Food Research International 100 (2017) 841–849

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

# The effect of adding a commercial phytosterol ester mixture on the phase behavior of palm oil

Eva Daels[a,b], Imogen Foubert[a,b,*], Bart Goderis[c]

[a] Research Unit Food and Lipids, KU Leuven Kulak, Department of Molecular and Microbial Systems Kulak, Kortrijk, Belgium
[b] Leuven Food Science and Nutrition Research Centre (LFoRCe), Heverlee, Belgium
[c] Polymer Chemistry and Materials, KU Leuven, Leuven, Belgium

ARTICLE INFO

*Keywords:*
Phytosterol esters
Polymorphism
Synchrotron radiation
Differential scanning calorimetry

ABSTRACT

The objective of this study was to investigate in depth the non-isothermal crystallization and melting behavior of binary blends of palm oil (PO) with a commercial, multi-component phytosterol ester (PE) mixture. DSC and time-resolved synchrotron X-ray diffraction (XRD) experiments were conducted on blends with a PE concentration from 0 to 100% at intervals of 10% for DSC and 20% for XRD. Based on XRD, two different ordered structures were identified in pure the PEs. The structure designated as $PE_x$ was truly crystalline and needed a very high degree of supercooling for its nucleation from the melt. The structure designated $PE_y$ formed without supercooling and showed long-range order with multiple reflections at small angles but only one broad reflection at high angle, typical of liquid crystalline samples. Furthermore, $PE_y$ had a high tolerance for molecules of different chemical nature. In the PE-PO blends no other ordered structures were formed other than the ones observed in the pure PEs and PO. The peaks in the DSC runs of the PE-PO blends were linked to transitions of the different polymorphic forms. All structural information of the binary blends as a function of concentration and temperature was collected in morphology maps. The binary blends exhibited eutectic characteristics visualized in the morphology maps with a eutectic point at 40% PEs.

## 1. Introduction

Phytosterols are triterpenes with a chemical structure similar to cholesterol. As shown in Fig. 1 they consist of a four-ring steroid nucleus with a hydroxyl group attached to the C-3 atom of the A-ring and an aliphatic side chain attached to the C-17 atom of the D-ring. Phytosterols include over 250 different plant sterols and stanols and related compounds, but the more abundant ones are β-sitosterol, stigmasterol and campesterol. Plant sterols only vary in their side chain. Possible side chain variations include the presence of a branched methyl or ethyl group on the C-24 atom and the presence and position of a single double bond (Piironen, Lindsay, Miettinen, Toivo, & Lampi, 2000). Plant stanols are the hydrogenation products of the sterols, in which the double bond in the ring is eliminated.

The richest natural sources of phytosterols are vegetable oils followed by cereal grains and nuts. Phytosterols are extracted for use in pharmaceutical, cosmetic and food applications (Fernandes & Cabral, 2007; Piironen et al., 2000). The two major raw materials used for large scale phytosterol extraction are vegetable oils and tall oil (a by-product of the kraft pulping process) (Helminen, Paatero, & Hotanen, 2006; Thompson & Grundy, 2005).

Epidemiologic and experimental investigations have shown that phytosterols may offer protection from the most common cancers in Western societies, but their main beneficial health effect lies in reducing circulating cholesterol concentrations. A meta-analysis of 41 trials showed that a 10% reduction in the blood levels of LDL-cholesterol can be obtained with an optimal intake of phytosterols of 2 g/day, beyond which only a marginal effect is observed (Mensink, Zock, Kester, & Katan, 2003). Furthermore there are indications that each 1% reduction in LDL-cholesterol corresponds to a 1% reduction in coronary heart disease relative risk, especially for subjects at high cardiovascular risk, like those affected by type 2 diabetes (Marangoni & Poli, 2010). The intake of phytosterols to lower LDL-cholesterol exhibits no side effects, except for children and pregnant women who risk a carotenoid deficiency (Nestel, Cehun, Pomeroy, Abbey, & Weldon, 1999; Sioen, Matthys, Huybrechts, Van Camp, & De Henauw, 2011). Given the fact that the average daily intake of phytosterols in adults only reaches between 150 and 400 mg/day, phytosterols have to be administered in a higher concentration in order to benefit from their health-promoting

*Abbreviations:* DSC, differential scanning calorimetry; FA, fatty acid; HMF, high-melting fraction; LMF, low-melting fraction; PE, phytosterol ester; PO, palm oil; SAXS, small angle X-ray scattering; TAG, triglyceride; $T_{offset}$, melting offset temperature; $T_{onset}$, crystallization onset temperature; WAXD, wide angle X-ray diffraction; XRD, X-ray diffraction

* Corresponding author at: Research Unit Food and Lipids, KU Leuven Kulak, Department of Molecular and Microbial Systems Kulak, Kortrijk, Belgium.
*E-mail address:* imogen.foubert@kuleuven.be (I. Foubert).

http://dx.doi.org/10.1016/j.foodres.2017.08.015
Received 22 March 2017; Received in revised form 19 July 2017; Accepted 4 August 2017
Available online 05 August 2017
0963-9969/ 

