Food Hydrocolloids 25 (2011) 639–646

Contents lists available at ScienceDirect

Food Hydrocolloids

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

Structuring in β-sitosterol + γ-oryzanol-based emulsion gels during various stages of a temperature cycle

Arjen Bot [a,*], Ruud den Adel [a], Christos Regkos [a], Hassan Sawalha [b], Paul Venema [b], Eckhard Flöter [a]

[a] Unilever Research and Development Vlaardingen, Olivier van Noortlaan 120, NL-3133 AT Vlaardingen, The Netherlands
[b] Laboratory of Physics and Physical Chemistry of Foods, Department of Agrotechnology and Food Sciences, Wageningen University, Bomenweg 2, NL-6703 HD Wageningen, The Netherlands

ARTICLE INFO

Article history:
Received 27 March 2010
Accepted 28 July 2010

Keywords:
Organogel
Emulsion gel
Edible oil
Self-assembly
Small-angle X-ray scattering (SAXS)
Plant sterol
Differential Scanning Calorimetry (DSC)

ABSTRACT

Water-in-oil emulsions were prepared, structured only by a mixture of sitosterol and oryzanol and without further emulsifiers, containing 16 and 32% total sterol(esters)s on lipid phase and 10, 30 or 60% water. Previously, mixtures of β-sitosterol + γ-oryzanol were shown to form self-assembled tubules in triglyceride oil with diameter 7.2 ± 0.1 nm and a wall thickness 0.8 ± 0.2 nm. At 16% total sterol concentration, the SAXS diffraction patterns only demonstrate the presence of sitosterol and oryzanol crystals, but not of tubules. At 32% total sterol concentration, the diffraction patterns reveal the presence of tubules next to the crystals of the individual compounds. However, the tubule structure in these emulsions was more complicated than in pure oil and changed during storage, revealing the formation of bigger structures in the emulsion over time. In the cooling stage of a temperature cycle, water droplets nucleate at the tip of the fibres that reappear as a consequence of crystallisation.

1. Introduction

The continuous quest for healthier products has led to an increased interest in alternatives to the traditional product structuring routes for oil-rich systems based on saturated fat (SAFA) (Marangoni, 2009; Pernetti, van Malssen, Flöter, & Bot, 2007; Rogers, 2009; Wassell, Bonwick, Smith, Almiron-Roig, & Young, 2010). It proved possible to find quite a number of alternative single components that can structure triglyceride phases: waxes (Dassanayake, Kodali, Ueno, & Sato, 2009; Morales-Rueda, Dibildox-Alvarado, Charó-Alonso, Weiss, & Toro-Vazquez, 2009; Toro-Vazquez, Alonzo-Macias, Dibildox-Alvarado, & Charó-Alonso, 2009; Toro-Vazquez et al., 2007), wax esters (Daniel & Rajasekharan, 2003), sorbitan monostearate (Murdan, Gregoriadis, & Florence, 1999), ceramides (Rogers, Wright, & Marangoni, 2009), fatty alcohols, fatty acids, dicarboxylic acids and derivatised fatty acids containing additional side groups (Daniel & Rajasekharan, 2003; Gandolfo, Bot, & Flöter, 2004, 2007; Rogers, 2009; Rogers & Marangoni, 2008, 2009; Rogers, Pedersen, & Quaroni, 2009; Rogers, Wright, & Marangoni, 2008a, 2008b, 2008c; Rogers, Wright, et al., 2009; Wright & Marangoni, 2006), and alkyl-12-hydroxyoctadecanamides (Toro-Vazquez, Morales-Rueda, Mallia, & Weiss, 2010). On top of this, three mixed systems were identified with the capability of structuring triglyceride oil phases: fatty acids + fatty alcohols (Gandolfo et al., 2004; Schaink, van Malssen, Morgado-Alves, Kalnin, & van der Linden, 2007), lecithin + sorbitan tristearate (Pernetti, van Malssen, Kalnin, & Flöter, 2007) and phytosterols + oryzanol (Bot & Agterof, 2006; Bot, den Adel, & Roijers, 2008; Bot, den Adel, Roijers, & Regkos, 2009; Rogers, Bot, Lam, Pedersen, & May, 2010; Sawalha, Venema, Bot, Flöter, & van der Linden, in press). Of these systems, the phytosterol + oryzanol system received specific attention because the structuring elements of the organogel differ considerably from regular triglyceride crystals, and because of its generally complex and interesting behaviour.

