Abstract

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Phenolic compounds are one of the most widely distributed groups of secondary plant metabolites. They are an integral part of the human diet and their consumption is associated with a number of positive effects. They include a wide range of substances, from simple molecules such as phenolic acids to large polymeric compounds such as tannins. Because of their structure, which includes, among others, free hydroxyl groups, they are able to interact with biogenic metals and form metal complexes with them. Under normal conditions, the homeostasis of these transition metals in the body is tightly regulated, but it can be disturbed in pathological conditions such as acute myocardial infarction, when the levels of free iron and copper increase as a result of a significant decrease in pH. Similar metal imbalance is observed as well as in inflammatory and neurodegenerative diseases, tumours, or diabetes mellitus.

The aim of this dissertation was to find out how these substances react with biogenic metals using *in vitro* and *ex vivo* methods, i.e., whether they can chelate or reduce ions of transition metals such as iron and copper, what effect they have on the production of hydroxyl radicals formed *via* the metal-induced Fenton reaction and how they impact copper-triggered lysis of red blood cells.

As part of this work, substances from the groups of dehydroflavonolignans, flavonoids, and their metabolites were tested. First, the ability of substances to chelate transition metal ions was determined, which is one of the mechanisms of action of antioxidants. The copper and iron-chelating activity of the substances was determined

under four (patho)physiologically relevant pH conditions by spectrophotometric competitive methods using bathocuproindisulfonate or hematoxylin to determine the amount of cuprous and cupric ions chelation, and ferrozine to determine the chelation of ferrous and ferric ions. Previous studies by our group showed significant chelating activity of some flavonoids, as well as of flavonolignan 2,3-dehydrosilybin. As part of this dissertation, these data were supplemented with 2 methyl metabolites of quercetin, isorhamnetin, and tamarixetin. Both showed high copper and iron chelating activity. The stoichiometry of their formed metal complexes was further determined using non-competitive spectrophotometric methods (Job´s method and the complementary method developed by our group) and the stability constants were calculated using spectrophotometric and potentiometric methods. Simultaneously, the ability of the flavonolignan 2,3-dehydrosilychristin to chelate both iron and copper ions was demonstrated.

The aforementioned competitive methods were also used in a slightly modified version to determine the ability to reduce copper and iron ions in a large group of 24 flavonoids to determine the relationship between structure and activity. Ions in a lower valence state can subsequently catalyse the formation of hydroxyl radicals from hydrogen peroxide and lead therefore to oxidative stress. The vast majority of the tested substances were able to reduce copper ions, and many of them achieved complete 100% reduction. 5-hydroxyflavone and chrysin, i.e. substances with a 2,3-double bond but not containing a 3-hydroxy group and also no hydroxyl group on ring B, were the only once able to decrease the spontaneous reduction of copper ions at pH 6.8 and 7.5.

For substances that are capable of both chelating and reducing metal ions, it is difficult to determine theoretically the effect on the Fenton reaction and the production of hydroxyl radicals. For that reason, the effect of the substances was determined using a sensitive HPLC method with coulometric detection. Both mentioned flavonoids and the effective chelators from 2,3-dehydroflavonolignan class were included in this study. The three tested flavonoids, 3-hydroxyflavone, 5-hydroxyflavone, and troxerutin, were able to block the copper-triggered Fenton reaction and had hence antioxidant activity with 5-hydroxyflavone demonstrating the strongest effect. The effect of the tested flavonolignans was variable depending on the pH and type of metal.

To verify the antioxidant or pro-oxidative action of substances under *ex vivo* conditions, the determination of the effect on copper-induced lysis of red blood cells was used. The vast majority of tested compounds had protective effects on red blood cells and proved to be antioxidant, alternatively neutral in few cases. Only the unsubstituted flavone increased hemolysis and had pro-oxidative activity. The addition of a hydroxyl group, at position 3, 5 or 7, not only blocked the pro-oxidative action but also led to significant protection of red blood cells. Analogously, 5 of 6 tested flavonolignans protected red blood cells from lysis induced by the addition of copper.

As part of this dissertation, a new method for screening potential cobalt chelators and evaluating their toxicity was also developed and published. This method enables very sensitive, rapid and cheap measurements in a wide range of pH (4.5-7.5), using the disodium salt of 1-nitroso-2-naphthol-3,6-disulfonic acid.

In conclusion, we can summarize that these published works concerning phenolic substances that are part of our food and their metabolites indicate possible protective effects on the human organism. Although some substances were shown to be pro-oxidative in *in vitro* tests, their action was protective in *ex vivo* conditions.