Fig. 1) [3] and acts as free radical trap or antioxidant playing an important role in quenching of toxic radicals including reactive oxygen species and protection of tissues from damage [4].

The characteristic conjugated double bond system of β-carotene and other carotenoids provides a wide range of properties i.e. absorption at the violet end of the visible spectrum, electrochemical activity, etc. But on the other side, there are many problems in work and manipulation with carotenoids that are associated with their instability especially towards light, oxygen, heat, acid and alkaline conditions [5–7]. Each factor may cause the degradation, oxidation and/or the *trans–cis* isomerization of β-carotene.

Different types of chromatography have been applied for the determination of β-carotene in real samples including high performance liquid chromatography (HPLC) with UV-detection [8,9], reversed-phase high pressure liquid chromatography [10], liquid chromatography with a coulometric electrochemical array detector [11] and normal-phase HPLC method [12]. The main advantages of chromatography are high versatility, sensitivity and selectivity providing reliable analysis of food samples.

The optic properties of β-carotene provide simple and cheap possibilities for its direct analytical determinations using near-infrared reflectance [13], xenon flash spectrometry [14] as well as resonance Raman and NMR spectroscopy, circular dichroism and mass spectrometry [15].

A simple spectrophotometric method has been developed for the quantitative analysis of total β-carotene in food additives such as powders, emulsions, and oily suspensions containing *E*/*Z*-isomers of β-carotene in different ratios. The approach is based on preliminary extraction with dichloromethane and ethanol and further measurement of the extracts absorbance at 421.0 nm [16].

The most important peculiarities of techniques applied to analyses of carotenoids including β-carotene and their impact on the reliability of the analytical results are discussed in review [17].

Although presence of conjugated double bonds in the structure of β-carotene provides its electrochemical activity, the analytical use of voltammetric oxidation and/or reduction processes, known to occur on the electrode surfaces [18], is rather limited. The amperometric detection of β-carotene in irradiated fruits after chromatographic separation has been described in [19]. HPLC with electrochemical detection has been developed for the determination of β-carotene in human plasma, blood cells and buccal mucosal cells [20]. Differential-pulse voltammetry on mercury

* Corresponding author. Tel.: +7 843 2337736; fax: +7 843 2337416.
E-mail address: Ziyatdinovag@mail.ru (G. Ziyatdinova).

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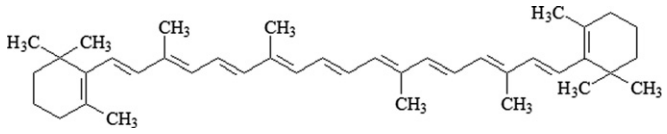

Fig.1. β-Carotene structure.

and glassy carbon (GCE) electrodes in dichloromethane has been applied for the determination of β-carotene in soya oil and brine reference concentrates involving preliminary extraction [21].

Several investigations are devoted to measurements of β-carotene oxidation potentials using various types of voltammetry [22–24] for further evaluation of its reactivity.

Voltammetry of β-carotene is usually carried out in aprotic organic media like hexane and dichloromethane due to hydrophobic nature and limited solubility of analyte in other solvents. Surfactants are known as substances able to effect on the electrochemical response of compounds due to facilitation of the adsorption and solubilization of various different electrochemically active compounds. This has been associated with changes like redox potential of the analytes, charge transfer and diffusion coefficients of species, as well as changes in the stability of the electrogenerated intermediates and electrochemical products [25–27]. Therefore, application of surfactant based media could be effective tool to increase solubility of β-carotene in polar solvents as it has been shown earlier for other liposoluble substances in particular, α-tocopherol [28,29] and retinol [30].

The aim of present work is research of voltammetric behavior of β-carotene in presence of surfactants and development of methods for its determination in raw vegetables and berries using preliminary extraction.

2. Experimental

2.1. Reagents

β-Carotene of 93% purity was purchased from Sigma (Germany). Its stock solution (0.01 M) was prepared daily by dissolving of accurately weighed amount in 5.0 mL of CH_2Cl_2. Model solutions were prepared by dilution of β-carotene stock solution aliquot portion in ethanol containing 0.1 M $LiClO_4$ and CH_2Cl_2. The CH_2Cl_2 portion was reduced to 10% (v/v).

