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Using of carbon electrodes in analysis of foodstuffs

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Abstract

This thesis is focused on preparing, characterization and utilizing of carbon electrodes in analysis of foodstuffs. The most it is devoted to properties, characterization and modifications of carbon paste electrodes for determination of target analytes. The acrylamide, lipophilic vitamins, ascorbic acid or flavonoids were determined in model and real samples. The carbon paste electrode was also used for evaluation of oxidative stability of edible oil. The cyclic voltammetry, square wave voltammetry and differencial pulse voltammetry were used as electrochemical tools for determination of analytes.

Abstrakt

Práce se zabývá přípravou, charakterizací a použitím uhlíkových elektrod v analýze potravin. Převážná část je věnována uhlíkovým pastovým elektrodám, jejich vlastnostem a modifikacím pro stanovení vybraných analytů. V modelových i reálných vzorcích byl stanovován akrylamid, dále vitamíny rozpustné v tucích nebo vitamín C a flavonoidy. Uhlíková pastová elektroda byla rovněž použita pro zhodnocení oxidační stability jedlého oleje. V práci byly využity různé elektrochemické metody, převážně však cyklická voltametrie, square wave voltametrie a diferenčně pulsní voltametrie.

Keywords

carbon paste electrode, modified electrodes, acrylamide, vitamins, flavonoids

Klíčová slova

uhlíková pastová elektroda, modifikované elektrody, akrylamid, vitamíny, flavonoidy

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1 Introduction

Electrochemical or electroanalytical methods are based on reactions between electrical quantities such as current, potential, or charge, and the determined substance, analyte. The resulting response of the measurement is proportional to the concentration of the substance. These reactions are most often carried out in solution where the analyte is in a contact with electrodes. Electrochemical measurements are specific by their speed and the instrumental and economical unpretentiousness in comparison to other analytical methods. Also, they are powerful tool for sensitive and reproducible determinations.

Voltammetric measurements are characterized by the time changing applied potential on the working electrode. The time dependence of the current on the applied voltage is recorded. Several voltametric techniques are known. The cyclic voltammetry, square wave voltammetry and differential pulse voltammetry were used in this work.

The cyclic voltametry (CV) is usualy used for characterization of oxidative reduction processes. The principle of this method is based on the insertion of time-varying potential with the triangular shape. The result voltammogram is characteristic for the analyte under the specific conditions, e.g. the type and composition of the electrolyte, the pH, setting of the measurement conditions, and also on concentration of the analyte.

The potential ramp in the square wave voltammetry (SWV) is different. The inserted potential is electro-modulated with alternating voltage of rectangular shape of small amplitude and frequency. The current is measured only for a short time period at the end of each pulse. The resulting voltamogram has a peak shape (the first derivative). It is record of differences of two successive measured values of the dependence of the current on the potential. SWV is significant by low charging current values, speed and number of parameters that can be optimize.

Pulse voltammetric techniques are similar to SWV. In differential pulse voltammetry (DPV), voltage pulses 10 to 100 mV are applied to the linear change potential over time for several milliseconds. The peak voltammogram is a result of dependence of potential and diferences between the current at the start and on the end of the pulse.

In this work was also used the method of stripping voltammetry (SV). The principle of SV is acumulation of the analyte on the electrode surface. The concentration of the analyte can be increased in several ways.The first method is pre-electrolysis. The analyte is concentrated on the surface of the electrode in a certain time (in minutes) due to the insertion of a constant potential, usually in a stirred solution. It lead to the reaction between the analyte and the electode material or electrolyte components and the result is compound that is captured on the electrode surface. Then, the result compound is cathodiccally (CSV) or anodically (ASV) dissolved back to the stationary electrolyte. Other method is adsorption stripping voltammetry (AdSV). As the name suggests, the analyte is being concentrated at the surface of the electrode by adsorption at a certain potential which may be equal to zero, or the electrode may be disconnected from the circuit. If the analyte is electrochemically active, the height of the corresponding peak is proportional to the concentration of the analyte in the sample. If the analyte is electrochemically inactive, the so-called tensometric peak is measured. This peak is result of the desorption of the

analyte from the surface of the electrode after reaching a certain one potential. It is also possible to meet the extraction stripping voltammetry (ExSV), where the analyte is extracted into an electrode material, most commonly carbon paste.

In the case of voltammetric measurement techniques, the three-electrode system is usually used. This system is consisting of a working, an auxiliary and a reference electrode. Ag/AgCl or calomel electrodes are usually used as a reference electrode and as a auxiliary it is most often a platinum wire. As a working electrode could be used a several types, from the metal electrodes as Pt or Au to different types and modifications of carbon electrodes. Electrochemical reactions running on the auxiliary electrode (reaction of basic electrolyte components, water oxidation, etc.) are not being recorded. The source of voltage is the potentiostat that keeps the stable the required value of voltage applied to the working electrode. Voltammetric techniques can be used to analyze aqueous, non-aqueous and mixed solutions. The basic electrolyte is often used for increasing the conductivity of the solution. As a basic electrolyte are used solutions of acids, bases and salts, or quaternary ammonium bases. In voltammetric measurements, the potential is linearly changed over the time and, in the presence of a substance which can be oxidized or reduced, occurs at the appropriate potential a reaction, called the depolarization of the electrode. This reaction manifests on the voltamogram as a voltametric wave, ie an increase in current. The height of this wave is the voltametric analytical signal, I_p, which is directly proportional to the concentration of the analyte (depolarizer) in the analyzed solution. Ip is a quantitative parameter. The peak potential, E_p, provides qualitative information characteristic of the analyte. If peak potentials of analytes present in the solution are sufficiently different, they can be under appropriate conditions determined side by side [1-3].

2 Carbon electrodes

Several types of carbon electrodes were used as working electrodes in this work. In general, carbon electrodes are more resistant against oxidation reactions of their surfaces compared with other electrodes. They are also characterized by a lower residual current. The surface of carbon electrodes is commonly renewal before every measurement, e.g. by cleaning or activating of the surface by applying potential or mechanicaly. Standard carbon electrodes used for common voltammetry measurements have the working surface size of milimetres. The electrode surface could also be of centimetres or micrometres in special types.

Carbon paste electrodes (CPE) were developed by coincidence by Ralph Norman Adams in 1958. He tried to develop electrode made of carbon which could replace the dropping mercury electrode (DME). However, this idea has not been realized and so after the increase of carbon powder content in the pasting liquid there was a carbon paste. In the recent years, the using of CPE and its various modification has gradually developed. CPEs are basically made of carbon powder and pasting liquid. Carbon powder represents an electrode material. The pasting liquid connects carbon particles and acts as an electric insulator. The spectrographic carbon powder is a basic type used for prepraing of CPE. Nowadays, glassy carbon powder (GC) or carbon nanotubes (single or multiwalled, SWCNTs, MWCNTs) are also commonly used. During preparation of CPE is important to pay attention for some characteristic properties of all compounds. The prticle size of carbon, their adsorption properties and the purity of the powder should be taken into account. As a paste binder could be used for example paraffin oil or wax, silicone oil, vaseline, ion liquids, and so on. In general, pasting liquid has to be non-volatile, insoluble in water, chemically stable and non-reactive. In recent years, the various ionic liquids were tested mainly for the production of biosensors. The resulting properties of CPE are significantly affected by the proportion of the components. The ratio of carbon powder and pasting liquid is usually 1.0 g C:0.4-1.0 mL of a binder. The carbon paste should have the correct consistency so that it is not too thin or stiff. The paste binder content influences the value of ohmic resistance (R) and thus conductivity of the electrode and baseline current. In general, the higher content of pasting liquid increases the capacitive current. The preparation of CPE is based on the precise homogenization of the basic components for about 10 to 30 minutes. Homogenization is most often done manually in the laboratory in a ceramic mortar. However, the comercial carbon paste are also available. Altough, "homemade" pastes are most commonly used, mainly because they could be easily modified and the composition is known. The resulting paste is then placed into the electrode holder. In our laboratories, a teflon holder with a conductive piston is used. CPE could be stored for several weeks. After some time, the paste starts to crumble and thus the electrode becomes unusable [2, 4-7].