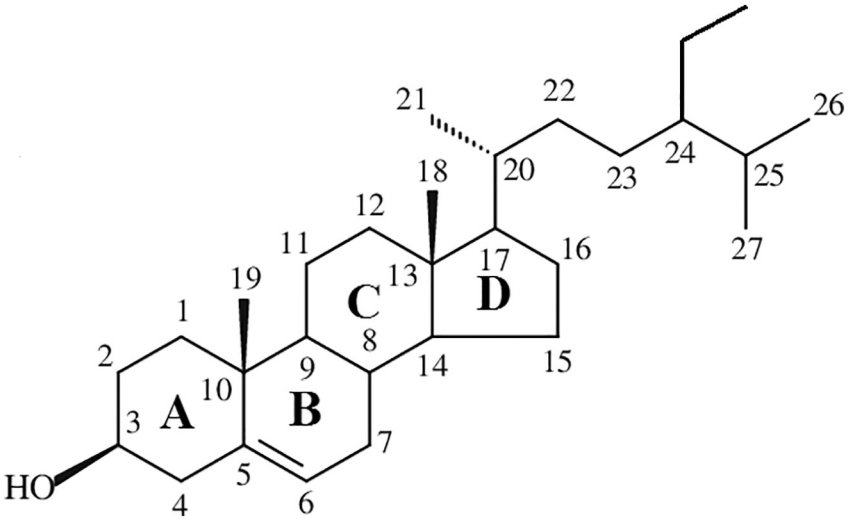


**Fig. 1.** Chemical structure of β-sitosterol (Soupas, Huikko, Lampi, & Piironen, 2005).

properties (Ntanios, 2001). One possible administration method is incorporation of phytosterols into a food matrix.

Enrichment of food products with free phytosterols is, however, difficult from a technological and food quality point of view since they are insoluble in water and poorly soluble in dietary fats while their melting points are rather high (about 140–150 °C) (Piironen et al., 2000). Consequently, much research was performed to find approaches to overcome these limitations. Esterification of phytosterols at the C-3 position of the A-ring with a fatty acid (FA) to obtain phytosterol esters (PEs) increases their lipid solubility, lowers their melting point and thus facilitates their incorporation into fat containing foods (Ostlund, 2007). PEs are currently already being used as a food ingredient in modern functional food formulations. Margarines and table spreads are ideal vehicles, although cream cheese, salad dressings, and yogurts are also used as delivery systems (Vaikousi, Lazaridou, Biliaderis, & Zawistowski, 2007). From an analysis of fat rich cholesterol lowering food products like margarines and spreads on the Belgian market it was deduced that the PE concentration that is applied in the fat phase in these products is in the range of 10 to 50% depending on the product type (e.g. light margarine, margarine with butter or olive oil, etc.).

However, addition of PEs to the fat phase of food products may influence its crystallization behavior and may thus lead to problems occurring during the production process or with the macroscopic properties of the end product (Rodrigues, Torres, Mancini-Filho, & Gioielli, 2007). Fundamental research on this topic is, however, very much lacking. To our knowledge, the influence of the addition of PEs on the physical properties of edible fats has only been reported twice in the literature. Vu, Shin, Lim, and Lee (2004) showed that self-made PEs added to liquid corn oil caused unwanted PE crystallization above a certain threshold concentration. Rodrigues et al. (2007) investigated how the crystallization behavior of milk fat was changed by blending with a commercial mixture of PEs. They showed that adding PEs to milk fat induced a softer consistency and a lower solid fat content. These authors however, did not report any information on the melting and polymorphic behavior of the pure substrates nor their blends since no differential scanning calorimetry (DSC) or X-ray diffraction (XRD) analyses were performed. Furthermore, PEs were only added in two different concentrations giving only a limited insight into the phase behavior of PE blends with milk fat.

The objective of this study was to investigate in depth the non-isothermal crystallization and melting behavior of a commercial PE mixture in blends with palm oil (PO) based on DSC and time-resolved synchrotron XRD measurements and to map the different polymorphic forms as a function of temperature and PE concentration. Although the concentration range of PEs that is applied in the fat phase of most of the existing cholesterol lowering fat rich food products is 10 to 50%, the full concentration range of 0 to 100% PEs in PO was investigated in this study to obtain insight into the phase behavior of the blends. In this study, PO was used as a 'host' fat for PE addition because recently PO has surpassed soybean oil to be the most consumed vegetable oil in the world (Tan & Nehdi, 2012).

## 2. Materials and methods

### 2.1. Materials

PO and a commercial PE mixture were donated by Unilever (Vlaardingen, The Netherlands). The degree of esterification in the commercial PE mixture was 97.7 ± 1.1% as was confirmed by solid phase extraction performed as described in (Panpipat, Xu, & Guo, 2013). Table 1 reports the sterol composition of the commercial PE mixture as measured by the method described in (Ryckebosch et al., 2012). Table 2 shows the FA composition of PO and the commercial PE mixture as determined by the method described in Ryckebosch, Muylaert, and Foubert (2012). Table 3 depicts the triglyceride composition of the PO used. It was analyzed in a high-performance liquid chromatograph (HPLC) (Waters, Belgium) on a NOVA-Pak C18 column (4 μm, 150 × 3.9 mm; Waters, Belgium) at 30 °C with acetonitrile–acetone (37.5:62.5, vol/vol) as the mobile phase. The HPLC was coupled with a refractive index detector (Waters, Belgium) set at 40 °C. PO was solubilized in a solvent mixture (1:1 methanol:chloroform) at a concentration of 20 mg/mL and injected in duplo (20 μL). The fatty acid profile and the TAG profile of PO were in good agreement with other studies (Tan & Nehdi, 2012).

### 2.2. Blend preparation

Binary blends of PEs and PO were prepared with a PE concentration from 0 to 100% with intervals of 10% for the DSC measurements and

**Table 1**
Sterol composition (%) of the commercial phytosterol ester mixture. The values show the average of two measurements. The error is 0.2%.

| Sterol | |
|---|---|
| Brassicasterol | 1.3 |
| Campesterol | 10.0 |
| Stigmasterol | 0.6 |
| β-Sitosterol | 78.5 |
| β-Sitostanol | 9.7 |