Surfactants Triton X100, Brij® 35 from Sigma (Germany), sodium dodecyl sulfate (SDS) from Panreac (Spain) and cetylpyridinium bromide (CPB) from Aldrich (Germany) were used. Their stock solutions (0.1 M) were prepared by dissolving a definite amount of the appropriate substance in 10.0 mL of water. More dilute solutions were prepared by exact dilution of the stock solution.

All other chemicals were of analytical reagent grade purity and used as received. Double distilled water was used for the measurements. The experiments were carried out at laboratory temperature (20–23 °C). All solutions were kept in glass vessels in the dark at laboratory temperature.

2.2. Apparatus

Voltammetric measurements were performed using voltammetric analyzer "Ecotest-VA" (Russian Federation). The electrochemical cell ($V=10$ mL) consisted of working GCE (3.14 mm^2 surface area), a silver–silver chloride saturated KCl reference electrode and a counter electrode (platinum wire).

The GCE was carefully polished with 0.05 μm alumina powder on polishing cloth. Then electrode was rinsed with acetone and double distilled water before use.

2.3. Procedures

2.3.1. Cyclic voltammetry

0.1 M $LiClO_4$ in ethanol containing 10% of CH_2Cl_2 was chosen as supporting electrolyte. After adding 10.0 mL of β-carotene model solution in electrochemical cell, cyclic voltammograms (CV) were recorded at potential scan rate of 100 mV s^{-1} and potential range from 0 to 1500 mV.

2.3.2. Sample preparation and extraction of β-carotene

Raw vegetables and fruits (carrot, pumpkin, parsley, rose hips and rowan berries) available on the local store were used. For the vegetables, outer most thin layer (skin) for carrot and pumpkin and central part (only for carrot and rose hips) were removed and remaining portions were sliced on small pieces of approximately 2 mm width and 3 mm length as described earlier [31].

Preliminary extraction of β-carotene with CH_2Cl_2 was used. A representative portion of the sliced sample (5 g) was accurately weighted and quantitatively transferred into separating funnel. Then 8 mL of CH_2Cl_2 were added and shaked for 10 min. The organic layer was collected and evaporated in quarter to 2 mL at 35 °C and then used for further measurements.

Analytical recoveries were determined by standard addition method using 500 and 750 μg β-carotene added to the extraction system together with sample. Five determinations were made for each concentration, and the recovery was calculated.

The standard addition and calibration graph methods were employed for the quantification of the β-carotene in real samples. One milliliter of the extract was transferred into the cell with 9.0 mL of the supporting electrolyte containing 0.01 M Triton X100. The additions of β-carotene stock solution were 50 μL. CVs in the range of 0–1500 mV at scan rate 100 mV s^{-1} were recorded. β-Carotene content was calculated per 100 g of the sample under investigation.

2.3.3. Spectrophotometric determination

Spectrophotometry as reference method has been emploied for determination of β-carotene in real samples owing to its absorbance at 446 nm [32]. Samples were extracted by hexane. The quantification of β-carotene has been performed applying a calibration curve for β-carotene solutions in hexane.

2.4. Statistical analysis

All the measurements were performed in five replications. Statistical evaluation was performed at significance level of 5%. All data were expressed as the $X \pm \Delta X$ with X as average value and ΔX as confidence interval.

3. Results and discussion

3.1. CV of β-carotene in presence of surfactants

Voltammetric behavior of β-carotene on GCE in 0.1 M $LiClO_4$ in ethanol containing 10% of CH_2Cl_2 has been studied. There are two irreversible steps of β-carotene oxidation at 500 and 920 mV on the CV (Fig. 2) that corresponds well with data reported earlier [21,33]. β-Carotene like other carotenoids is oxidized with the formation of cation radicals and dications. The dications decay by the loss of a proton and undergo a comproportionation reaction with the parent carotenoid forming two cation radicals [34].

Increase of β-carotene concentration above 50 μM leads to formation of heterogeneous system that makes impossible measurement in alcoholic media. Moreover there is no linear dependence between oxidation current and concentration of β-carotene under conditions noted above.