Glassy carbon (GE) is a less porous form of carbon produced by pyrolysis of hydrocarbons. Due to low porosity, it is not necessary to use pasting liquids for the preperation of GCE. The GCE surface is renewing by polishing of wet filter paper with a small amount of alumina. This procedure causes mirror polish of the surface which is very homogeneous, uniform and solid. Glassy carbon powder could also be used to prepare carbon paste electrodes (glassy carbon paste electrode, GCPE). These pastes electrodes are usable in aqueous-organic solvents up to content 80 % of organic compound. The individual particles of GC have the shape of beads, the spectrographic

carbon particles are scales. The shape of carbon particles, especially spatial arrangement of individual particles, affects the content of pasting liquid and thus also the electrical conductivity and the stability of the paste. In general, standard CPE from spectrographic carbon is better conductive than GCPE. This is due to better contact between spectral carbon scales than between glassy beat [2, 8, 9].

In this work has also been used a special type of carbon electrode, the pencil graphite electrode (PGE). Basically it is ordinary pencil. The tufts of pencils are made of graphite (electrode material) and clay, which works as a binder. The contact between the graphite and potentiostat is mediated by a conductive wire connected to the metal part of the pencil. During the measurement, only a graphite part of the pencil is immersed in the electrolyte, not the metal tip of the pencil. This type of electrodes have already been used in several determinations of varied substances. It has been experimentally found that not all commercially available pencils are suitable for electrochemical measurements. From the practical experience from the laboratory, the best are pencils from KOH-I-NOOR HARDTMUTH a.s. (České Budějovice, Czech Republic). These pencils exhibits sufficient conductivity of the material. On the other hand, for example pencils Q-CONNECT (Gent, Belgium) cannot be used due to they are not conductive [10, 11].

Standard CPEs have the great advantage of simple modification. The pastes can be modified either by directly mifing together with other compounds during preparation (bulk modification), or the surface of prepared standard CPE can be modified. Both of them have their own advantages. Bulk modificiation includes modification by special paste binder or adding of some substance. However, these electrodes are usable only for the target purpose. Surface modification is based on applying of some substance on it, e.g. carbon nanotubes, graphene for improving of transmission of electrones, or also there could be make metal or bio films. Biologically modified electrodes are called biosensors. These electrodes contain a biological component, e.g. enzymes, microorganisms, antibodies, DNA, parts of plants, and so on. A special type of bulk modification is using of surfactants. A special type of bulk modification is using of surfactants. These substances are able to protect the carbon paste against effects of organic solutions in supporting electrolyte. They prevent the dissolution of the paste binder. Electrodes modified by surfactant are called by their designer "Adams electrodes" (AE). They are characterized by very low background currents in organic electrolytes, even in comparison with for example platinum electrodes. Surfactants are bipolar substances containing two parts - hydrophilic and hydrophobic. Depending on the ionic nature of the hydrophilic group, the surfactants are divided into ionogenic and nonionic. The ionic surfactants are further divided into cationic, anionic or ampholytic, which behave as anionic or cationic depending on the pH of the medium. In practice, several substances, such as anionic sodium dodecyl sulphate (SDS), cationic hexadecylpyridinium chloride (CPC), nonionic Triton X-100®, Triton®, Tween®, etc. have been used. Surfactants are usually used for bulk modification, but they also could be a part of the supporting or stripping electrolyte. Standard composition of AE is carbon powder, paste binder and surfactant in ratio 50:20:30 % [2, 12, 13].

3 Acrylamide

Acrylamide (AA) is an industrially produced substance used primarily for the synthesis of polyacrylamide. Polyacrylamide polymers and copolymers are used in the cosmetics, paper and textile industries, for purification of waste and drinking water, or in laboratories for the preparation of polyacrylamide gel for electrophoretic separation of proteins [14, 15]. In 2002, Swedish scientists from the University of Stockholm, in cooperation with the Swedish National Food Administration (NFA), published a finding of high acrylamide content in food. The highest AA content was found in fried potato chips and potato products. In general, acrylamide is formed mainly during Maillard reaction between aminoacids, especially asparagine, and reducing sugars, mainly glucose, under temperature higher than 120 °C. However, foodstuffs can contain AA also from a packaging material [16-18]. AA can be even found in wheat products like bread and crisps, in the roasted coffee, olives, almonds, and so on. AA can be even found in wheat products like bread and crisps, in the roasted coffee, olives, almonds, and so on.

AA has a neurotoxic and carcinogenic effects in the human body. According to the Globally Harmonized System (GHS) it is classified to group 6 - toxic substances, and to group 8 - substance hazardous to health. The International Agency for Research on Cancer (IARC) sorts AA to the class 2A as a substance probably carcinogenic for humans [19-22]. The main symptoms of AA intoxication are numbness or weakness of limbs, tremor, loss of balance, instability, sweating or skin peeling. There were also cases of weight loss or speech disorders. Intoxication of AA could be caused by dermal, oral or parenteral way [23].

Due to effects of AA on the human organism, it is desirable to develop a reliable, rapid and simple method of determining its content in food. AA cannot be determined by UV spectroscopy because its molecule does not have eighter the sufficient strong conjugated double or triple bonds or aromatic ring. The common method for determination of AA is gass or liquid chromatography with mass or tandem mass spectrometry detection (LC-MS, LC-MS/MS, GC-MS, GC-MS/MS). The gass chromatography can be used after derivatization. Acrylamide can also be determined by infrared spectroscopy (IR), capillary electrophoresis (CE), imunoenzymatic test ELISA or by electrochemical methods [18, 24, 25]. In general, determination of AA in real samples includes extraction by water, sunsequent purification by solid phase extraction (SPE), addition of internal standard and analysis. The most often the analysis is made by GC-MS after bromination or directly by LC-MS/MS. Although, these methods are reliable and fully funcional, they have considerable disadvantages, such as long analysis time or complex sample preparation. In the case of using IR, CE, ELISA or electrochemical methods, the complex preparation of sample is not necessary.

3.1 Electrochemical methods for determination of acrylamide

The polarographic method is the only direct electrochemical method for determination of AA. This method is based on measuring of current on dropping mercury electrode (DME) which is used as the working electrode. It was investigated in aqueous, mixed and purely organic electrolytes [26-28]. Due to the solubility of AA in water, it is advantageous to use the aqueous medium to extraction of AA from the sample. The differential pulse polarography was used for determination of AA in

0.5 mol.L^{-1} LiCl. Acrylamide provides the current response depending on the used electrolyte at about -1.9 V. Although, the polarographic methods are reliable, reproducible and sensitive for this use, due to mercury toxicity the DME is not desirable to using.

