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Formulation of phytosterols in emulsions for increased dose response in functional foods

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Abstract

Phytosterols can significantly reduce cholesterol levels in humans. However, their dose response is strongly formulation dependent. Due to their insolubility in water and poor solubility in oil and their surface activity, the formulation in functional foods of unesterified non-crystalline phytosterols with an expected dose response even higher than the esterified phytosterols commonly applied today proved problematic. Supersaturating phytosterols with a crystallization inhibitor in the oil phase of an o/w emulsion in a special process combines high phytosterol concentrations with the potential of strongly increased dose response. In two formulations, no crystallization was observable in stability investigations over a period of 60 days.

Keywords: Phytosterols; Water-dispersible; Serum-cholesterol; o/w Emulsion

Industrial relevance: Compared to actual formulations as phytosterol fatty acid esters in fat-based foods, the method describe here for formulating free, solved and water-dispersible phytosterols allows for their incorporation into almost all kinds of foods as well as beverages. Additionally, the expected to dose–response to phytosterol formulated according to this method is expected to exceed by far that of phytosterol fatty acid esters. Combined with the comparably high concentration in the new, water-dispersible formulations, the method yields the opportunity of producing a wide variety of functional foods with phytosterols highly effective in lowering the risk for cardiovascular diseases.

1. Introduction

Phytosterols are present in all foods of plant origin, especially seeds and oils, in concentrations of up to 5%. They are essential constituents of the plant-cell membrane due to their membrane-stabilising effect. On a molecular level, they are composed of the same C_{27}-backbone of dimethylsterane and a branched aliphatic side-chain like cholesterol. Different phytosterols, like β-sitosterol, stigmasterol or campesterol, and cholesterol are distinguishable by corresponding variations of the side-chain or its additional methyl groups.

Since the 1950s, phytosterols are well known for their cholesterol lowering and anti-carcinogenic effect that became evident in many both animal and human studies (Awad & Fink, 1998; Pollak, 1953). In 1985, it was additionally discovered that regular intake of phytosterols can reduce number and size of gallstones as well as improve symptoms of prostatitis (Pollak, 1985). Above that, expected negative side effects like e.g. hormone-like action could not be observed (Jones, MacDougal, Ntanios, & Vanstone, 1997; Ling & Jones, 1995).

Depending on dose and formulation, phytosterols not only lower the total serum-cholesterol concentration but the ratio of the concentrations of low-density lipoprotein to high-density lipoprotein bound cholesterol in the serum as well (Mattson, Grundy, & Crouse, 1982; Miettinen, Puska, Gylling, Vanhanen, & Vartiainen, 1995; Pouteau et al., 2003).

Although the mechanisms of action are not yet fully understood, the following mechanism is suggested for the reduction of total serum cholesterol. It seems likely that

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phytosterols compete with cholesterol for absorption into mixed bile salt and acid micelles, the vehicles for resorption of lipophilic compounds in the intestine. Due to their higher hydrophobicity, phytosterols are more readily absorbed into the micelles than cholesterol, a great part of which is therefore not available for resorption and excreted with the faeces. The phytosterols, however, are only taken up into the serum to a very small extent either since the intestinal lumen precisely differentiates between cholesterol and phytosterols. In consequence, the phytosterols, too, finally leave the intestine with the faeces. Therefore, the phytosterols' cholesterol-lowering effect is not based on their bioavailability but on their availability for absorption into the mixed bile salt and acid micelles (Piironen, Lindsay, Miettinen, Toivo, & Lampi, 2000).

Phytosterols are produced from vegetable oils in the process of refining. They are extracted together with other unsaponifiable matter in a steam distillation, which yields, after a purification step, a mixture of different phytosterols in the form of a white crystalline powder. For the formulation of free phytosterols as a functional ingredient in foods, their practical insolubility in water and their poor solubility in oil at room temperature pose the main obstacle.