To avoid this limitation, surfactants of different nature have been investigated. These are cationic (CPB), anionic (SDS) and nonionic (Triton X100 and Brij® 35) surfactants. All of them are electrochemically inactive at anodic potentials under investigation.

The addition of different surfactants to the system changes the first oxidation potential and peak current of β-carotene depending on the surfactant type and concentration (Fig. 3).

Anionic SDS does not affect on β-carotene voltammetric characteristics within the whole range of concentrations excluding 10 mM concentration leading to decrease of oxidation potential to 483 mV (Fig. 3A).

Cationic CPB decreases the β-carotene oxidation potential to 474 mV (Fig. 3B) for the highest surfactant concentration (1×10^{-3} M) but peak current decreases on 15% (1.12 ± 0.05 vs. 1.40 ± 0.09 μA in the absence of surfactant). The peak current changes insignificantly at lower CPB concentrations. In general, both surfactants have no significant effect on the peak current.

Nonionic surfactants (Triton X100 and Brij® 35) at high concentration of 10 mM increase β-carotene oxidation current as well as lead to cathodic shift of oxidation potential (Fig. 3C and D).

Thus, high concentrations of surfactant facilitate the electrooxidation of β-carotene independently of surfactant type. Similar trends in effect of surfactant at high concentration on voltammetric characteristics of β-carotene allow to expect that surfactants provide solubilization of analyte molecule. Nonionic surfactants have shown better results due to structural affinity with molecule under investigation which is neutral highly hydrophobic compound. Similar effect has been observed earlier in Triton X100 micellar media [35].

The best voltammetric characteristics (E_p=482 mV and I_{pa}=1.55 μA) have been observed in 10 mM Triton X100 media which has been used for further investigations.

Cyclic voltammograms of β-carotene in 10 mM Triton X100 in supporting electrolyte are shown in Fig. 4.

There are linear relationship between oxidation current of β-carotene and its concentration in the range 10–380 μM. The corresponding calibration equation is $I_{pa}=(0.06\pm0.03)+(15.5\pm0.2)\times10^3C$ with $R^2=0.9997$, where intercept is expressed in μA and slope—in μA L mol^{-1}. The limit of detection (LOD) and quantification (LOQ) has been calculated using statistic treatment ($3SD_a/b$) and ($10SD_a/b$), respectively, where SD_a is the standard deviation of the average arithmetic of 10 voltammograms of the blank solution obtained in the same potential oxidation of β-carotene and b is the slope of the calibration graph. The LOD and LOQ are 2.5 and 8.3 μM of β-carotene, respectively. Both parameters indicate satisfactory sensitivity of the proposed method. The repeatability of the method has been evaluated by ten successive measurements of 50 μM of β-carotene. The obtained relative standard deviation is 3.4%.

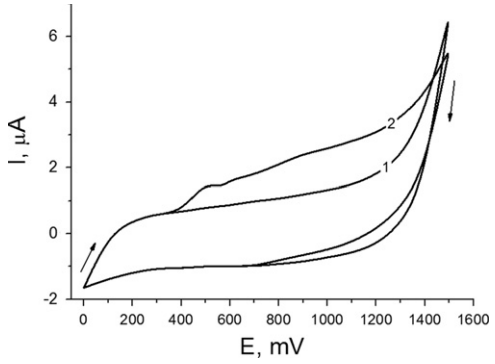

Fig. 2. Cyclic voltammogram of 50 μM β-carotene (curve 2) on GCE in 0.1 M $LiClO_4$ in ethanol containing 10% of CH_2Cl_2 (curve 1). Potential scan rate is 100 mV s^{-1}.