Other electrochemical methods for determination of AA are indirect, some mediator has to be used. The complexes of transition metal cations can be used. The method of AA determination by creation of complex with Ni(II) ions was investigated by Veselá and Šucman (2013). The AA-Ni complex have been adsorbed on the surface of the hanging mercury drop electrode (HDME) and the analysis was done by adsorption stripping square wave voltammetry (AdSSWV) in amonium buffer (AMB) of pH 9.5 with 500 µmol.L^{-1} Ni(II). The desorption of AA-Ni provided current response at about -0.30 V with shifting to higher potential values with increasing content of AA and Ni(II) [29]. The similar principle have been used in the study of Zargar et al. (2009). In this study, complex with Co(II) ions have been used for the determination of AA content [30].

The HDME was used in both of these works as working electrode and current responses were recorded in reduction area. Based on above mentioned studies, the CPE modified by ruthenium dioxide (CPE/RuO_2) has been investigated in our study [31]. Generally, ruthenium salts can improve current responses of various organic compounds [32]. Acrylamide cannot be determined on bare CPE due to the fact that CPE can be used in potential window aproximately only from -1.0 to +1.4 V. However, the using of bare CPE for measuring complex of AA-Ru in the solution has not been possible due to its limited solubitity. CPE/RuO_2 provided reduction response at +1.03 V in the 1.0 M LiCl as a supporting electrolyte. The using of electrolyte containing Cl^- is important due to its capability of creating unspecified complex with ruthenium in the carbon paste. The current response of this complex were increasing until the 10th cycle of CV. It could be explained by the presence of unreacted ruthenium on the surface until that. The current response were same after the tenth cycle [33]. The presence of AA caused linear decreasing of the current response at +1.03 V. This behavior could be likely to be explained by the competition between AA and Cl^- in the electrolyte for unreacted ruthenium in the paste. The limit of detection (LOD) 0.94 µmol.L^{-1} has been found. This method has been successfully applied for the determination of AA content in real sample of potato crisps with recovery 97.7 %.

This CPE modification was also used in another our study [34] The basic difference between these two works was using also of another cation of transition metal, e.g. Ni(II) or Co(II). However, the bare CPE has been used too. In case of determination of AA by creating complex with Ni(II), the ammonium buffer solution with pH 9.0 was used. The current response was recorded at about -0.310 V. Unfortunatelly, no change was determined in the presence of AA. Although, the results of AA-Co(II) was satisfactory at bare CPE, based on previous experience, the modified CPE/RuO_2 was used due to the improvement of the current response in the presence of Cl-. The AA-Co provided anodic oxidation peak at about -0.225 V in Britton-Robinson buffer (BR) of pH 3.0. Current values linearly increased with increasing content of AA. However, the applying on the real sample has not been successfull probably due to reaction of Co(II) with used Carrez reagents during sample preparation.

Biosensors for determination of AA has been also investigated. One of the first biosensors to determine AA in aqueous food extracts is that with hemoglobin (Hb) immobilized on a surface of working electrode. As is known, Michael's reaction produces adducts between the AA and the amino group of valine at the N end of the hemoglobin polypeptide chain [14, 21]. Hemoglobin consists of four heme groups containing Fe(III). Electrode with Hb then exhibits a quasi-reversible oxidation-reduction reaction of Hb-Fe^{3+}/Hb-Fe^{2+}. The interaction between Hb-Fe and AA being monitored on the reduction current response. Although the electrode reaction is very slow, the method provided very low detection limits ($1.2 \cdot 10^{-10}$ mol.L^{-1}) and is suitable for determining AA in food matrices. GCE, PGE, Au or glass electrodes can also be modified by Hb [35-37].

Acrylamide is able to bind to the purine and pyridine DNA bases by hydrogen bonding and form stable adducts. Li et al. developed an biosensor based on DNA immobilization on the GCE surface with a graphene layer (DNA / GO / GCE). DPV measurements were performed in pH 7.0 phosphate buffer, where two anodic oxidation peaks of DNA were recorded (+0.700 V corresponding to guanine and +1.000 corresponding to adenine). Due to the fact that these purine nucleic bases are part of the DNA macromolecule, the electron transfer of this reaction is slow. For this reason, graphene was used to modify GCE, which facilitates the transfer of electrons. This biosensor has proved to be stable (30 days) and suitable for the determination of AA in the linear range of $5.0 \cdot 10^{-8}$ to $1.0 \cdot 10^{-3}$ mol.L^{-1} [38]. Similar results were obtained by modifying of the gold electrode with single stranded DNA (ssDNA), with a guanine peak at +0.740 V [39]. Based on these findings, the optimization of AA determination by CPE/DNA using CV and DPV was performed. The working electrode was prepared by adsorption of DNA to the surface of standard CPE at a potential of +0.500 V for 300 seconds. The acetate buffer of pH 4.5 (ACB), phosphate buffer of pH 7.5 (PBS), and ammonium buffer of pH 9.5 (AMB) were used as supporting electrolytes. Two oxidation peaks at +0.665 and +0.918V were observed in CVs in the PBS, which is similar result as in previous studies. No measurable current responses were recorded in AMB, whereas two oxidative waves of DNA were recorded in the ACB at potentials of +0.930 V and +1.070 V. The second wave disappeared in the presence of 0.2 mg/mL of AA. This work was carried out in the laboratories of the Karl-Franzens University of Graz (Uni Graz) during one-month foreign internship. The optimization measurement was not completed due to the short timeframe for work. Unfortunatelly, the same results were not obtained in laboratories of the University of Pardubice (UPce). Different types of electrodes (CPE, GCE, printed electrodes commercially available and prepared in the laboratory) and two types of instrumentation devices (PalmSens, Autolab) were tested. However, the corresponding current response of the probable AA-guanine adduct was not observed.

It has been proved that amino acids lysine, cysteine and glycine can inhibit AA formationn. The amino group of glycine (Gly) and lysine, and sulfanyl group of cysteine are able to form adducts with double bond of acrylamide [14]. Acrylamide also forms adducts with taurine (Tau), a derivate of cysteine. The principle is the reaction of double bond in the AA molecule with the sulfanyl group of taurine. The resulting compound was detectable by liquid chromatogramy [40]. However, the formation of this compound has not been proved electrochemically. An indirect AA determination study was conducted using CV and SWV on CPE where AA and

Tau were present in solution. The 0.1 mol.L^{-1} H_2SO_4, NaOH and KCl were used as supporting electrolytes. However, no oxidation or reductive peaks were detected in the CV or SWV. Data from these measurements are not published.

As it was mentioned above, acrylamide is able to react with the amino group of glycine, as well as with polyphenolic substances [41]. Glycine, a nonessential amino acid with a nonpolar side chain, reacts with the AA double bond to form the adduct. Polyphenolic substance quercetin (Q), belonging to the flavonoid group, has antioxidant properties and providing an oxidative peak in an acidic medium using PGE [42]. The reaction kinetic of AA with Gly is temperature dependent. No response was recorded in the SWV of mixture of AA and Gly. However, two oxidation peaks (-0.110 V and +0.088 V) were recorded when the mixture (AA/Gly, both of concentration 1.10^{-3} mol.L^{-1} in BR buffer of pH 7.0) had been heated to 100 ° C for 60 minutes. The oxidation peak of the mixture was observed at +0.160 V and the reduction peak at -0.590 V in the BR buffer of pH 2.0. However, the proposed method was low sensitive for the determination of AA in real samples. The using of Q for determination of AA was also studying. The adduct provided a reduction peak at +0.128 V for the unheated AA/Q mixture in BR pH 7.0. When the mixture has been heated to 100 °C for 60 min, the peak at the same potential was observed but with almost twice time lower current value. Q alone in the fresh solution did not provide a peak. However, after the heating the reduction peak of quercetin at +0.140 V had been observed. No current respones were observed in BR buffer of pH 2.0 or 10.0. It is clear that the AA/Q mixture in an acidic medium exhibits electrochemical behavior that can be attributed to the formation of a polymeric structure on the surface of the working electrode [41, 43].

