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Organogel-Emulsions with Mixtures of β-Sitosterol and γ-Oryzanol: Influence of Water Activity and Type of Oil Phase on Gelling Capability

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ABSTRACT: In this study, water-in-oil emulsions were prepared from water containing different salt concentrations dispersed in an oil phase containing a mixture of β-sitosterol and γ-oryzanol. In pure oil, the β-sitosterol and γ-oryzanol molecules self-assemble into tubular microstructures to produce a firm organogel. However, in the emulsion, the water molecules bind to the β-sitosterol molecules, forming monohydrate crystals that hinder the formation of the tubules and resulting in a weaker emulsion-gel. Addition of salt to the water phase decreases the water activity, thereby suppressing the formation of sitosterol monohydrate crystals even after prolonged storage times (∼1 year). When the emulsions were prepared with less polar oils, the tubular microstructure was promoted, which significantly increased the firmness of the emulsion-gel. The main conclusion of this study is that the formation of oryzanol and sitosterol tubular microstructure in the emulsion can be promoted by reducing the water activity and/or by using oils of low polarity.

KEYWORDS: *Organogel, emulsion, tubules, water activity, polarity, self-assembly*

INTRODUCTION

Structuring of edible oils and food-emulsions with crystalline fats (i.e., triacylglycerols, TAGs) increases the risk of cardiovascular diseases due to the high levels of saturated fatty acids in these fats.[1] Researchers have proposed several structurants to replace TAGs[2−6] of which plant sterols are one.[1,7] Plant sterols are not only free of saturated fatty acids but have also been found to lower blood cholesterol by interfering with cholesterol absorption in the intestine and by enhancing fecal extraction of cholesterol.[8,9] Bot et al. showed, through rheological experiments, the organogelling role of mixtures of γ-oryzanol and β-sitosterol in edible oils and found that a stable and translucent organogel in triglyceride oil can be obtained.[10−12] The β-sitosterol and γ-oryzanol molecules assemble in curved one-dimensional aggregates because they cannot stack perfectly parallel because of the presence of an intermolecular hydrogen bond.[1,7] On a supramolecular level, this leads to the formation of tubular microstructures (∼7 nm in diameter and with ∼1 nm wall thickness). These tubules form a network that gels the oil phase and that may serve as an alternative to the network of small fat crystallites.[10−13]

When oryzanol and sitosterol mixtures are used for structuring water-in-oil (w/o) emulsions, the situation becomes different from the case of structuring pure oil. At low total sterol concentrations (i.e., 16% w/w), tubules were not formed and only crystals of sitosterol and oryzanol were observed.[14] At higher total sterol concentrations (i.e., 32% w/w), the tubules seem to partially form next to the crystals of the individual compounds, but the structure of these tubules was found to be different (i.e., water molecules are possibly involved in the tubule structure) and less stable than those formed in pure oil.[14] In addition, relative to the pure oil systems, the firmness of these gelled emulsions dropped dramatically. The absence of the tubules (at 16% w/w) and the distortion of their structure (at 32% w/w) were attributed to the formation of monohydrate crystals of β-sitosterol in the presence of water, which hinders the self-assembly of the two compounds into tubules.[15] The water molecules bind to the β-sitosterol molecules, which causes a transition of the crystals from anhydrous and hemihydrate into monohydrate forms and consequently interferes with the self-assembly into tubules in the emulsion. It is also believed that the hydration of the β-sitosterol molecules prevents the hydrogen bonding between β-sitosterol and γ-oryzanol molecules, weakening the tubules and eventually reducing the firmness of the gelled emulsion.[14,16] Heating these emulsions to temperatures above that of the transition between sitosterol monohydrate and hemihydrate allows the formation of these self-assembled supramolecular tubules.

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The formation and stability of the tubular microstructure in the presence of water is crucial for using the plant sterols in structuring emulsions. To promote the self-assembly process of the sterol compounds in the emulsion, the availability of water molecules in the oil phase needs to be reduced in order to prevent the formation of monohydrate β-sitosterol crystals. The present paper discusses two possible routes in this regard. The first route is to reduce the water activity through the addition of a salt or sucrose to the aqueous phase.[17] The other option is to use oils with lower polarity, thereby reducing the water solubility in the oil phase. To investigate the feasibility of the first route, water/sunflower oil emulsions with different NaCl or sucrose concentrations in the water phase were prepared. To study the effect from the type of oil, emulsions were prepared with a series of oils that have a different polarity and chemical structure. The resulting emulsions were characterized by small-angle X-ray scattering (SAXS), wide-angle X-ray scattering (WAXS), differential scanning calorimetry (DSC), and texture analysis (TA).