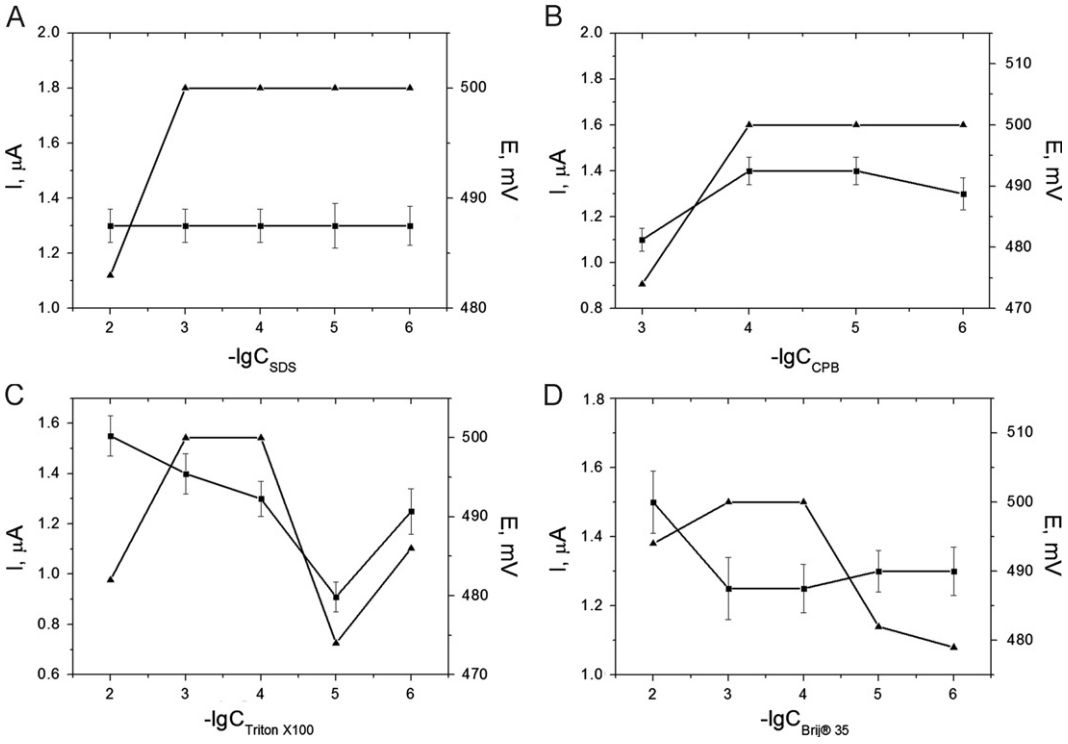

Fig. 3. Effect of surfactants on voltammetric characteristics of β-carotene oxidation. (A) SDS, (B) CPB, (C) Triton X100, and (D) Brij® 35. ▲, oxidation potential, mV; ■, oxidation current, μA. E_{p0}=500 mV, $I_{pa0}=1.40\pm0.09$ μA. $C_{\beta\text{-carotene}}$=100 μM.

The approach developed for β-carotene determination using Triton X100 media has shown improvements in comparison to other voltammetric method described for foodstuff. The lower detection limit and comparable linearity of calibration graph have been observed in present study using cyclic voltammetry in comparison with data in [21] for differential pulse voltammetry on GCE and dropping mercury electrode. The less volume of supporting electrolyte (10 mL vs. 25 mL) has been used for the

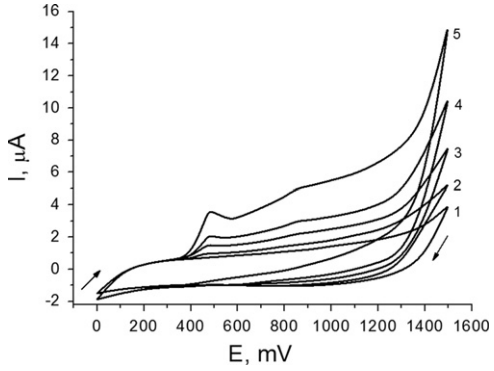

Fig. 4. Cyclic voltammograms of β-carotene on GCE in 10 mM Triton X100 in 0.1 M $LiClO_4$ in ethanol containing 10% of CH_2Cl_2: 1–0, 2–10, 3–50, 4–89, and 5–200 μM. Potential scan rate 100 mV s^{-1}.

Table 1
Voltammetric determination of β-carotene in model solutions ($n=5$; $P=0.95$).

Added (μg)	Found (μg)	RSD (%)
53.7	53 ± 2	3.7
268	268 ± 5	1.6
483	482 ± 3	0.4
1073	1071 ± 5	0.4
2147	2143 ± 6	0.2

Table 2
Recovery of β-carotene ($n=5$; $P=0.95$).

