Gastrointestinal stability of phytosterols encapsulated in liposomes

Magdalena Rudzińska[1], Anna Grygier[1], Joanna Igielska-Kalwat[1], Katarzyna Cieślik-Boczula[2]

[1]Poznań University of Life Sciences, Faculty of Food Science and Nutrition, Poznań, Poland

[2]Wrocław University, Faculty of Chemistry, Fryderyka Joliot-Curie 14, 50-383 Wrocław, Poland

The bioavailability of many biologically active compounds in **the digestive tract** can be enhanced by encapsulating them into liposomes used as direct delivery system. Liposomes are a very attractive system for delivering drugs and bioactive substances into the human body, as they provide **protection** of these compounds from thermal and oxidative degradation as well as from the effects of light, pH, and enzymes. They are widely used in the pharmaceutical industry and their properties have been thoroughly studied and described. However, the use of liposomes in **food** involves research of a much broader type.

Our previous study have shown that **phytosterols and their esters** with fatty acids can be encapsulated with liposomes. During storage and frying tests, stigmasterol encapsulated with liposomes degraded to form oxyphytosterols, dimers, and oligomers. Some of these degradation products have adverse effects on cell cultures. The health and safety of foods is crucial for consumers and, in the case of sterols, it is prudent to establish a safe form of delivery that will prevent thermo-oxidative degradation and will heighten absorption in the gastrointestinal tract. **PEGylated** liposomes show many positive properties over conventional liposomes: they have longer effects in the body, better accumulation in cells, reduced toxicity to healthy tissue, and increased stability. They also have some disadvantages: they reduce cellular uptake, they induce the so-called accelerated blood clearance (ABC), they can lead to pseudo-allergies, and they are unevenly distributed in tissues. These challenges indicate that **further research and optimization is required to improve the safety and efficacy of PEGylated liposomes**.

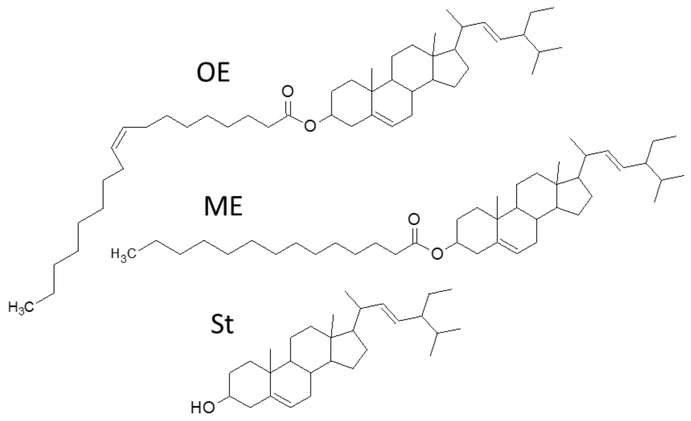

Figure 1 Chemical structure of stigmasterol (St), stigmasteryl myristate (ME) and stigmasteryl oleate (OE)

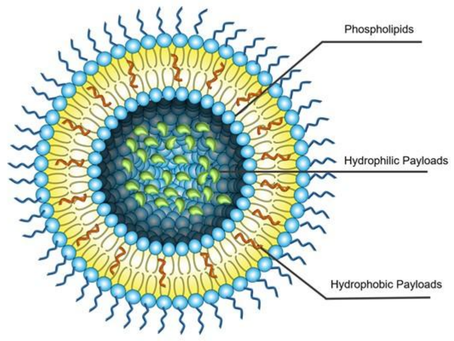

Figure 2 Structure of liposome

Figure 3 Gastrointestinal tract)

Aim: The aim of this work was to investigate the stability of stigmasterol and its esters encapsulated in liposomes during *in vitro* digestion. Two types of liposomes (conventional and PEGylated) were used.

Method: Chemical esterification was used to obtain the stigmasteryl esters with myristic and oleic acids (Neises & Steglich, 1978; Raczyk et al., 2017). The standard of stigmasterol and its esters were encapsulated in liposomes. The structure of both lipid membranes of liposomes and encapsulated molecules and interactions between lipids and encapsulated components were qualitatively and quantitatively studied using Fourier transform infrared (FTIR). The analysis of thermodynamic properties of lipid membranes in the obtained liposomes were done by estimation of enthalpy, partial heat capacity, cooperativity of the phase transition associated with the differential scanning microcalorimetry (DSC) peak width for exo- and endothermic peak transitions of lipid membranes measured in different scan rates and incubation time The thermo-oxidative stability of stigmasterol encapsulated in liposomes was determined after heating at 60 and 180°C. The products formed during degradation of liposomes and stigmasterol were determined using HPLC-SEC/ELSD and GC-FID methods. Stigmasterol oxidation products were identified by GC x GC/MS ToF method.

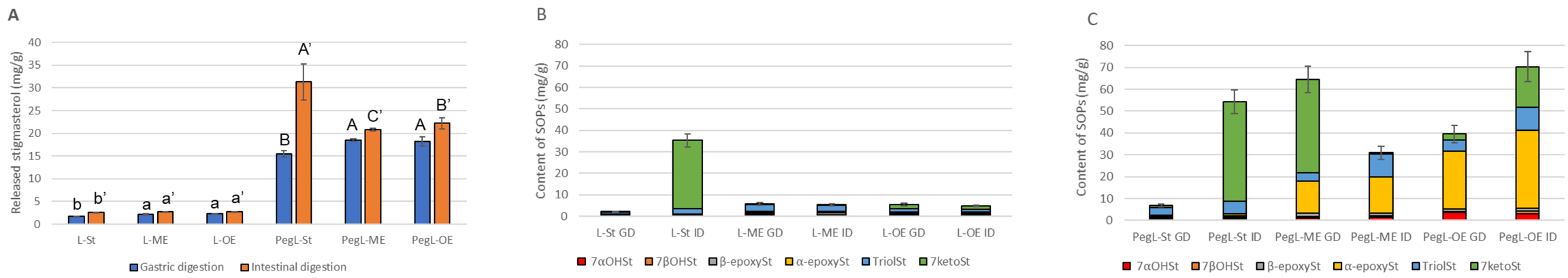

Figure 4: Changes in stigmasterol during digestion. A: stigmasterol released during digestion of conventional and PEGylated liposomes; B: amount and composition of stigmasterol oxidation products formed during digestion of conventional liposomes; C: amount and composition of stigmasterol oxidation products formed during digestion of PEGylated liposomes. GD: gastric digestion; ID: intestinal digestion; St: stigmasterol; ME: stigmasterol myristate; OE: stigmasterol oleate; PegL: PEGylated liposomes. Data are expressed as the mean ± standard deviation of three replicates. Different superscript letters in (A) denote statistically significant differences ($p < 0.05$).

Results: PEGylation of liposomes and the chemical structure of the encapsulated compounds affect the stability of the liposomes following gastrointestinal digestion. Esterification of stigmasterol with fatty acids affects the release of stigmasterol encapsulated in liposomes, especially from PEGylated liposomes. The level of fatty acid saturation has no significant effect on this release. PEGylation of liposomes did not inhibit sterol oxidation, but in fact can induce the formation of SOPs.

The use or nonuse of additional substances such as polyethylene glycol (PEG) in liposomes and the esterification of stigmasterol with saturated or unsaturated fatty acids both have significant impact on the stability of the encapsulated substances during gastrointestinal digestion in different parts of digestion tract.

The use of liposomes as carriers of plant sterols in foods still requires much research, including on their bioaccessibility and bioavailability.

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