EXPERIMENTAL SECTION

Materials. In the present study, γ-oryzanol (Tsuno Rice Fine Chemicals, Wakayama, Japan) and tall oil sterol (78.5% β-sitosterol, 10.3% β-sitostanol, 8.7% campesterol, and 2.5% of other minor sterols, Unilever, The Netherlands) were used as structurants. Sunflower oil (Reddy, NV Vandemoortele, Breda, the Netherlands), decane (purity >99%, Sigma-Aldrich, The Netherlands), eugenol (purity 99%, Aldrich, The Netherlands), castor oil (Sigma, The Netherlands) and limonene (purity 97%, Sigma-Aldrich, The Netherlands) were used as a solvents (see Figure 1 for the chemical structures of these oils). Sodium chloride (NaCl, purity >99%, Merck, The Netherlands) or sucrose (purity >95% (GC), Sigma, The Netherlands) dissolved in Milli-Q water solution was used as the aqueous phase in the w/o emulsion. All materials were used as received.

Preparation of the Emulsion. The oil phase was prepared by dissolving the structurants in the oil at elevated temperatures (~100 °C). The structurant concentration in the oil phase was kept constant at 32% (w/w) with a fixed γ-oryzanol to β-sitosterol ratio of 60% to 40% w/w. Aqueous solutions of different NaCl concentrations (0%, 1.5%, 5%, 10%, 12%, 15%, 17%, 20%, and 25% w/w) were prepared and heated to 90 °C. To prepare the emulsion, the two phases were mixed at a fixed weight fraction of the aqueous phase (10% w/w) in a closed container at 90 °C and stirred at 1300 rpm for ~2 min using a magnetic stirrer. The resulting w/o emulsion was cooled to room temperature and stirred until gelling occurred. The solidified emulsion was subsequently stored at 5 °C for 1 week before characterization. Note that no additional emulsifiers or surfactants were added during emulsification and that the final w/o emulsion was stabilized by solidification of the oil phase. Furthermore, note that high concentrations of structurant are not necessary to form an organogel but were chosen to have a very clear scattering signal in the X-ray scattering experiments.

X-ray Scattering. 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 setup are given elsewhere.[11,18] SAXS data were collected in the range 0.079 $nm^{-1} < q < 4.4$ nm^{-1} and WAXS data in the range 2.7 $nm^{-1} < q < 22.6$ nm^{-1} (at 10 °C) and 2.7 $nm^{-1} < q < 35.0$ nm^{-1}, where q is the scattering vector defined by $q = 4\pi \sin\theta/\lambda$ (with θ the scattering angle and λ the wavelength of the incoming X-ray beam). The scattering experiments were performed at 10 °C and at 70 °C. Scattering data from the separate water and different oil samples were subtracted from the scattering data of the emulsions. The SAXS experiments were performed after 4, 29, or 50 weeks of storage at 5 °C for the samples with different salt concentrations, and after 2 and 23 weeks of storage at 5 °C for the samples with various oils.

