

## 1. Reason for choosing the research topic

Plant origin sterols, also referred to as phytosterols, are being applied as ingredient with increasing intensity to our daily diet and have become consciously searched for by consumers. These compounds are naturally present in edible oils, vegetables, seeds, nuts and other plant origin products. Their biological properties are connected with a reduction of blood cholesterol level in humans and are playing an increasingly important role in the prevention of cardiovascular heart diseases. Initially applied in the form of medicines, then as dietary supplements, now added to food products as functional ingredients. In 1995 the Finnish company Benecol first introduced the margarine with addition of phytosterols. Today we are dealing with a large variety of high- and low-fat products enriched with these compounds. Phytosterols have been approved as functional ingredient by many organizations world-wide including European The Food and Drug Administration consented the GRAS status for phytosterols. As unsaturated chemical compounds, similar structurally to cholesterol – the main animal sterol, undergo oxidative degradation. Oxidized derivatives of cholesterol (oxysterols) have mutagenic, atherogenic and cancerogenic properties. However data related to the toxicological effect of phytosterols oxidation derivatives (oxyphytosterols) are lacking. Oxyphytosterols were detected in raw materials and food products, also are formed during production and storage. Thereby reduces the level of sterols, which, in addition to the oxidized derivatives, can also be subject to disintegration or oligomerization. The literature data on forming of low molecular weight compounds, volatiles and oligomers are limited. In the last 5 years 700 papers were published on cholesterol oxidation products however only 10 on the oxidation of phytosterols. In the era of widely food fortified with plant sterols, as well as in relation to their presence in vegetable oils, there is a need to assess the biological properties and understand mechanisms of action of thermal oxidation products formed from phytosterols and its esters. It is particularly important since products containing these compounds are often used for cooking, baking and frying.

## 2. Research objective

Phytosterols and their fatty acid esters are endogenous oils and vegetable fats components, where they account for as much as 90% of unsaponifiable fraction. As functional components reducing blood cholesterol levels in humans these compounds are added in relatively large amounts to various food stuffs to observe their activity, and these products are frequently subjected to thermal processes. Studies carried out under previous research projects showed that during thermal processing or storage of food products containing plant sterols and its esters formed oxidized derivatives, dimers, oligomers and many low molecular compounds including volatile components. It should be noted that the cytotoxicity of thermally oxidized soybean and rapeseed oils was reported by Totani et al. (2008), but the influence of individual compounds or group of components like phytosterol thermo-oxidative derivatives is lacking. It is crucial for consumers' health to test toxicity of these compounds and proposed research will provide understanding which compounds are responsible for adverse activity.

The aim of the study is assessment of toxicological properties of isolated products formed during thermo-oxidative degradation of stigmasterol and its esters based on model system and food products.

## 3. Material and methods

Stigmasterol will be the main phytosterol used in the planned model study, also its esters with oleic and linoleic acids will be produced and tested. Cholesterol will be applied as control sample.

3.1. At the first stage of this project, the stigmasterol standard will be esterified with oleic and linoleic acids following the method proposed by Neises and Steglich (1978). Then standards of cholesterol (as control sample), stigmasterol and its esters will be thermo-oxidized at a temperature of 60°C and 180°C. Degradation rate of heated compounds formation and type of products formed will be qualitatively and quantitatively analyzed using chromatographic methods such as GC/MS, GC x GC/MS, HPLC/MS.

3.2. Products formed during thermo-oxidation of stigmasterol and their esters will be fractionated by SPE and/or HPLC, TLC, column chromatography techniques according to their polarity and molecular weight. Stigmasterol oxidation products will be also separated from heated samples.

3.3. In the next step the total effects of cholesterol, stigmasterol and its esters before and after heating on viability and proliferation of cells, apoptosis in cells, genotoxicity, potential mutagenic or promutagenic activity and formation of reactive oxygen species in normal cells will be assessed.

3.4. Rapeseed oil and margarine will be enriched with stigmasterol and its esters and heated at 60°C and 180°C. Then isolation of phytosterols and products of their degradation will be carried out. Then degradation products will be isolated, separated into fractions containing polar and non-polar compounds, oxyphytosterols, dimers and oligomers as well as quantitative and qualitative analyses will be attempted.

## 4. Expected results

Proposed research seems to be a fundamental task due to the prevalence of the use of plant oils and margarines enriched in phytosterols and their esters in daily diet. Taking into consideration the toxicity of products formed during thermo-oxidation of phytosterols and their esters, an important issue is to understand and define the relationship not only at the level of research using model experiments, but mainly in food products enriched in plant sterols or their esters. Only limited data are present in literature about biological activities of phytosterols oxidation products and no data are available about oligomers. Research planned in this project will provide a new information on the toxicity of compounds formed during thermo-oxidative degradation of phytosterols and its esters with fatty acids.

The main objective of the research conducted within the proposed project is to obtain new and lacking knowledge about biological activity and action mechanisms of compounds formed during thermo-oxidative degradation of phytosterols and its esters. Its realization will significantly enhance the knowledge base dealing with chemical composition, formation mechanism and biological activity of compounds formed in the functional food intended to help consumers with current civilization diseases. The application of highly efficient analytical methods will allow separation into specific fractions containing different products formed during thermo-oxidative degradation of stigmasterol and its esters. This information will lead to understanding mechanism of products formation and its toxicity. The assessment of these properties is fundamental to development protection mechanism to

prevent detrimental degradation of phytosterols in foods enriched in these compounds during cooking and frying. The analysis of biological activity of degradation compounds is important for food safety and assessment of potential health implications to the consumers, from other side protection of food industry.

Literature:

Neises B., Steglich W. (1978) Simple method for esterification of carboxylic acid. *Angew Chem Int Edit* 17, 522-524.

Totani N., Burenjargal M., Yawata M., Ojiri Y. (2008) Chemical properties and cytotoxicity of thermal oxidized oil. *J Oleo Sci* 57, 153-160.