It was found that mixtures of sitosterol with oryzanol self-assemble in triglyceride oil to form a helical ribbon, and that these tubules can aggregate to form a network (Bot et al., 2008). Ergosterol, stigmasterol, cholesterol, cholestanol self-assemble with γ-oryzanol too. The tubule diameter varies between 6.7 and 8.0 nm, the wall thickness between 0.8 and 1.2 nm (Bot, den Adel, et al., 2009).

Most potential applications of this specific alternative structurant mixture are foreseen in emulsion systems, as many fat-based food products are based on emulsion technology (de Bruijne & Bot, 1999). Recently, the sensitivity of the structuring elements to water was demonstrated (den Adel, Heussen, & Bot, in press). On the other hand, sitosterol + oryzanol mixtures had been shown to be capable of structuring w/o emulsions (Bot, Veldhuizen, den Adel, & Roijers, 2009) and of forming structures in o/w emulsions (Duffy et al., 2009). It was not clear, however, whether the fibres in emulsions were identical to those in pure triglyceride oil: the observation of

* Corresponding author. Tel.: +31 10 460 6449; fax: +31 10 460 6000.
E-mail address: arjen.bot@unilever.com (A. Bot).

0268-005X/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodhyd.2010.07.026

fibres by light microscopy (Duffy et al., 2009) suggested that these fibres were not the same as the ~7 nm diameter tubules in pure triglyceride oil (Bot et al., 2008; Bot, den Adel, et al., 2009), which should not be visible by optical microscopy (and which are indeed not visible by light microscopy in pure triglyceride oil).

The present paper aims to investigate the state of sitosterol and oryzanol in emulsions as compared to their state in pure oil. To do this, the effects of total sterol(ester) concentration and of amount of water in the emulsion is studied. In addition, the effects of ageing, heating and cooling of the emulsions are established in order to identify the opportunities and limitations of this particular oil structuring system in applications.

2. Material and methods

2.1. Materials

In the present experiments γ-oryzanol (Tsuno Rice Fine Chemicals) and tall oil sterol (Unilever, 78.1% β-sitosterol, 10.3% campesterol, rest minor but structurally very similar sterols (Bot, den Adel, et al., 2009)) were used in combination with refined sunflower oil.

Stock solutions of β-sitosterol + γ-oryzanol mixtures (16 or 32% on oil phase, 40% β-sitosterol + 60% γ-oryzanol) were prepared by heating during stirring until all powder had dissolved in the sunflower oil (at ~100 °C). Emulsions were prepared by mixing the components using a high shear mixer (Ultraturrax) in the appropriate amount of pre-heated water (10, 30 or 60% on emulsion). Note that no further emulsifiers were used in the present procedure, and that the resulting emulsions should be seen as Pickering-type emulsions. Production of such emulsions requires the presence of some solids during the emulsification process, because the solids stabilise the emulsion by means of the Pickering mechanism. Therefore, temperature during mixing is a compromise between having much solids present (i.e. low temperature) and preventing gelling (i.e. high temperature). The resulting emulsions were cooled and stored overnight at 5 °C and stored for 1 week at 10 °C.

2.2. Measurement techniques

Small-angle and wide-angle X-ray scattering (SAXS, WAXS) experiments were performed at the high-brilliance ID2 beamline of the European Synchrotron Radiation Facility (ESRF) in Grenoble, France. Details of the experimental set-up are given elsewhere (Bot et al., 2008; Narayanan, Diat, & Bösecke, 2001). SAXS data was collected in the range $0.069 < q/nm^{-1} < 4.3$ and WAXS data in the range $3.6 < q/nm^{-1} < 33.1$, where $q = 4\pi \sin\theta/\lambda$ is the wave vector (and θ the scattering angle and λ the wavelength of the incoming X-ray radiation). Separately obtained scattering data from water and sunflower oil reference samples were subtracted from the emulsion and dispersion data to obtain curves representing the sitosterol and/or oryzanol. Data analysis was described elsewhere (Bot, den Adel, et al., 2009; Deutch, 1981).