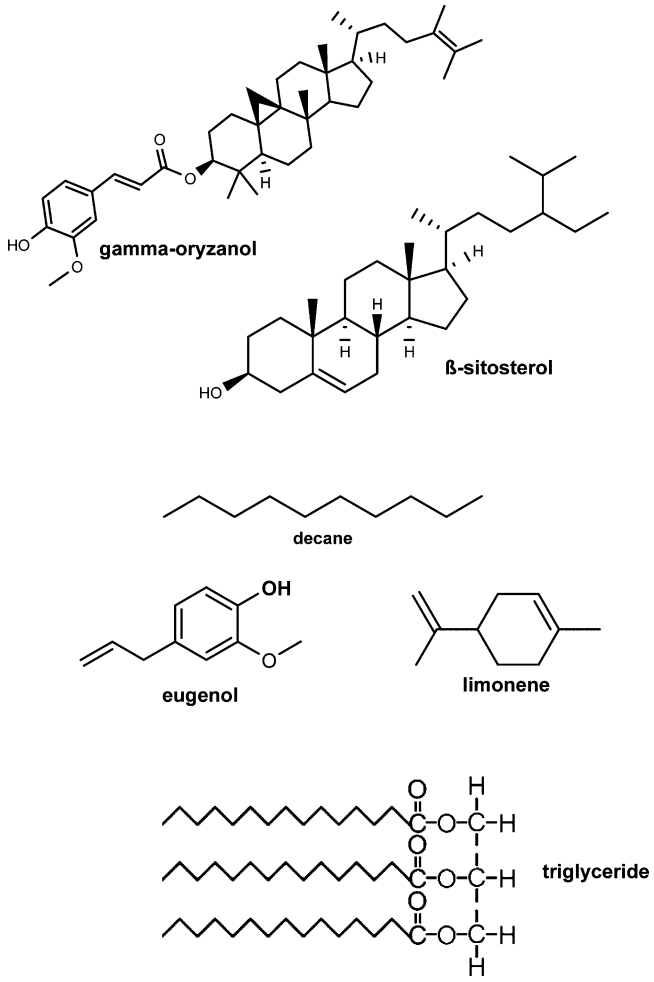

Figure 1. Chemical structures of β-sitosterol, γ-oryzanol, and some of the oils used in the present study. Sunflower oil and castor oils are essentially mixtures of triglycerides; therefore, the general chemical structure of triglycerides is given.

DSC. DSC scans of the emulsions were performed using a Perkin-Elmer Diamond DSC (Perkin-Elmer Co., Norwalk, CT). A 7−15 mg amount of the emulsion was added to stainless steel pans, sealed, and loaded in the differential scanning calorimeter. The DSC scans were performed by first heating the sample from 0 °C to 120 °C, followed by cooling the sample from 120 °C to 0 °C and a second heating from 0 °C to 120 °C. The results from the second heating run were used. A constant heating/cooling rate of 10 °C/min was used.

Firmness Measurements. The firmness (hardness) of the emulsions was measured using a Texture Analyzer T2 (Stable Micro Systems Ltd., Surrey, UK). The emulsion was prepared and kept in glass cups with an internal diameter of 25 mm. A texture analyzer probe of 2 mm (diameter) penetrated into the sample to a maximum depth of 8 mm at a constant speed of 1 mm/s. The firmness was defined as the peak force (N) at the maximum penetration depth.[12] For the majority of formulations, two samples (cups) were prepared independently. For the series concerning the effects of the oils, each sample was only prepared once. All measurements were taken at least at three different locations in the same cup and subsequently averaged.

Water Activity. The water activity (a_w) of the aqueous solutions with different NaCl concentrations was determined using Novasina LabMaster-a_w (Lachen SZ, Switzerland). Water activity values were taken after at least 6 min of equilibration in the sample holder. Most of the samples were measured in duplicate.

Water Solubility in the Oil Phase. The water content in different oils was measured using a Karl Fischer Titrator, Mettler Toledo DL 38 (Mettler Toledo, The Netherlands). Samples were prepared by mixing

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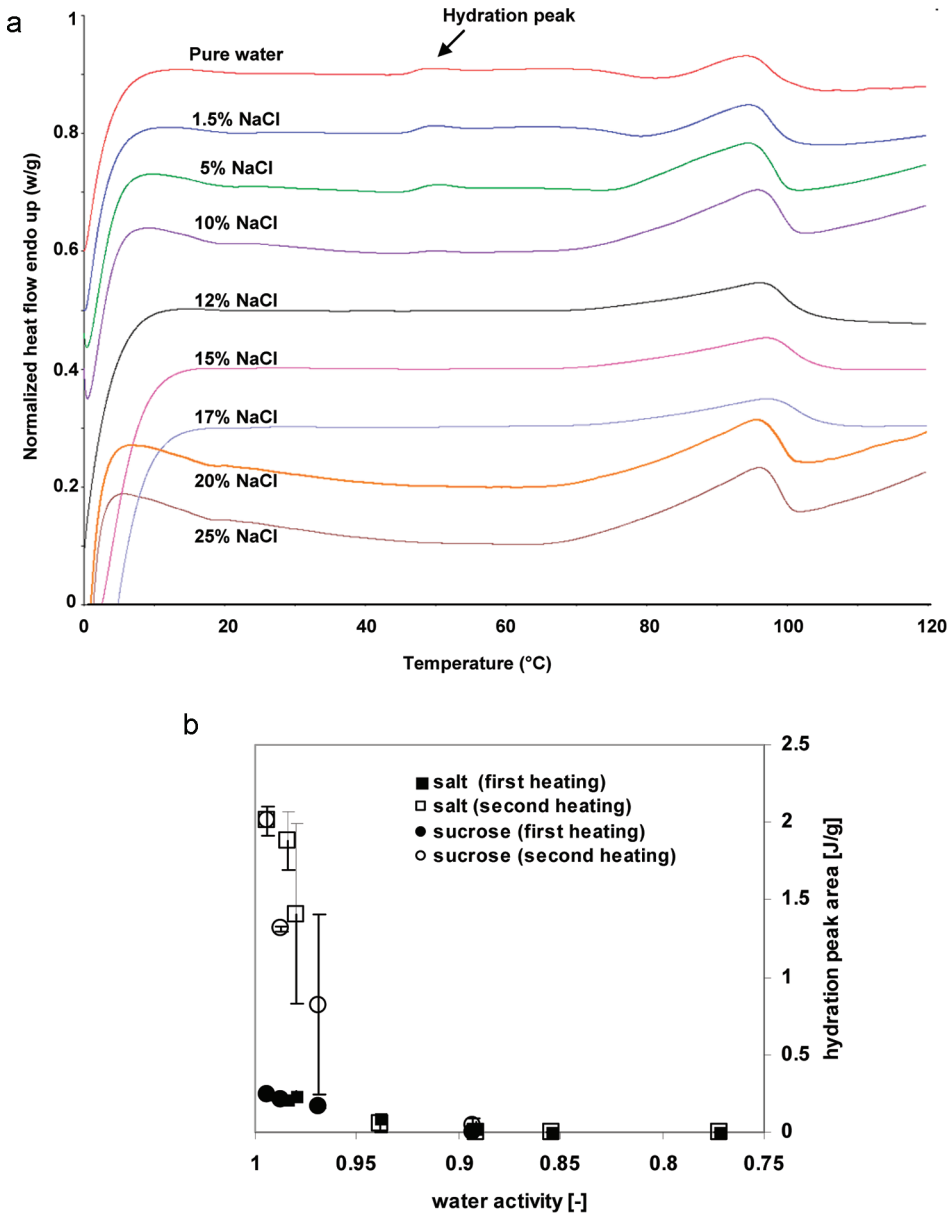