4 Vitamins

Vitamins represent a diverse group of organic substances having a character of essential nutrients. Generally, the human organism is not able to produce them itself. Therefore they must be obtained from food. Functions of vitamins in the human body are several. They ensure catalytic processes in the body, thus helping to absorb substances in the organism. They participate also metabolic conversions of sugars, fats and proteins. Vitamins have a demonstrable effect on immune system. They are essential for the proper functioning of the body and can also affect treatment of diseases. Vitamins are divided to two group, watersoluble and lipophilic.

4.1 Lipophilic vitamins

Fat-soluble vitamins are generally classified into four main groups such as vitamin A (carotenoids), D (cholecalciferols), E (tocopherols), and K (phylloquinones).

Retinol (vitamin A_1) is essential substance which our organism cannot create therefore it must be taken from diet. In a narrower sense, it belongs to group known as retinoids. Retinol can be degraded by oxygen, high temperature or light. The content of vitamin A in foodstuffs depends on good storage and packaging, thus the content of retinol can be an indicator of freshness of food or of the good storage conditions. Synthetic retinoids (retinyl acetate and retinyl palmitate) are usually used as food additives due to chemical stability at presence of oxygen and light exposure. Deficiency (avitaminosis) of vitamin A leads to the night blindness or keratinization of cells. It is often disease of children, especially occurring in the developing countries. On the other side, its surplus (hypervitaminosis) can cause a poisoning. Especially, teratogenic properties were declared. However, it should be mentioned that the content of vitamin A in foodstuff and food supplements is very low. For that reason, poisoning caused by overdosing by this vitamin A_1 is rare. [44, 45]. Nowadays, retinol is predominantly determined by UV spectrometry or HPLC, which require long and difficult sample preparation and also very expensive instrumentation [46].

Cholecalciferol (D_3) and ergocalciferol (D_2) together create a vitamin D group. Cholecalciferol is produced in skin after exposure to sunlight from provitamin 7-hydrocholesterol. The dietary need of vitamin D is therefore associated with the exposure of the organism to sunlight. Its shortage is more often manifested in the winter. Ergokalciferol is produced in plants also by UV light from provitamin ergosterol. Vitamin D_3 is predominantly present in food of animal origin. Ergosterol, from whos ergocalciferol are create, are present in plant food, e.g. carrots, cabbage, spinach, mushrooms. There is a vitamin D decrease during food processing, similar to other lipophilic vitamins. The degree of degradation is dependent on both, the type of food and the way of storage and heat treatment. Great influence on the degradation of vitamin D also has UV radiation. Vitamin D deficiency leads to rickets, skeletal changes, deformation or softening of already developed bones. It also affected the absorption of calcium and phosphorus in the body. It is necessary to remove matrix effect for determination of vitamin D in food, ie to extract vitamin from fatty substances. This mainly includes saponification of fats with alkaline hydroxide and subsequent extraction with organic solvents with possible purification of sample extract. LC-MS / MS can also be used to determine individual vitamin D analogues with each other, but only after using saponification and extraction after protein

precipitation [47]. Vitamin D can be determined electrochemically in organic, aqueous and aqueous-organic solutions where it undergoes an irreversible single electron oxidation reaction.

Vitamin E contains tocol derivatives exhibiting vitamin activity, tocopherols and tocotrienols. Both of these substances can be referred as α, β, γ and δ in dependence on the functional groups attached to the 5 ', 7' and 8 ' positions of chromane ring in the molecule. α-tocopherol exhibits the highest vitamin activity. It's natural stereoisomer occurring predominantly in foods of plant origin. Its content in food of animal origin is dependent on its content in animals feed. Other isomers of tocopherol and tocotrienol occur predominantly in plant foods and have a lower level of vitamin activity compared to α-tocopherol. Tokotrienols have a lower bioavailability activity compared to α-tocopherol due to the presence of double bonds in the side chain. Tocopherols and tocotrienols are very nonpolar substances, relatively stable in absence of oxygen and oxidative lipids, and have high antioxidant properties. Synthetic α-tocopherol acetate is used to fortify foods, mainly plant oils and cosmetics. It is a racemic mixture of all possible α-tocopherol stereoisomers, also called all-rac-α-tocopherol. α-tocopherol is used to reduce production of nitrosamines in sausages during smoking. There are losses of vitamin E during storage and processing of food depending on conditions such as time, temperature and humidity. The largest part of his degradation during storage is attributed to the natural oxidation of lipids and its antioxidant properties. Generally, losses of vitamins can occur when the food is cooking, baking, frying, drying, freezing, etc. Vitamin E is used to prevent cardiovascular diseases, slows down the aging process. The nutritiopn needs of vitamin D is predominantly covered by consuming of vegetable oils, but also an important source is meat, milk and dairy products, fruits and vegetables [48, 49]. The saponification is used for the separation of fats from vitamin E. The alkaline hydrolysis causes the transformation of all vitamin E esters on α-tocopherol. The oxidation reaction during sample preparation may need to be taken into account. The oxidized tocopheroxyl radical, which can be convert back to the α-tocopherol by ascorbic acid (vitamin C), is formed during reaction between free radicals and vitamin E. Generally, the liquid chromatography for the determination of vitamin E in foods, biological matrices and cosmetic products, are mainly used with combination of UV, fluorescence or amperometric detection [45, 46, 50, 51].

Vitamin K consist of menadione derivates, phylloquinone (K_1), and menachinone (K_2). These substances are composed of naphthoquinones with a side chain isoprene units in the 3´ position. Menadione is an unsubstituted naphthoquinone (at position 3 has only a methyl group). Sometimes it can be referred to as vitamin K_3. It's synthetic form of vitamin K which is used for fortification of food and feed. Phylloquinone is formed in plants, large amount is contained in leafy vegetables including spinach or cabbage. Menachinon is a product of intestinal microflora. Vitamin K contributes to the treatment of osteoporosis, supports hardness of the bones. Vitamin K deficiency is very rare in healthy individuals due to its formation in the colon. It may occur in case of a liver disease or during treatment of blood coagulation. The vitamin K deficiency is manifested as the blood clotting disorder. Vitamin K is relatively stable to high or low temperature. The degradation can occur under the influence of light. Determination of vitamin K can be done by using of spectrophotometric methods. However, the HPLC method is used to determine the

individual forms of vitamin K with UV, fluorescence, electrochemistry, chemiluminescence and mass detectors [45, 52, 53].

4.1.1 Electrochemical determination of lipophilic vitamins

It is desirable to perform the determination of lipophilic vitamins in mixed aqueous-organic or purely organic electrolytes due to the nature of lipophilic vitamins. However, in this case the use of CPE and some other electrodes is limited by the content of the organic component in the electrolyte.