By esterifying phytosterols with fatty acids, their solubility in oil increases approximately 10-fold. Therefore, incorporation of phytosterols into oily or fatty foods by means of their fatty acid esters is today's state of the art formulation. However, the formulation dependence of the daily intake of phytosterols required to obtain a certain decrease of the serum-cholesterol level is very important. Between 10 and 20 g/day of crystalline phytosterols are required for a serum-cholesterol reduction of approximately 10%, whereas only 2 to 3 g/day of esterified phytosterol are necessary to achieve a comparable effect. Most probably, the same effect can be achieved with an even smaller amount of solved free phytosterols. (Jones et al., 1997; Mattson et al., 1982).

Therefore, the aim of this project is to find a formulation of free phytosterols from which they are readily absorbed into the bile salt and acid micelles in the intestine. Above that, this formulation should be low in dietary fats and water-dispersible in order to make it suitable for a broader range of different foods.

2. Materials and methods

Crystalline technical β-sitosterol, a mixture of phytosterols containing at least 75% β-sitosterol, 10% campesterol and other phytosterols, was obtained from Acros Organics BVBA, Geel, Belgium. The solubility of technical β-sitosterol in a middle chain triglyceride (MCT) oil, Miglyol, purchased from Berg+Schmidt GmbH KG, Hamburg, Germany, was taken as a reference to determine the influence of temperature on the solubility of phytosterols in oil. The temperature–solubility dependency

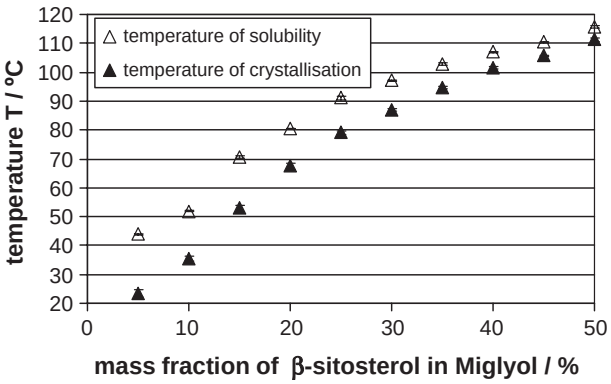

Fig. 1. Temperature–solubility correlation for technical β-sitosterol in Miglyol. Symbols represent triple measurements; S.D. within symbol size.

for technical β-sitosterol in Miglyol is depicted in Fig. 1. Besides with Miglyol, some of the following experiments were carried out with three different single acid triglycerides, namely tricaprylin, triolein and tristearin, all purchased from Sigma-Aldrich Chemie GmbH, Schnelldorf, Germany.

Oil soluble additives should stabilise a supersaturated solution of phytosterol in oil by increasing its solubility and thus preventing crystallization. Two substances were tested for this crystallization-inhibiting purpose, namely a distilled monoglyceride, 'Dimodan P Pel/B', of Danisco Cultor Deutschland GmbH, Quickborn, Germany, as well as a lecithin, Emulfluid F30, of Degussa Texturant Systems, Hamburg, Germany.

In order to increase further the supersaturation already achievable at room temperature by the use of a crystallization inhibitor, another effect preventing crystallization of supersaturated components was applied. If the supersaturated solution is dispersed into smaller droplets, the volume of each single droplet will be reduced. With decreasing droplet volume, the number of nuclei for crystallization per droplet can thereby be so much reduced that crystallization barely occurs anymore (Bunnell, Driscoll, & Bauernfeind, 1958). For the system regarded here, this can be done by dispersing the oily phytosterol solution into a continuous watery phase yielding an oil-in-water (o/w) emulsion. In order to form stable emulsions and promote the formation of small droplets, emulsifiers have to be added to the continuous phase.

However, as described in Stauffer and Bischoff (1966) and Jandacek, Webb, and Mattson (1977), the presence of an interface to a watery phase induces crystallization of sterols at that interface into the watery phase even from unsaturated solutions with organic solvents. Therefore, during the formation of emulsions, crystallization of technical β-sitosterol from the dispersed phase into the continuous phase had to be prevented. This could be achieved by dispersing the phytosterol solution into the watery phase at 90 °C as well as choosing an emulsifier that quickly absorbed to the oil–water interface and sufficiently masked it for the phytosterols. Therefore, the emulsifiers