Figure 2. (a) DSC thermographs of emulsions prepared with 90% oil phase consisting of 32% sterols (60% oryzanol–40% sitosterol) in sunflower oil and 10% aqueous phase that contains different NaCl concentrations. (b) Hydration peak area of sitosterol (part a) as a function of water activity of different salt and sucrose concentrations in the water phase.

oil and water with equal mass ratio in a vessel for 1 h at 800 rpm using a magnetic stirrer. To ensure saturation of the oil phase, samples were left to equilibrate for at least three days before measurements. To determine the water content, a certain amount of the saturated oil phase (0.7–1.5 g) was added and titrated. Samples were measured at least in duplicate.

Scanning Electron Microscopy (SEM). The tubular microstructure of the organogels was visualized using a field emission scanning electron microscope (Magellan 400, FEI, Eindhoven, The Netherlands). The organogel sample was placed in a beaker filled with pure hexane for ~5 min to partly extract the oil. The sample was dissected, fit onto a SEM sample holder by carbon glue (Leit-C, Neubauer Chemicalien, Germany), and subsequently frozen in liquid nitrogen. The samples were transferred to a nondedicated cryopreparation system (MED 020/VCT 100, Leica, Vienna, Austria) with a sample stage at −95 °C. In this cryopreparation chamber the samples were freeze-dried for 1 min at −95 °C at 1.3×10^{-6} mbar to remove water vapor. Thereafter, the sample was sputter-coated with a layer of 3 nm tungsten at the same temperature. Finally, the samples were cryoshielded and visualized with SEM at −120 °C and 4×10^{-7} mbar.

RESULTS AND DISCUSSION

Effect of Salt. *DSC.* To investigate the effects of the addition of salt on the formation of sitosterol monohydrate crystals, w/o emulsions with different NaCl concentrations in the aqueous phase were prepared and tested using DSC. Figure 2a shows the DSC thermographs of emulsions prepared with sunflower oil containing 32% (w/w) sterols (60% oryzanol–40% sitosterol) and 10% (w/w) aqueous phase containing different NaCl concentrations (0%, 1.5%, 5%, 10%, 12%, 15%, 17%, 20%, and 25% w/w). The emulsions prepared with low salt concentration (<10% w/w) show two transition peaks in the DSC curve, one peak at ~50 °C and a second peak at 95 °C. The peak at 50 °C corresponds to the transition of monohydrate sitosterol crystals into hemihydrate crystals,

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which is in agreement with a previous study on sitosterol-based w/o emulsion systems.[16] The size of the hydration peak decreases with increasing salt concentration (see Figure 2b). Samples containing high salt concentration (>12% w/w) behave like a water-free organogel and only show one transition peak at 95 °C. The peak at 95 °C corresponds to the melting transition of the emulsion.

The water activity of different NaCl and sucrose solutions is given in Table 1. The table shows that addition of sufficient amounts of salt (i.e., >10% w/w) effectively reduces the water activity, thereby decreasing the formation of monohydrate crystals.