A number of complementary X-ray diffraction (XRD) measurements were performed at Unilever R&D Vlaardingen using a Bruker D8 Discover in a θ/θ configuration. Data was collected in the range $0.64 < q/nm^{-1} < 7.7$ (Bot, den Adel, et al., 2009). Data in these complementary experiments was not corrected for the contribution of oil or water.

Differential Scanning Calorimetric (DSC) experiments were done by filling aluminum sample pans with 15–30 mg emulsion gel structured with the oryzanol + sitosterol mixture, sealing and loading them in a Perkin–Elmer Pyris 1. DSC curves were obtained at a scanning rate of 10 °C/min during a heating–cooling–heating cycle.

Microscopic images of emulsions gels were obtained with a Zeiss Axioskop microscope (Zeiss lenses, magnification ×4/0.10 Achroplan and ×10/0.25 DIC LD Epiplan) equipped with a Linkam PE 94 temperature stage and a Sony DFW-SX 900 camera. Samples were imaged at room temperature or during temperature cycling from 5 to 90 °C and subsequent cooling to 5 °C at a rate of 10 °C/min. Images were processed using Camera Firei software.

3. Results and discussion

3.1. Structuring of the emulsion on a molecular scale

A previous paper addressed the relation between the structuring elements occurring in the organogel and in an emulsion gel. It was shown that the tubules that self-assemble in pure triglyceride oil do not form in emulsion gels, by comparing emulsion gels and organogels at 16% total sterol concentration on oil phase and at room temperature. In fact, the organogel was found to be the odd one out: systems containing water or only one of both components from the sitosterol + oryzanol mixture were found to show 'normal' crystals as characterised by sharp crystallographic reflections (den Adel et al., in press). The present study continues this line of research, except that a wider range of conditions were investigated.

The molecular organisation of the structuring elements in a number of emulsions was assessed by means of small-angle X-ray scattering (SAXS). The curves in Fig. 1 for emulsion gels containing 16% total sterols show the same behaviour as the data for the emulsion gel in the previous study: the crystallographic reflections can be found at positions that can be traced to either sitosterol crystals or oryzanol crystals in an aqueous environment (den Adel et al., in press). Major sharp peaks can be found at $d = 2\pi/q_i = 5.19$, 3.59, 2.72, 2.59, 1.80, 0.60 nm. No evidence is found of tubular structures. The amount of water does not affect the shape of the diffraction pattern much.

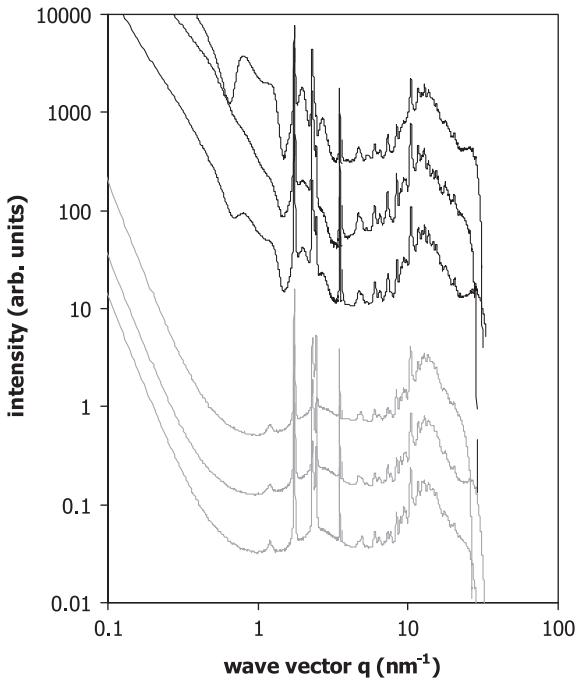

Fig. 1. SAXS data for a 40:60 mixture of sitosterol:oryzanol dissolved/dispersed in emulsions after 1 week storage at 10 °C. From top to bottom: three black curves for 32% total sterols on oil in emulsions containing 10, 30 and 60% water, respectively; three grey curves for 16% total sterols on oil in emulsions containing 10, 30 and 60% water, respectively.