Sample	Spiked (μg)	Expected (μg)	Found (μg)	Recovery (%)
Carrot	0		271 ± 10	
	500	771	768 ± 8	99.6
	750	1021	1022 ± 9	100.1
Pumpkin	0		69 ± 9	
	500	569	566 ± 10	99.8
	750	819	818 ± 9	99.9

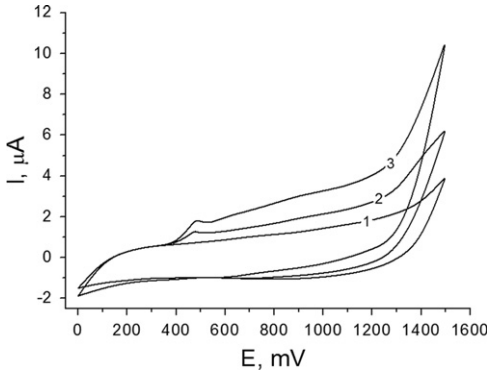

Fig. 6. Cyclic voltammograms of 1 mL rowan berries extract in the absence (curve 2) and in the presence of 49.8 μM β-carotene (curve 3) in 10 mM Triton X100 in 0.1 M $LiClO_4$ in ethanol containing 10% of CH_2Cl_2 (curve 1). Potential scan rate 100 mV s^{-1}.

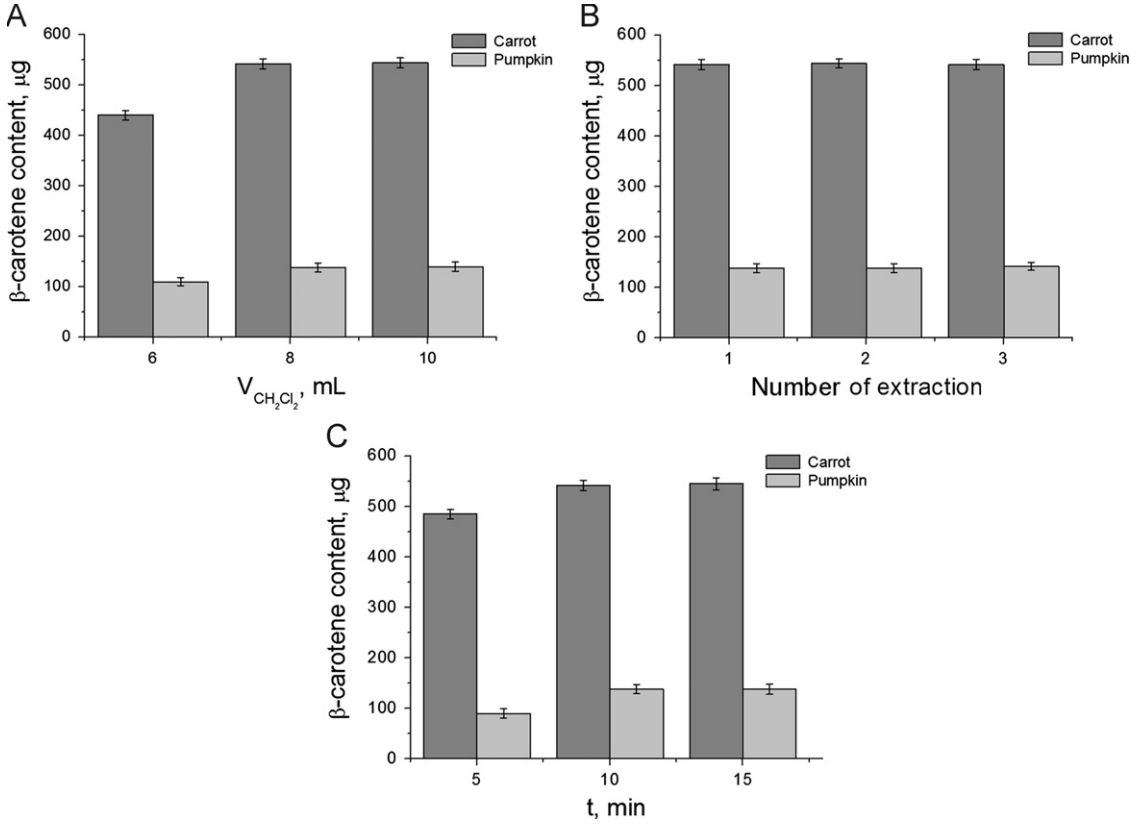

Fig. 5. Efficiency of β-carotene extraction from vegetables with CH_2Cl_2. (A) Effect of extractant volume; (B) effect of number of extractions; and (C) effect of extraction time.