Table 1. Water Activity of Different NaCl and Sucrose Aqueous Solutions[a]

solution	water activity (a_w)
water	0.994 (0.004)
% w/w NaCl	
1.5	0.984
5	0.980 (0.007)
10	0.939 (0.001)
15	0.891 (0.002)
20	0.855 (0.002)
25	0.772 (0)
% w/w sucrose	
20	0.987 (0.003)
40	0.969 (0.007)
60	0.893 (0.001)

[a]The values in parentheses stand for the standard deviation of the measurements.

Sugar (sucrose) was also used to reduce the water activity and to make sure that the water activity is the main factor for suppressing the formation of monohydrate sitosterol crystals and not electrostatic interactions. The results show that addition of sucrose (i.e., >60% w/w) reduced the water activity to below 0.9 and hindered the formation of sitosterol monohydrate (see Figure 2 and Table 1).

SAXS. X-ray scattering measurements were performed to investigate whether the addition of salt also enables the formation of tubular microstructures in water/sunflower emulsions, as opposed to the situation in the presence of pure water. Figure 3 shows the diffraction patterns of water/sunflower oil emulsions prepared with different NaCl concentrations (0%, 1.5%, 5%, 10%, 20%, and 25% w/w) in the water phase. Clear differences in the diffraction patterns of the emulsions can be observed. The emulsion prepared with pure water shows sharp crystallographic reflections at $d = 2\pi/q_i$ = 3.59, 2.72, 2.59, and 1.80 nm, equivalent to the individual sitosterol hydrate crystals in aqueous media.[14,16] Besides the individual crystals, an interference pattern (in the range ~0.5−3 nm^{-1}) resulting in a double peak was detected. This interference pattern is in accordance with observations in a previous study for emulsions prepared under similar conditions, and a hypothesis was formulated to explain the scattering pattern in terms of a more complex tubule wall structure (e.g., bilayer structure involving water molecules) in the emulsions.[14] The interference pattern of the emulsion deviates from the simpler pattern that is observed for the pure organogels.[11] At increasing salt concentration, the intensities of the sharp crystallographic reflections decreased whereas the intensity of the interference pattern increased and the double peak

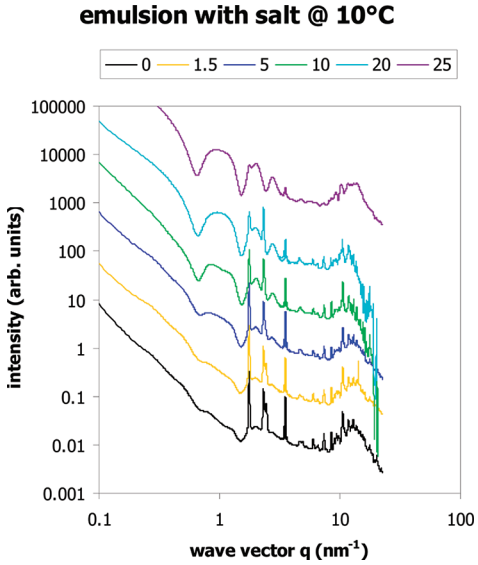

Figure 3. SAXS data for 32% total sterols in sunflower oil (60:40 mixture of oryzanol:sitosterol) in emulsions containing 10% of different salt−water solutions. Data taken at 10 °C after 1 week storage at 5 °C. From top to bottom: 25%, 20%, 20%, 5%, 1.5%, and 0% salt, respectively.

gradually transformed into a single peak (i.e., at salt concentration >10%), analogous to what is observed in water-free organogels.[11] These findings show that the presence of salt in the emulsion allows the formation of a tubular network similar to the one found in the pure oil system. This result is in line with the DSC results (Figure 2), showing that the addition of salt interferes with the formation of sitosterol monohydrate and allows, at adequate levels, the oryzanol and sitosterol to self-assemble in a tubular microstructure. SAXS measurements of the emulsions at 70 °C, in which the sitosterol crystals were already dehydrated, showed that the tubule interference pattern is already present in the absence of salt (see Figure 4), supporting the conclusion that once the hydration of sitosterol is prevented, the self-assembly process of sterol compounds into tubular structure can proceed.

The tubular structure of the organogels predicted with SAXS measurements was visualized with SEM. SEM images of organogel samples prepared with a 32% w/w sitosterol−oryzanol mixture in sunflower oil are shown in Figure 5. The images support the presence of a tubular microstructure as indicated by the SAXS data. The tubules seem to form bundles, as seen in Figure 5a, with an average size of ~60 nm/bundle. The image made using a higher magnification (Figure 5b) shows that the diameter of the single tubule ranges between 7 to 15 nm (including the tungsten coating (3 nm), which is on the same order of magnitude as the tubule size calculated with SAXS. The image also shows that most of the tubules are linear with an average length ranging from 5 to 10 μm.

