SUMMARY

In the last decade, reactive oxygen and nitrogen species (RONS) and free radicals have become important subject of study in many fields including medical sciences. The state, in which the equilibrium between generation and degradation of free radicals in the organism is shifted towards their generation, is called oxidative stress. The excessive generation of free radicals in the organism can lead to the impairment of important biomolecules and to tissue injury, which may result in the disease onset. Oxidative stress plays an important role in the pathogenesis of a number of diseases such as diabetes mellitus, atherosclerosis, rheumatoid arthritis, Alzheimer's disease and many others as well as in the physiological process of aging. Various supplements containing vitamins and antioxidants of natural origin are used in order to slow down the process of aging and to prevent the disease onset.

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and it is always accompanied by oxidative stress. Glucose as well as various intracellular sugars (e.g. fructose, ribose, glyceraldehyde) covalently bind to the free amino groups of proteins and cause the impairment of their structure. This process is called non-enzymatic glycation. Advanced glycation end-products (AGEs) arise in subsequent reactions during which different cross-links between adjacent molecules are formed. The most important AGEs are represented by N-ε-(carboxymethyl)lysine, pyrraline, pentosidine, and argpyrimidine. Free radicals and a variety of reactive intermediates (e.g. α-dicarbonyl compounds), which are generated during the autoxidation of glycating sugar and AGEs, further contribute to the progress of non-enzymatic glycation. The whole process can be termed glycoxidation due to the participation of oxidative processes, which are often catalyzed by transition metal ions.

One of the goals of the presented work, which is a continuation of several preceding diploma theses, was to introduce and to optimize *in vitro* model of glycation of aspartate aminotransferase (AST). The optimization started with the selection of a reliable, commercially available enzyme preparation and continued with choosing a sufficiently active glycating agent in a suitable concentration. The

extension of the palette of methods used for monitoring the course of glycation about the methods used for the quantification of the generated products, e.g. fluorescent methods, seemed to be suitable. Direct interactions of the individual antioxidants with the molecule of protein as well as the effect of antioxidants on the protein glycation were monitored in the introduced model of non-enzymatic glycation.

The preparation obtained from Serva Electrophoresis, which demonstrated all spectral characteristics of AST described in scientific literature and possessed also the lowest initial concentration of fluorescent AGEs, was selected as the most suitable source of AST for following the experiments based on the spectroscopic and fluorimetric methods. D-Fructose in 50 milimolar concentration was used as the glycating agent in the first model of glycation because this compound is an important intracellular sugar with high reactivity. The ions of copper and iron, which catalyze radical reactions, were added to this model in order to support the oxidative processes. Optimal concentration of these ions seemed to be 1 micromolar concentration. This model of glycation was slowly abandoned because of the long duration of each experiment (up to 21 days) and optimization of a new model of glycation currently proceeds. Methylglyoxal, which is a highly reactive dicarbonyl compound formed as an intermediate of non-enzymatic glycation, is used as a glycating agent in the new model.

Fluorescence detection for total AGEs as well as pentosidine formation was successfully introduced. Total AGEs and pentosidine were measured at excitation and emission wavelengths $\lambda_{exc}/\lambda_{em}$ 370/440 nm and $\lambda_{exc}/\lambda_{em}$ 335/385 nm, respectively. The methods for the evaluation of oxidative stress in patients with rheumatic diseases can be useful tools for the investigation of the course of protein glycation in our model.

Antioxidants of natural origin tested in this work exerted both positive antiglycation effects and negative direct effects on the catalytic activity of AST to various extents. Hydroxycitric acid, *o*-coumaric acid and uric acid belonged to the group of compounds with the most pronounced antiglycation properties and also the least effects on the catalytic activity of a model protein. In contrast, flavonoid