Table 3
β-Carotene content in raw vegetables and berries (n=5; P=0.95).

Sample	Scientific name	Cyclic voltammetry				Spectrophotometry	
		β-Carotene content[a] (mg 100 g^{-1})	RSD (%)	β-Carotene content[b] (mg 100 g^{-1})	RSD (%)	β-Carotene content[a] (mg 100 g^{-1})	RSD (%)
Carrot	*Daucus carota*	9.9 ± 0.3	2.5	9.7 ± 0.2	1.9	9.8 ± 0.4	3.2
Pumpkin	*Cucurbita pepo*	2.94 ± 0.05	1.8	2.95 ± 0.06	2.2	2.90 ± 0.07	2.5
Rowan berries	*Sorbus aucuparia*	7.82 ± 0.08	1.0	7.86 ± 0.08	1.0	7.9 ± 0.1	1.3
Rose hips	*Rosa canina*	2.90 ± 0.08	2.8	2.9 ± 0.1	3.4	2.8 ± 0.1	3.6
Parsley	*Petroselinum crispum*	5.5 ± 0.2	2.2	5.6 ± 0.2	3.2	5.4 ± 0.2	3.1

[a] Calibration graph method.
[b] Standard addition method.

measurements. The application of Triton X100 media allows to perform measurements in 10% dichloromethane instead of pure solvent characterized by high volatility and toxicity.

Quantitative determination of β-carotene in model solutions with Triton X100 media was carried out. The accuracy of results obtained was evaluated by added-found method (Table 1).

3.2. Extraction of β-carotene with dichloromethane

Dichloromethane is one of the best solvents for β-carotene [36] and used for its liquid extraction. The efficacy of extraction has been checked by voltammetry. The effect of extractant volume, time and order of extraction has been evaluated on the carrot and pumpkin samples as examples with relatively high and low content of β-carotene (Fig. 5). The best results have been observed for single extraction for 10 min at 8 mL CH_2Cl_2 for 5 g of sample with further evaporation of solvent to 2 mL.

In order to check the extraction accuracy, a known amount of crystalline β-carotene has been spiked in the extraction system and the recovery has been tested (Table 2). The values of recovery are in the range from 99.6% to 100.1% indicating an absence of matrix effects in these determinations.

3.3. Determination of β-carotene in real samples

Based on the results obtained, voltammetric method for β-carotene determination in raw vegetables and berries has been developed using preliminary extraction with CH_2Cl_2.

As one can see from Fig. 6, there is well-defined signal at 482 mV on the cyclic voltammograms of extract corresponding to oxidation of β-carotene that has been checked by standard addition method as well (Fig. 6, curve 3). The addition of β-carotene standard solution leads to increase of oxidation current at the same potential proportionally to the inserted amount of analyte.

Different types of raw vegetables and berries have been tested for β-carotene concentration (Table 3). As one can see, data correspond well in both methods of calculations. Comparison of the results with reference spectrophotometric determination shown good correlation. The results obtained for different types of samples agree well with nutrient database for standard reference [37] and HPLC determination [8,38].

4. Conclusion

Sensitive and selective method of β-carotene analysis in the solubilized analyte systems has been developed in current work. Combination with preliminary extraction allows quantitative determination of β-carotene in raw vegetables and berries. The analytical results obtained by calibration graph and standard addition methods are adequately accurate and precise and in good agreement with those obtained by other techniques. The main advantage of approach developed is the use of less hazardous surfactant media permitting to decrease portion of dichloromethane. Consequently the proposed method has a high potential of a good analytical alternative for determining β-carotene.

Acknowledgments

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