Firmness. The firmness of the gelled emulsion was measured as a function of salt concentration (Figure 6). This figure shows that the firmness increases as the salt concentration is increased in the water phase. The firmness of the emulsion prepared with 20% salt was at least 3 times higher than that of the emulsion prepared with pure water. The results also show that the firmness of the emulsion varies inversely to the size of the hydration peak shown in Figure 2. This indicates that the firmness is dependent on the structure in the emulsion. The

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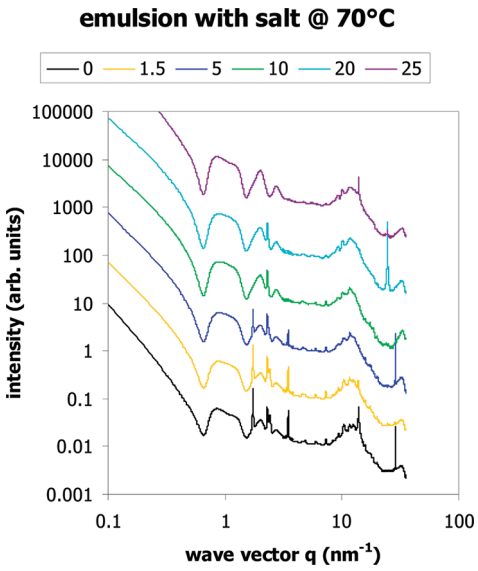

Figure 4. SAXS data for 32% total sterols in sunflower oil (60:40 mixture of oryzanol:sitosterol) in emulsions containing 10% of different salt−water solutions. Data taken at 70 °C, in water after 1 week storage at 5 °C. From top to bottom: 25%, 20%, 20%, 5%, 1.5%, and 0% salt, respectively.

differences in the firmness shown in Figure 6 were in line with direct observations of the firmness of the emulsions.

Effects of the Type of Continuous Oil Phase. *DSC.* Figure 7 shows the DSC thermographs of emulsions prepared with 32% (w/w) sterols (60% oryzanol−40% sitosterol) in different oils (decane, limonene, sunflower oil, castor oil, and eugenol) and pure water as aqueous phase. The ratio of oil to aqueous phase was kept constant at 90:10 w/w. The results reveal that the emulsions prepared with decane and limonene did not comprise hydration peaks of sitosterol whereas with other oils such as castor oil and sunflower oil the hydration peak was clearly present. For eugenol, the sitosterol hydration peak is superimposed as a shoulder on the melting peak of the emulsion. The absence of the hydration peak for the emulsions prepared with decane and limonene might be explained by the

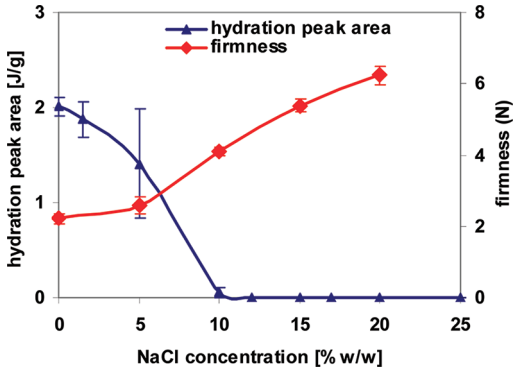

Figure 6. Firmness of emulsions prepared with 90% oil phase consisting of 32% sterols (60% oryzanol−40% sitosterol) in sunflower oil and 10% aqueous phase that contains different NaCl concentrations.

fact that these oils are less polar than the other oils, and therefore the water solubility in these oils is lower than in the others (see Table 2 for solubility of water in different oils). This reduces the water transport through the oil phase to the sitosterol, which may delay or hinder the formation of sitosterol monohydrates. Another possible explanation might be that the interaction between sitosterol and low polarity oils is stronger than that of sitosterol with water, which makes the formation of sitosterol monohydrates energetically unfavorable. The solubility of water is higher in the case of eugenol, castor oil, and sunflower oil (see Table 2), which is in line with the formation of the hydrate crystals in these oils.