As representatives of lipophilic vitamins (retinol (vitamin A_1), cholecalciferol (vitamin D_3), α-tocopherol (vitamin E), phylloquinone (vitamin K_1) has been chosen as main analytes for their sensitive simultaneous electrochemical detection at GCE. The adsorptive stripping voltammetry (AdSV) was used as method for measurements. The AdSV is a typical very sensitive electroanalytical method which is realized in ex-situ mode. In the first step, an electrode is immersed into mixed solution containing certain analyte (aqueous organic mixture, pure organic solvent, or their mixtures). Accumulation of this analyte is mediated by adsorption onto nonpolar surface of working electrode (unmodified graphite or glassy carbon). In the second one, the enriched electrode by analyte is replaced into selected supporting electrolyte (pure aqueous solutions) and electrochemical detection is done. The 50% acetonitrile solution with the content of 50 $\mu mol.L^{-1}$ of vitamins standards was used as adsorption medium. The ACB of pH 4.5 was used as supporting electrolyte. It is necessary to realize that vitamin K is present in its oxidized form and therefore it has to be reduce it before its own analysis. This was done by applying a potential of -0.6 V for 120 s. The current responses of chosen vitamins were determined in one analysis by DPV at +0.015 V, +0.337 V, +0.737 V and +1.074 V for phylloquinone, α-tokoferol, retinol and cholekalciferol, respectively. These potential values are in suitable distance so all of these vitamins can be determined in one analysis. The method was applied on the real sample of margarine. Vitamin K and E were determined. This phenomenon can be caused by the composition of the margarine which is consist of emulzicated plant oils [54].

Our next study was focused to the electrochemical determination only of retinol (vitamin A1) at different working electrodes was done. The adsorptive stripping voltammetry (AdSV) at solid glassy carbon electrode (GCE), carbon paste electrode (CPE) covered by thin layer of single layer graphene (CPE/Graphene), CPE covered by thin layer of multi-wall carbon nanotubes (CPE/MWCNTs) was compared. Moreover, mentioned types of AdSV were compared with an extractive stripping voltammetry (ExSV) at glassy carbon paste electrode (GCPE) based on silicone oil as lipophilic binder. In this study, it was found that retinol extraction into silicone oil provided better analytical parameters than others [3]. Based on this finding, the method was applied for the determination of retinol in real samples of milk, where extraction was realized directly from sample matrix. Results was satisfactory but with low reproducibility due to no sample preparation. These data has never been publicated.

The aim of following studies was describe electrochemical properties of carbon paste electrodes modified with surfactants and their using in a voltammetric determination of lipophilic vitamins with antioxidant properties. These substances are used as additives in food and cosmetic products due to their nutrition and preservative

properties. Carbon paste electrodes modified with the anionic surfactant sodium dodecyl sulphate (CPE/SDS) or the cationic surfactant hexadecylpyridinium chloride monohydrate (CPE/CPC) represent specific sensors which can be used for voltammetric measurements in pure organic solvents. These substances were tested as possible modifiers for the carbon paste. It has been obtained that selection of type and content of the surfactant used as modifier for carbon paste electrode significantly influences electrical conductivity and mechanical stability in pure organic solvents, which plays a significant role in the voltammetric determination of mentioned vitamins. Despite the positive correlation between the content of CPC and ohmic resistance of CPEs, this surfactant cannot be used due to high current values of the base line. However, the using of SDS as modifier of CPE provided satisfied current responses during determination of lipophilic vitamins. Although CPE with 40 % of SDS provided better plotting of peaks, the corresponding current responses were decreased. Nevertheless, it has been proved that CPE with 30 % of SDS provides satisfactory results for the determination of lipophilic compounds in pure organic solvent. All-trans-retinol (vitamin A), retinol acetate or palmitate, tokoferol acetate and α-tocopherol (vitamin E) have been chosen as typical examples of analytical samples. In previous studies, it was found that α-tocopherol provides only one oxidation peak in a non-aqueous solution [55]. On the other hand, retinol acetate provides three oxidation steps under the same conditions [56]. However, potential values of these three oxidation peaks of the retinol acetate are sufficiently distance from the potential value of α-tocopherol peak. Thus, the peaks of retinol acetate and α-tocopherol can be distinguished well.

The electrode oxidation reaction of retinol has not been clearly elucidated yet. Initially, $2e^-/2H^+$ was mentioned [57]. However, due to the similar electrochemical behavior of retinol esters, it is likely the $2e^-$ and H^+ irreversible reaction. Retinoids provided an oxidation peak in the range of +0.500 V to +0.900 V depending on the used electrolyte and a working electrode [58, 59]. Vitamin A contains a conjugated double bonds system in its structure for which it is possible to determine it spectrophotometrically in the UV/VIS region. It has been found that the highest electron density is located in this dual system linkages between C5 to C14 carbons, so the oxidation of retinol is likely to occur at these sites [60].

The CPE with 30 % of SDS was successfully applied on determination of tocopherol acetate in cosmetic products [61]. The DPV was carried out in 0.1 M $LiClO_4$ in 99.8 % acetonitrile. All three forms of vitamin A provided the first oxidation peak in the +0.800 V range, so they can not be distinguished from each other, just like individual forms of vitamin E [60].

4.2 Watersoluble vitamins

Vitamin B, the so-called B-complex, includes vitamins B_1 (thiamin), B_2 (riboflavin), B_3 (niacin), B_5 (pantothenic acid), B_6 (pyridoxine), B_7 (biotin), B_9 (folate, folic acid), B_{12} (cobalamin) and PP (nicotinic acid). None of these vitamins were determined in any of our studies, so they are not in attention in following pages.

4.2.1 Vitamin C – ascorbic acid

Due to their antioxidant activity the L-isoascorbic acid and L-ascorbic acid (ascorbic acid-AAc) are used as food additives. They serve as substances suppressing enzymatic browning, e.g. fruit or vegetables. Isoascorbic acid has no nutritional significance as well as D-ascorbic acid. AAc is used as a fortification agent in the meat industry for reduction of production of nitrosamines, toxins arising from meat processing, e.g. smoking or addition of nitrites and nitrates. Vitamin C can also be used to fortify oils and oil emulsions in combination with tocopherol or in the form of a lipophilic ascorbyl palmitate derivative. The sodium salt of L-ascorbic acid can also be used for the fortification. Lack of vitamin C causes disease scurvy. It is manifested by increased bleeding, inflammation of the gums and teeth. AAc is naturally presents in fruits and vegetables, in food of animal origin in a small extent due to metabolic transformations in living organism. AAc is highly soluble in water, which may make it significant loss during handling of fruit and vegetables. Vitamin C is easily oxidized by the environmental conditions such as high temperature, water content, light, pH, other accompanying substances, etc. Oxidation of L-ascorbic acid leads to dehydroascorbic acid (DHAAc), which is further hydrolyzed to nutritionally insignificant products. These reactions are dependent on the pH of the medium, the oxygen concentration, temperature, and also the presence of catalysts such as metal ions, e.g. copper. Several methods are used to determine vitamin C. Pure AAc absorbs in ultraviolet spectrum at 245 nm. However, it can not be used in the food industry to a large matrix effect. Traditional titration methods can be used for the determination of AAc, but there is a high risk of interference. The most accurate method is HPLC in combination with a spectrophotometric, fluorescence, mass or electrochemical detector. The HPLC method also makes it possible to determine individual forms of ascorbic acid side by side [45, 62-64].

