1. PHD PROJECT DESCRIPTION (4000 characters max., including the aims and work plan)

Project title:
Study of the oxidative stress influence on the biosynthesis and durability of plant origin xanthophylls, including lutein and zeaxanthin and determination of their degradation products using hyphenated chromatographic techniques

1.1. Project goals

The main aim of the project is to identify the degradation products of lutein and zeaxanthin (belong to xanthophylls group) due to photo-and radiolysis and determining potential fragmentation pathways of test compounds and their metabolites. The study will need to use thin layer chromatography (TLC), high performance liquid chromatography (HPLC) with detection by UV-Vis and Corona-CAD and tandem mass spectrometry (MS^n). An important part of the study will be determination of the effect of UV and gamma radiation on the stability of lutein present in the cells of Brassicaceae plants (kale, green cabbage) and Amaranthaceae plants (spinach).

1.2. Outline

Carotenoids, including xanthophylls, are an important group of biologically active compounds because of its antioxidant properties, as well as participation in the process of photo-protection and photosynthesis. The source of carotenoids are fruits and vegetables. Carotenoids are photostable and undergo changes under the influence of UV radiation. They take part in protecting living organisms against the effects of oxidative stress, related with disorder associated with reducing the state. The consequence of oxidative stress is the increased production of peroxides and free radicals that cause oxidative damage to all cell components. In the case of plants, oxidative stress may be a response to various external factors and growing conditions. The effect of external factors on plant cells may be impaired production of many compounds, including carotenoids and their degradation.
Reaction products formed by photo-and radiolysis of carotenoids have not yet been fully recognized. Therefore, it is important to study the degradation routes of these compounds and to develop appropriate methods for isolating and determining, using modern analytical techniques. On the basis of previous studies assessing the impact of UV radiation on the carotenoids, it was established that due to absorption of radiation, the excitation products of carotenoids and triplet oxygen are formed. Carotenoid molecules react with both triplet and singlet oxygen. UV radiation on plant cells generates the formation of free radicals, which in turn attack the carotenoid molecules in the locations of double bonds in the aliphatic chain.

One of important nutrients is lutein, a yellow plants pigment. In human health it plays an important role. Lutein is considered as very strong antioxidant, which can destroy free radicals. This is connected with the influence of lutein for the protection of eye and skin. High concentration of lutein can be found in retina, where it works as a filter from blue and UV light and as an antioxidant in the scavenging of photo-induced free radicals. It can be produced in high amount because of increased exposition of retina on light. The similar

protection (lutein as a filter), found in skin, reduces risk of skin cancer. Also, lutein plays a protective role from cataract and AMD (Age-related Macular Degeneration). The macula has a high concentration of photoreceptor cells – the cells that detect light. They send signals to the brain, which interprets them as images. The rest of the retina processes our peripheral, or side vision. The retina is exposed to UV radiation, which destroys the photoreceptors. The xanthophylls located in the retina protect sensitive cells by absorption of UV radiation. The radial absorption maximum for lutein: $\lambda = 450$ nm.

1.3. Work plan

1. The determination of degradation products of lutein under UV radiation and gamma radiation, by means of HPLC coupled with MS using different ionization methods: ESI and APCI.
2. The impact study of UV radiation effect on lutein biosynthesis in case of plants grown under abiotic stress conditions.
3. Determining the impact of selected growing conditions such as the excess or deficit of water, salt stress, temperature, mechanical damage and fertilization on the concentration of carotenoids in plants, kale will be grown in laboratory conditions.
4. The method developed for the qualitative and quantitative analysis in order to specify the content of lutein and other carotenoids in the examined samples. Development of various extraction methods will be used (PLE, ASE, SFE) and chromatographic techniques (TLC and HPLC) coupled with UV, Corona-CAD and MS^n.
5. The impact study of UV radiation on the stability of lutein and zeaxanthin present in the cells of Brassicaceae and Amaranthaceae plants, depending on the time and storage conditions.
6. Ascertainment the kind of effects of gamma radiation on the stability of lutein, for example vegetables, which are designed for long term storage.
7. The final stage of research the validation proposed methods and statistical verification of obtained results.

1.4. Literature (max. 10 listed, as a suggestion for a PhD candidate)

[1] A.V. Rao, L.G. Rao; Carotenoids and human health, Pharmacological Research 55 (2007) 207-216.

[2] H. Jackson, C. L. Braun, H. Ernst; The chemistry of novel xanthophyll carotenoids. The American Journal of Cardiology 101(10A) (2008) 50-57.

[3] A. Lienau, T. Glaser, G. Tang, G. G. Dolnikowski, M. A. Grusak, K. Albert; Bioavailability of lutein in humans from intrinsically labeled vegetables determined by LC-APCI-MS. Journal of Nutritional Biochemistry 11 (2003) 663-670.

[4] A. Alves-Rodrigues, A. Shao; The science behind lutein. Toxicology Letters 150 (2004) 57-83.

[5] J.M. Holden, A.L. Eldridge, G.R. Beecher, I.M. Buzzard, S. Bhagwat, C.S. Davis, L.W. Douglass, S. Gebhardt, D. Haytowitz, S. Schakel; Carotenoid content of U.S. foods: an update of the database. Journal of Food Composition and Analysis 12 (1999) 169–196.

[6] R. Aman, R. Carle, J. Conrad, U. Beifuss, A. Schieber; Isolation of carotenoids from plant materials and dietary supplements by high-speed counter-current chromatography. Journal of Chromatography A 1074 (2005) 99–105.

[7] M. Ligor, B. Buszewski; Study of xanthophylls concentration in spinach leaves by means of HPLC coupled with UV-Vis and Corona-CAD detectors. Food Analytical Methods 5(3) (2012) 388-395.

[8] M. Ligor, B. Buszewski; Effect of kale cultivation conditions on biosynthesis of xanthophylls. J. Food Research 1(4) (2012) 74-84.

[9] M. Ligor, J. Kovácóvá, R. M. Gadzała-Kopciuch, S. Studzińska, Sz. Bocian, J. Lehotay, B. Buszewski; pt. „Study of RP HPLC retention behaviours in analysis of carotenoids", Chromatographia 77 (2014) 1047-1057

1.5. Required initial knowledge and skills of the PhD candidate

- Skills in analytical chemistry, knowledge of chromatographic techniques and extraction methods (i.e. SPME, SPE, MSPD, ASE, SFE).
- The ability to analytical thinking.
- The PhD candidate should have experience in laboratory work, planning experiments, performing qualitative and quantitative analysis and conducting method validation.
- Required initial knowledge and skills in ChemStation (Agilent), MassHanter (Agilent), Statistica.

1.6. Expected development of the PhD candidate's knowledge and skills

- The candidate will have the opportunity to develop new methods for the isolation (e.g. QuEChERS, MSPD) and determination of carotenoids using HPLC in combination with various MS, UV and Corona-CAD detection systems.
- Broadening the knowledge and skills to the preparation of raw plant's material and biological samples.
- Broadening the specialized knowledge of plant biology and chemical sciences.
- Proposed studies provide the opportunity to acquire specialist knowledge in the field of analytical chemistry and statistical methods.
- Developing the ability to present posters and oral presentations at conferences. Also, the PhD student will be the author and co-author of publications quartile category Q1.
- The PhD student will have the opportunity to develop methodologies and procedures recommended for the routine food or pharmaceutical analysis.