The type of oil influenced not only the hydration peak but also the melting of the emulsions. Emulsions prepared with eugenol and limonene showed a melting peak at ∼45 °C and ∼65 °C, respectively, lower than that of the emulsions prepared with the other oils (melting at ∼90 °C). This could be related to the difference in the polarity of the oils and their mutual interactions with water and the structurants. Besides, the solubility of water in the eugenol phase is higher than in decane and therefore the efficiency by which water is able to interfere

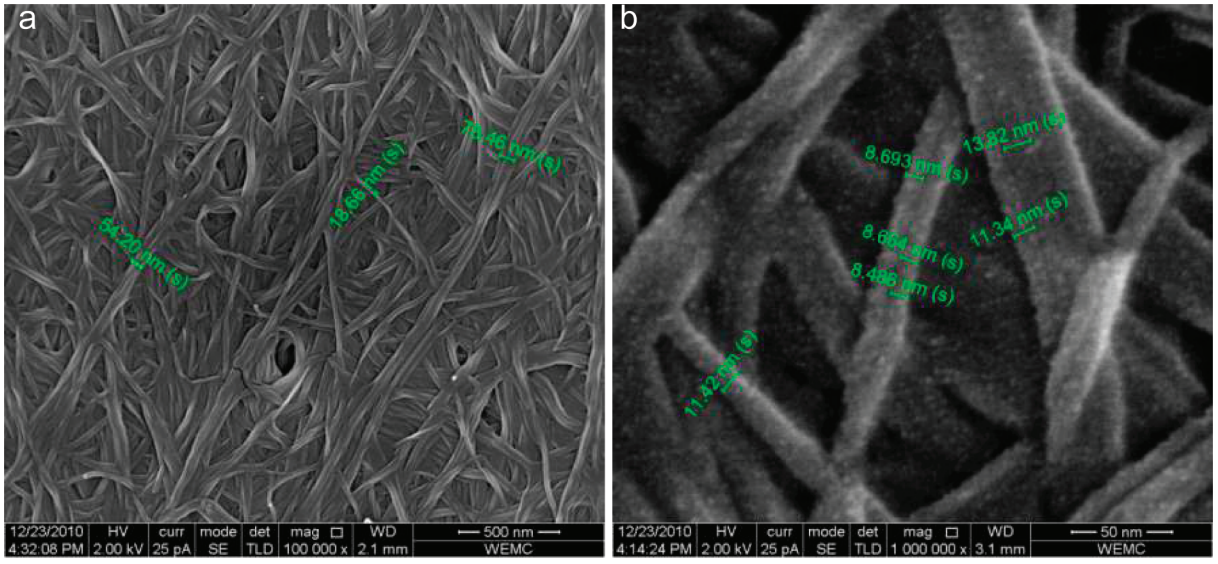

Figure 5. SEM images of organogel prepared with 32% total sterols (60:40 mixture of oryzanol:sitosterol) in sunflower oil.

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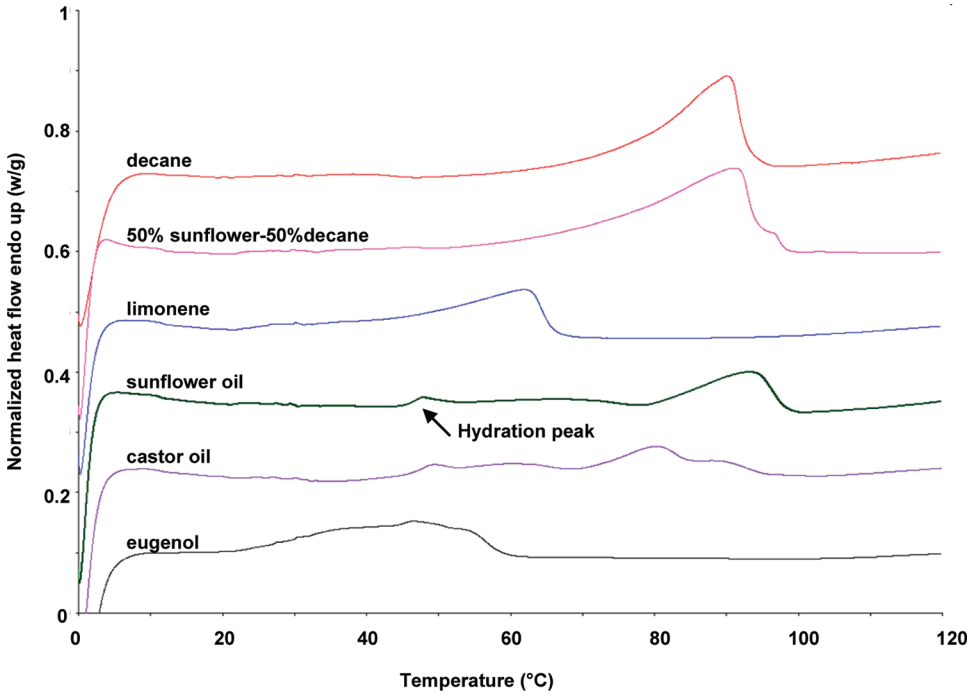

Figure 7. DSC thermographs of emulsions prepared with 90% continuous oil phase consisting of 32% sterols (60% oryzanol−40% sitosterol) in different oils and 10% pure water aqueous phase, ordered by polarity.