4.2.2 Electrochemical determination of vitamin C

Ascorbic acid is an electroactive substance and can therefore simply be determined by using electrochemical methods. Electrochemical oxidation of AAc is diffusion controlled process dependents to the pH of the solution. Two protons and two electrons are exchanged during oxidation of AAc to DHAAc. It is a reversible reaction. The 2,3-diketo-L-gulonic acid is formed by irreversible reaction at a pH of less than 4. Electrochemical oxidation of ascorbic acid can be measured on different electrodes (GCE, CPE, Pt, etc.) using voltammetric methods. Oxidation peak potential is variable depending on the type of working electrode and used electrolyte. The CV and DPV method for the determination of AAc in fresh samples was investigated fruit juices and vitamin supplements. The very simple sample preparation was an advantage. The tablet of vitamin C was simply dissoluted, followed by filtration and centrifugation. Juice sample was diluted in water. DPV method on GCE showed

very good signal stability [65]. The DPV method for determining AAc was also applied on wine samples using Pt and CPE [66].

Determination of AAc using CPE modified by MWCNTs and brilliant cresyl blue (BCB) was performed in our study [67]. Modified CPE (BCB-MWCNTs / CPE) was made from 0.5 g of CR-5 carbon powder (Maziva Týn nad L., s.r.o., Czech Republic) and 130 μL of mineral oil (M5904, Sigma-Aldrich, Germany) with an adding of 1.0 % MWCNTs (40-60 nm, Shenzen Nano Tech Port Co., China) and 3.0 % of BCB (Sigma-Aldrich, Czech Republic). BCB in the paste improved charge transfer. MWCNTs increased the surface of the electrode. The BCB showed a reversible oxidative reduction reaction in CV in BR buffer of pH 5.0. The reaction has been diffusion-controlled. AAc on BCB-MWCNTs/CPE in CV provided an oxidative-reduction response with anodic peak at +0.125 V and cathodic at +0.075 V. Amperometric detection was used to determine the AAc content. The method showed a linear range from 1.0 to 350 $\mu mol.L^{-1}$ with a detection limit 0.05 $\mu mol.L^{-1}$. Multivitamin juice and a dietary supplement, both commonly available in stores, were used as a samples. The juice was not modified before the analysis. The vitamin C tablet was only dissolved in 250 mL of redistilled water. Amperometric detection on BCB-MWCNTs/CPE achieves comparable values with HPLC-UV (juice: amperometry - 120.1±5.2 $mg.L^{-1}$, HPLC-UV - 120.5±4.7 $mg.L^{-1}$; tablet: amperometry - 25.8±0.5 $mg.L^{-1}$, HPLC-UV - 25.2±0.4 $mg.L^{-1}$). The electrode stability was also monitored. In normal storage at room temperature, the electrode showed almost identical values even after 45 days.

5 Other analytes

In addition to the electrochemical determination of acrylamide and vitamins, this dissertation thesis also includes the determination of flavonoids and electrochemical evaluatio of the oxidative stability of edible oil.

5.1 Electrochemical determination of flavonoids

Flavonoids are organic phenolic plant substances formed from the amino acids of phenylalanine and tyrosine and malonic acid salt. The basic structure of flavonoids is the flavon nucleus containing a fifteen carbons. The nucleus consists of three rings A, B and C. Rings A and B are aromatic, ring C is heterocyclic. Hydrogen atoms in all three rings can be replaced by three major substituents, hydroxyl (-OH), acetyl (-OR) or sugar to form a glycoside. Flavonoids are divided to flavones, flavanones, flavonols, flavanonols, isoflavones, catechins and anthocyanins depending on the functional substituents of the flavon nucleus, degree of hydroxylation, conjugation or polymerization. Flavonoids are naturally occurred in plants, including fruits and vegetables, in seeds, nuts, spices, herbs, legumes, or sprouts. They can also be found in tea, beer, wine or herbal beverages. They are responsible for coloring the plants into shades of orange, red and yellow. From the point of view of human nutrition, flavonoids are interesting mainly for its antioxidant properties. However, flavanoids have proven anti-inflammatory or anti-viral and anti-allergic properties. They also contribute to strength blood capillaries, exhibit chelating properties. Daily intake of flavonoids is around 1-2 g and is mostly taken via fruit and vegetables. Considering their beneficial effect on health, the great deal of attention is paid to flavonoids in the pharmaceutical industry. Different foods are enriched with flavonoids or there exist dietary supplements that help fight diabetes, cardiovascular disease, cancer, or menopause symptoms [68-70].

Flavonoids are usually extracted from the samples with solvent followed by solid phase extraction (SPE). Liquid-liquid or Soxhlet extraction can also be used [71]. The thin film chromatography can be used for qualitative determination of flavonoids. Colorimetric method, where the flavonoid complex with aluminum chloride is formed, can also be used for determining of total flavonoids content in the methanolic or ethanolic sample extract [72]. The most often used methods for its quantitative determination are chromatographic methods with various detections or capillary electrophoresis (CE) [73, 74]. Flavonoids are easily subjected to oxidative-reduction reactions [160]. Oxidation reduction reactions of flavonoids are used in their electrochemical determination. Electrochemical methods used for the determination of flavonoids include coulometry, voltammetry, but also electrochemical detection in chromatography or biosensors. The electroactivity of flavonoids is linked to circles A and especially B and the phenolic groups present. Another reason for flavonoids electroactivity may be the delocalization of electrons influencing the radical intermediates, which may caused several electrode reactions occur [75-77].

5.1.1 Quercetin

Quercetin (Q) is the best described flavonoid. It belongs to the group of flavonols. The highest content can be found in fruits (apples, berries) and vegetables (broccoli, onion, parsley). It occurs predominantly in the form of a glycoside (with rhamnose as quercitrin or isocquercitrin, with rutinose as rutin).

Quercetin has the most powerful antioxidant effects of all flavonoids, is capable of neutralizing peroxonitrile and hydroxyl radicals. It also has antifibrotic, anti-tumor, anti-inflammatory or antibacterial properties, can protects against osteoporosis, has a positive effect on the cardiovascular system. Together with the catechins present in a tea quercetin is able to regenerate α-tocopheroxyl radical to α-tocopherol [78, 79].

Generally, methods developed for determination of flavonoids can be used for determination of Q. The mechanism of electrochemical oxidation of the quercetin can take place on all five present -OH groups. One to four oxidation peaks can be observed in potential areas of +0.150 V, +0.300 V, +0.600 V and +0.800 V depending on the pH and type of used medium and on the working electrode. First two oxidation peaks being best visible in the acidic and neutral pH medium, the remaining two appear only as tiny waves and completely disappear in an alkaline medium. The reversible behavior of the first of the peaks is attributed to catechol on circle B. It is the reaction of two electrons and two protons. The irreversible behavior of the second peak corresponds to the hydroxyl group at position 3 on ring C. The remaining two peaks are result from reversible reactions of the hydroxyl groups at positions 5 and 7 of circle A. The resulting oxidation product of the quercetin is able to adsorb on the electrode surface and thereby disable the surface for another reaction [75].

