The objective of the research project:

The objective of the proposed project is to develop a method to obtain structured lipids being a hybrid of acylglycerols with a plant sterol (s-TAG), to assess their physicochemical and biological properties and thermoxidative stability.

In order to improve nutritional value and functional parameters of lipids they are subjected to various modifications. Acylglycerols and phytosterols are natural components of vegetable oils. Generation of a hybrid of these compounds may affect thermal oxidation stability of phytosterols, particularly if glycerides containing saturated fatty acids are applied.

Justification for the proposed research:

Sterols are important components of fats serving important metabolic functions, they are sources of hormones, vitamin D precursors and essential components of cell membranes. This group of compounds includes: (1) animal origin sterols, with cholesterol being the most important representative; (2) plant origin sterols, referred to as phytosterols, which over 200 representatives have been identified in plant fats, represented particularly by β-sitosterol, avenasterol, campesterol and stigmasterol, as well as (3) sterols produced by microorganisms, particularly mould fungi, e.g. ergosterol.

Plant sterols are considered to be antagonists of cholesterol, since they cause a reduction of its level in the human organism. These compounds are found naturally in vegetable oils, vegetables and seeds of fruits, while they are also added to food products as functional components. Apart from their positive properties, phytosterols administered at large doses result in decreased levels of carotenoids and vitamin E in the organism. Plant sterols in their structure have from one to several double bonds. This affects their oxidation stability and the formation of oxidized derivatives, which have an adverse effect on the human organism, including cytotoxic properties. They additionally cause atherogenic lesions and contribute to the development of oxidative stress. Elevated levels of oxyphytosterols were detected in diabetics. Literature data concerning these compounds are scarce. Studies on the effect of phytosterol degradation products on normal cells of the alimentary tract are being conducted at the laboratories of the Poznań University of Life Sciences in cooperation with the Poznań University of Medical Sciences. It results from the obtained data that the compounds formed during thermal degradation of stigmasterol have a negative effect on examined cells. In view of the current offer of foodstuffs enriched with phytosterols it is essential for the phytosterols to be supplied in the form which is absolutely safe for consumers. As it is now known that both free sterols and their esters are oxidized, forming plant oxysterols and other derivatives, the development and tests on the new form, which may be found for the structured acylglycerol with a phytosterol may facilitate the formation of an absolutely safe form of this bioactive substance.

Research method:

1. In the first stage of the study the method of chemical hybridization of acylglycerols with stigmasterol will be elaborated. Structures of s-TAG will be established based on spectroscopic methods (NMR, IR, HR-MS).
2. Obtained compounds as well as pure stigmasterol and its ester will be heated in the presence of oxygen at temperatures of 60°C and 180°C, which correspond to the storage test and frying of fats.
3. Degradation products of stigmasterol and acylglycerols as well as fatty acids will be analyzed quantitatively and qualitatively using such chromatographic techniques as GC-FID, GC-MS, GCxGC-MS, HPLC-SEC/ELSD, HPLC-MS.
4. Thermal oxidation products of stigmasterol and its esters will be fractionated using SPE and HPLC according to their polarity and molecule size into low molecular weight compounds, monomers, dimers and oligomers. Among thermal oxidation products the fraction containing oxidized derivatives of stigmasterol will also be fractionated.
5. Next *in vitro* studies will be conducted to evaluate cytotoxicity, genotoxicity and mutagenicity before and after their thermal oxidation. Cytotoxicity will be analyzed in relation to human cells isolated from the small intestine epithelium, the mucosa of the large intestine, the liver and vascular endothelium.
6. Additionally, in order to explain the molecular mechanism of biological activity for the newly obtained compounds studies will be conducted on the interactions of these compounds with different models of biological membranes and biomolecules.
7. At the next stage of the project the obtained compounds will be subjected to *in vitro* digestion in order to investigate their metabolism in the digestive tract. Their contents as well as levels of stigmasterol and its oxidized derivatives will be determined in epithelial cells of the digestive tract.
8. At the last stage of the project we will implement s-TAG into food fats and investigate their influence on its quality during storage and processing.