

## ABSTRACT

This PhD Thesis addresses the feasibility of applying electrochemical methods, e.g. cyclic voltammetry (CV) and differential pulse voltammetry (DPV), for determination of the reducing activity of phenolic compounds in the *in vitro* model studies and the antioxidant/reducing capacity of foods of plant origin rich in bioactive compounds. The electrochemical methods which are based only on the redox properties of antioxidants, belong to the direct methods and for this reason present an attractive alternative to the classical methods commonly applied for the determination of the antioxidant activity/capacity of food.

This study was aimed at finding a relationship between the antioxidant activity of the tested phenolic compounds and values of their oxidation potentials derived from electrochemical measurements, and at comparing their reducing activity derived from the electrochemical methods with their antioxidant activities obtained from the spectrophotometric methods (DPPH and FRAP). An attempt was undertaken in the study to identify which elements of the structure of phenolic compounds are responsible for their electrochemical properties as well as reducing activity under *in vitro* conditions.

Due to the high content and multiple forms in which phenolic compounds are found in food, those which are commonly present in our diet were selected for the research. Therefore, the research material included phenolic compounds well-known as antioxidants and belonging to different subclasses due to their structures and reactivity of their functional groups. The experimental material included also a wide spectrum of low-molecular phenolic acids being metabolites of flavonoids after colonic degradation. The utility of the developed electrochemical methods was verified when compared with the classic ones used for the evaluation of the antioxidant/reducing capacity of apples of local origin.

Electrochemical properties of the phenolic compounds were determined from CV and DPV voltammograms recorded in buffered solutions at pH 2.5, 6.0, and 7.5. It was shown that the position and shape of peaks appearing on voltammograms and resulting from oxidation and/or reduction of the phenolic compounds were closely related to their chemical structures. The presence of a reversible oxidation/reduction peak on CV voltammograms was only observed for the phenolic compounds with a 3',4'-dihydroxyl group. In contrast, phenolics without di- or trihydroxyl groups showed only anodic oxidation peaks on CV voltammograms, thus corroborating the irreversible electrooxidation process. Moreover, a significant influence of the pH value of electrolyte on the oxidation potential of the phenolic

compounds was observed in the study. With increasing pH of electrolyte, the shift of oxidation peaks into the lower values of potentials was noted for all investigated compounds.

The reducing activity of the phenolic compounds was determined based on oxidation potentials and area under anodic current wave (AUC) derived from CV and DPV voltammograms. It was demonstrated that phenolic compounds with a low oxidation potential and high AUC values revealed a strong reducing activity. The values of reducing activity of phenolic compounds were confirmed by comparison with the values provided by classic spectrophotometric methods based also on the electron transfer mechanism (SET).

In this study, a strong positive correlation was found between the reducing activity of phenolic compounds determined with the CV method and that provided from the spectrophotometric FRAP and DPPH assays. Moreover, high values of a correlation coefficient were also obtained between values of oxidation potentials and data derived from FRAP and DPPH assays.

Based on the results obtained, it was demonstrated that the reducing activity of the tested phenolic compounds was closely related to their chemical structure, mainly due to the number and position of hydroxyl groups, and presence of catechol or galloyl moieties in the molecule. It was also noted that Fe(II)-chelating activity of phenolic compounds depended on their structure and pH value. The lowest Fe(II)-chelating activity of phenolic compounds was observed in the solution at pH 2.5 whereas the highest ones at pH 6.8 and pH 7.5.

The usefulness of the applied electrochemical methods was evaluated in relation to food samples, e.g. individual parts of apple (peel and flesh). The high correlation of the reducing/chelating activities of extracts from apple peel and flesh provided by electrochemical methods and classical spectrophotometric assays confirmed the benefits of cyclic voltammetry (CV) and differential pulse voltammetry (DPV) for determination of the antioxidative/reducing properties of food of plant origin.