In our study, Q was determined in samples of commonly available juices by using of anodic stripping differential pulse voltammetry (ASDPV) at a pencil graphite electrode (PGE) [42]. The bare pencil lead was modified before every measurement by applying potential +1.450 V for 60 s in 0.1 M PBS of pH 7.0 with 0.1 M KCl. This modification is applicable also for other types of carbon electrodes. It serves to oxidize the surface layer of carbon of the electrode material and thus it becomes more hydrophilic, repels lipophilic binding molecules and exposes the hydrophilic surface of the unoxidized carbon. Resulting hydrophilized PGE surface is more similar to compact carbon materials, such as glassy carbon. It has a better sorption and kinetic properties, thereby increasing the reversibility of electrode reaction. Quercetin showed a quasi-reversible oxidative reductive reaction, so this electrode modification was desirable. The scan rate, pH of the supporting electrolyte, accumulation potential and time has ben optimized. Electrochemical behavior of Q on PGE has been investigated using DPV in a BR buffer with pH 7.0 at different scan rates. The current responses of quercetin at +0.370 V increased linearly with an increasing scan rate, which suggests that the electrode response is dependent on Q adsorption on the surface of the PGE. During optimization of the pH of the electrolyte it has been found that it affects both the resulting peak potential and the magnitude of the current response of Q. Peak potential of Q increased with increasing pH toward negative values. The current response of Q was highest in BR buffer of pH 3.0 at potential +0.370 V. The oxidation peak of the quercetin was not recorded at pH 8 and above. The acumulation potential 0.1 V and acummulation time 120 s were found as optimal. Extracts of blackcurrant and cranberries juices were used as samples. The sample was prepared by centrifugation for 20 minutes at 2650×g followed by extraction of the supernatant into 2.0 mL of methanol. For the measurement, 50 μL of extract was placed into the supporting electrolyte (0.1 M BR buffer of pH 3.0) in an electrode measuring cell. This sample modification before the analysis is necessary due to the presence of sediment and colorants in patterns that cause significant increasing of the background current. The calibration curve method

was used for quantification of Q. The linear range of the method was from 0.001 to $1.5 \cdot 10^{-6}$ mol.L^{-1} with limit of detection (LOD) $3.0 \cdot 10^{-10}$ mol.L^{-1}. This method has achieved good results compared to other voltametric assays for determination of Q using other types of electrodes.

5.1.2 Rutin

The molecule of rutin consists of quercetin and disaccharide rutinoses at position 3. It is a glycoside of quercetin. It belongs among flavonols, naturally occurring in fruits, potatoes, onions, tomatoes, spices, herbal beverages and teas. Rutin (R) is recommended for diabetics. It works as an inhibitor of carbohydrate absorption in the intestines, stimulates insulin production, increases the absorption of sugars into tissues. R has the ability to bind hydroxyl, peroxyl and the superoxide radical, acts anticancer and antimicrobial. Rutin has the significant effect as an antithromboticum. The daily intake of rutin is changing depending on dietary habits between 1.5-70 mg/kg bodyweight per day. The method of rutin encapsulation into phosphatidylcholine has been proposed acilitate solubility and bioavailability for the fortification of food and food supplements [80-82].

Determination of rutin can be done by several methods, same as like other flavonoids. Various methods have also been proposed for the electrochemical determination of the rutin using different types of electrodes and their modifications. Rutin provided in CV quasi-reversible two-electron oxidation-reduction reaction. This reaction is adsorption-dependent. The potential of the oxidation peak was at +0.440 V and a reduction at + 0.220 V. It correspond to the catechol reaction on ring B [83]. Relatively frequent modifications of CPE for rutin determination include modification by ionic liquid [84, 85].

The determination of rutin at CPE modified by silica gel (SG) was investigated in our study [86]. SG was choose as modifier due to its properties to increase the surface of the electrode. The cyclic voltammetry was used to evaluate the behavior of R and also CPE/SG. The SG content in the paste was optimized. The CPE with a SG content of 15 % was selected for further measurements due to the highest current values for rutin. Several electrolytes were also used to optimize the method. After statistical evaluation of the measured data (two-factor analysis ANOVA), the 0.1 M HCl was selected for further measurements as the most suitable electrolyte. The CV and SWV results also shows that the current responses decreased with the increasing pH and peak potential shifted toward negative values. Linear dependence of peak potential at the pH of the electrolyte indicates that the ratio of exchanged protons and electrons in the reaction was 1: 1, which corresponds to the previously proposed rutin electrode reaction mechanism ($2e^-/2H^+$).

Due to the fact that rutin is the glycoside of quercetin, and also that both of these flavonoids are present in real samples together, the evaluation of interferences of Q for R determination has been done. Quercetin in 0.1 M HCl medium at CPE/SG provided a peak in the same potential area, i.e. around +0.500 V, as a rutin. If the both compounds were present in the solution, the two peaks were combined in one with two vertices. Both of the vertices were shifted to negative values compared to peak potentials of pure substances. Statistical evaluation of the calibrations of the rutin in the presence of different of the quercetin content was done. It was found that the presence of $5.0 \cdot 10^{-6}$ mol.L^{-1} Q already affects the determination of R. We are able to

deduce that although these two flavonoids have peak potentials in sufficient distance, if both of them were present in the sample we were not able to distinguish them.

5.2 The evaluation of oxidative stability of edible oil

Rapeseed oil is produced from a Brassica napus L. This plant occupies a significant part of agricultural production not only in the Czech Republic (394 thousand ha for year 2017 [87]). Rape seeds contain about 45 % of oil, which is mainly obtained by pressing, extraction techniques may also be used. The method of oil extracting from oil seeds influences not only the properties and composition of the final product but also the corkscrews remaining that are used as animal feed. The basis of obtaining vegetable oils from oil plant seeds is generally a cleaning of seeds from different admixtures with a series of sieves and magnets, peeling, and subsequent extraction oil from seeds and refining the extract. Rape oil is the third most produced edible oil. It is characterized by the composition. It has the most advantageous ratio ω-3 and ω-6 unsaturated fatty acids acids, the lowest content of saturated fatty acids compared to other vegetable oils and the highest content of phenolic substances [88]. Vegetable oils are easily oxidized. The product of the oxidation process are aromatic compounds that give the oil an unpleasant taste and smell, and radicals. This oxidation process is referred to as rancidity. The rate of oil oxidation is depending on the type and composition of the oil, the storage and production conditions (e.g. temperature, air access and light), the content of antioxidants and metal ions, but also on the method of oil obtaining. Fatty acids bonded to triacylglycerols (TAG) are responsible for the oil oxidation. The process of oxidation of TAG includes the cleavage of fatty acids double bonds to form primary products - hydroxy peroxides, tasteless and odorless. However, these primary oxidation products are unstable and secondary oxidation products are create of them. Secondary TAG oxidation products are aldehydes, ketones, esters, alcohols and others. Hydroxy peroxides are formed by autooxidation, photooxidation or enzymatically. The mechanisms of these types of oxidation are similar, the difference is in the kind of formed radicals. Several methods for determination of rancidity has been develoepd. The sensorical analysis is considered as the most important. The peroxide value and p-anisidine value or Totox are also used for it. The peroxide number (PV) indicates the peroxide content in the sample, which is the primary oxidation products, thus it indicate the degree of oxidation of the oil. The principle of PV determination is dissolving of a fat sample in chloroform with acetic acid addition and excess potassium iodide followed by titration of the resulting iodine with a sodium thiosulphate solution . On the other hand, the p-anisidine number (AV) indicates the content of aldehydes, the secondary products of the oxidation. They react with anisidine to produce color products which absorbance is measured at 350 nm. AV value is an important for evaluation the overall quality and the course of production and distribution of oil. It is posibble due to the fact that secondary oxidation products are hardly removable. The Totox value is calculated according to the equation AV + 2 PV and it gives the overall oxidation state of the oil. As much as this value is lower, the oil quality is better. Primary lipid oxidation products can also be detected polarographically, chemiluminescence or HPLC. Secondary oxidation products are often determined by HPLC with UV detection after derivatization with 2,4-dinitrophenylhydrazine. The gass

chromatography can also be used for determination of the volatile secondary oxidation products [89-91].

The above mentioned methods express the current degree of fat oxidation. It is necessary to measure characteristic oxidation products at different times intervals at different temperatures for evaluation of the oxidative stability of fats and oils. Nowaday, this measurement are realized by Rancimat from the Methrom company. This machine is based on the principle of controlled accelerated oil oxidation by higher temperature and air access to the sample followed by detection of secondary volatile oxidation products. These volatile substances are accumulated in the distilled water tank, thereby changing its conductivity. The disadvantage is the acceleration of oxidation, which does not work exactly the same as in the case of standard oil storage [92, 93].

Relative novelty in the analysis of volatile substances in various industries including food industry is the use of so-called electronic noses (ENs) and tongues (ETs). Electronic noses and tongues are a set of electrical and chemical sensors that measure individually different properties (conductivity, current, resistance, etc.) of volatile compounds. The individual results are evaluated together by mathematical and statistical methods [94-96].

Electrochemical methods found use in oil analysis mainly for detection and the determination of the content of present synthetic and natural antioxidant and phenolic compounds [97, 98]. The Clark oxygen electrode for the determination of dissolved oxygen cab be used [99]. However, methods for detection and determination of primary oxidation degradation products of oils can rarely be found in literature. Adhoum et al. (2008) applied the glassy carbon electrode modified by prussia blue for detection of hydroperoxides in vegetable oil samples. The method was an alternative to titration determination of the peroxide number [100]. The SWV at Au and Pt electrode was used to determine the oil type and to evaluate its oxidation state. The method was not recommended for species oil resolution but was appliable for resolution of oxidized and unoxidized samples [101].

Determination of the oxidative stability of rapeseed oil by the CV method using CPE have been studied in our work [102]. The method is based on the modification of the CPE by oil sample similar to that of Apetrei et al. (2005) [103]. The electrode was prepared by mixing of 0.5 g of carbon powder (CR-5, Maziva Týn n. L. s., Czech Republic) with 140 μL of rapeseed oil sample. Three types of oil were used as samples. Two samples were commercially available in Czech stores. The third sample was purchased from Sigma Aldrich spol. s.r.o. (Prague, Czech Republic) and is not intended for human consumption. 150 mL of each sample was put into a glass beaker and left stand at room temperature under air and daylight for 40 days. Peroxide and p-anisidine values of every sample were determined after 0, 7, 28 and 40 days. Totox values were then calculated from the results. A carbon paste was also prepared from each of the samples and CV measurements were made. The pH of supporting electrolyte has been optimized and 0.1 M HCl of pH 1.0 was selected. Anodic oxidation waves occurred in the area of potential +0.575 V to +0.600 V, cathodic reduction waves in the +0.400 V to +0.425 V range. The increasing pH leads to a significant shifting of various phenolic substances signals. It was observed that the oxidation-reduction response dissappeared at pH higher than 3.0. For that reason, the HCl was choose for the next measurement. This was also a sign of the fact that the

oxidative reduction responses were not the result only of the reaction of the phenolic substances present in the sample. With the increasing oxidation time of the samples, the current responses increased. The obtained data were then statistically evaluated using Origin Pro Software using the Spearman Correlation Coefficient (r). No dependency was detected for current responses and AV values. This is related to the fact that AV denotes the value of secondary oxidation products whose content varies with time, and is therefore indicator of the momentary degree of oil oxidation. For PV values for individual samples over time and corresponding current responses of anodic and cathodic current values, some correlations have been found, but not significant. For Totox values calculated from PV and AV and the corresponding data from the CV records, a positive correlation was found. Observed shifts in peak potential values did not show any correlation. Although connections have been found between Totox and current values, it could not be mathematically described and applied to all samples of rapeseed oil. For this reason, the data were evaluated for each sample separately. Each one of them had a linear correlation for both Totox and anodic and cathodic current responses.

6 Conclusion

Several types of carbon electrodes, namely glassy carbon electrode, carbon paste and glassy carbon paste electrodes and their various modifications, and pencil graphite electrode have been tested in the work. A few methods have been developed to determine the selected analytes predominantly in food matrices. Electrochemical methods have been optimized using the above mentioned electrodes for the determination of acrylamide, selected vitamins, flavonoids, and also the CPE for evaluation of the oxidative stability of rapeseed oil was used.

To the present day, several methods have been developed to determine acrylamide. Mercury electrodes are for determination of acrylamide the most suitable because of the high potential of acrylamide reduction. However, these methods are undesirable for use in the food industry. Considering to the impossibility of using unmodified carbon electrodes at such wide potential window such as mercury electrodes, it is necessary to modify the another electrodes or to use the reaction of acrylamide with other substances in the solution and thus allow its determination. Reactions of acrylamide with taurine, DNA molecules which form electrochemically detectable adducts with AA, reactions with flavonoids, transition metal cations, e.g. with Ni(II) or Co(II) and modification of CPE by admixing RuO_2 into carbon paste were tested. No reaction with taurine or DNA with AA was detected. The reaction of acrylamide with quercetin or glycine showed a very low sensitivity ($1.0 \cdot 10^{-3}$ mol.L^{-1}), therefore could not be applied to real samples. The Ni(II) ions were not sensitive to AA concentration. In contrast, AA with Co(II) provided satisfactory results. However, the method could not be applied to real samples, probably due to the reaction of cobalt ions with the Carrez reagents used in the sample preparation. The using of CPE modified by RuO_2 was succesfully optimized. This method showed a detection limit of AA of 0.94 mol.L^{-1} with recovery 97.9 %, and was successfully applied to real samples of potato chips.

Several methods for the electrochemical determination of lipophilic vitamins have also been developed . The stripping voltammetry methods were tested for their determination on both solid and paste carbon electrodes. The accumulation step was performed in aqueous-organic mixture and the detection was done in a purely aqueous medium. The method of adsorption stripping voltammetry has been successfully applied to the determination of all lipophilic vitamins in margarine in one analysis. The AdSV method at a glassy carbon electrode and a modified paste electrodes was then compared with extractive stripping voltammetry on a glassy carbon paste electrode. It was found that ExSV into the paste is the more effective method for determination of vitamin A with a limit of detection $4.5 \cdot 10^{-7}$ mol.L^{-1}. Due to the insolubility of lipophilic vitamins in aqueous solutions as well as the impossibility to use paste electrodes in an organic medium, the surfactant modified CPE were tested for determination of lipophilic vitamins. After optimization, CPE with 30 % of sodium dodecylsulfate was selected This electrode has been successfully used for the determination of retinoids and vitamin E in real samples of cosmetic products. Although the detection limit was only 17.0 mg.L^{-1}, the method can be applied to determine the vitamin E in cosmetics with respect to its relatively high concentration. Vitamin C was determined in a juice sample and a dietary supplement using CPE modified by brilliant cresyl blue. The detection limit was 0.05 μmol.L^{-1}.

Flavonoids quercetin and rutin were determined in this work. Quercetin was successfully determined in juice samples using anodic stripping differential pulse voltammetry at PGE,where the detection limit of the method was 0.0003 μmol.L^{-1}. CPE modified by silica gel was tested for determination of rutin. Due to the fact that the rutin is glycoside of quercetin, these substances are both preset in real samples. Therefore, an quercetin interference study has been conducted to determine the rutin. It was observed that when both substances were present in the sample solution, its peaks were merged into one and can not be distinguished from each other. For this reason, CPE/SG was not applied to real samples. To evaluation of the oxidative stability of rapeseed oil, carbon paste electrode modified by the sample was used. Satisfactory results have been achieved and it can be stated that CPE modified by the sample of oil as a pasting liquid can be used for these purposes.

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8 List of Students' Published Works

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