Table 2. Water Solubility in Different Oils[a]

oil	water solubility (mg/g)	dielectric constant
decane	0.23 (0.05)	2.0[19]
limonene	0.62 (0.02)	2.4[20]
sunflower oil	1.04 (0.08)	3.1[21]
castor oil	8.56 (0.58)	4.5[22]
eugenol	19.64 (0.08)	10.4[23]

[a]The values in parentheses stand for the standard deviation of the measurements.

with structure formation is higher in the eugenol phase than in decane.

SAXS. To assess the effect of oils on the structure of the emulsions, the emulsions were studied with SAXS. Figure 8 shows the interference patterns for organogels prepared with a 32% w/w sitosterol−oryzanol mixture in various oils (decane, limonene, sunflower oil, castor oil, and eugenol). For all oils, patterns very similar to those for the tubules in sunflower oil observed in previous studies are present.[10,11] This shows that in all cases very similar self-assembled tubular structures are formed. The SAXS diffractograms of emulsions prepared with the aforementioned oils are shown in Figure 9. It was found that the type of oil strongly influences the diffraction patterns of the emulsions. Emulsions prepared with decane and limonene showed interference patterns (in the range ∼0.5−3 nm^{-1}) similar to those observed in the organogels (Figure 8) and in water/sunflower oil emulsion with high salt concentrations, (i.e., >20% w/w salt, Figure 3), indicating the formation of the tubular structure. Emulsions prepared with eugenol and castor oil did not show any interference patterns related to tubular structures and only showed sharp crystallographic reflections corresponding to sitosterol or oryzanol hydrate crystals. These results are in agreement with the DSC data shown in Figure 7, which show again that the polarity of the oil plays an important role in the self-assembly of sterol

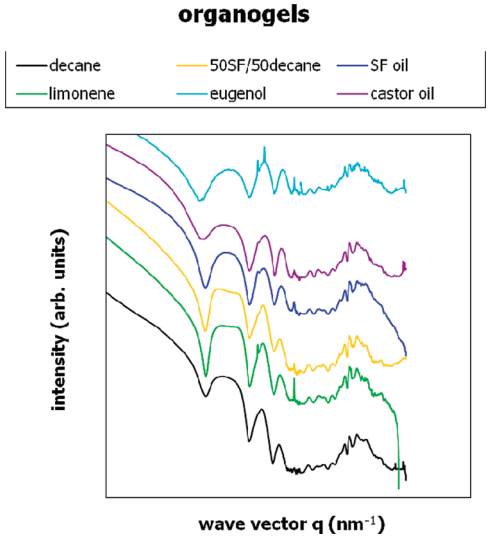

Figure 8. SAXS patterns of organogels prepared with 32% total sterols (60:40 mixture of oryzanol:sitosterol) in different oils. Measurements were performed at 10 °C, after 1 week storage at 5 °C. From top to bottom: eugenol, castor oil, sunflower oil, 50% sunflower oil/50% decane, limonene, decane.

molecules into tubules in the presence of water. The polarity of the oil seems to influence the transfer rate of water to the sterol molecules and, at the same time, the thermodynamic interactions between sterols and water. To study the kinetics of the hydration of sitosterol and the stability of the tubules in more detail, the emulsions shown in Figure 9 were tested with SAXS over various storage times as will be discussed in the following sections The difference in the structures of the emulsions is expected to influence the firmness of these emulsions.

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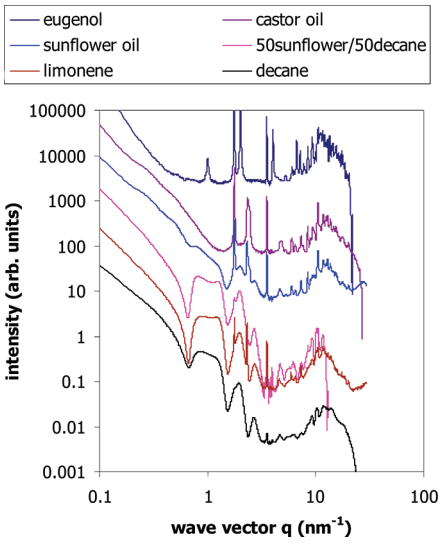

Figure 9. SAXS data for 32% total sterols (60:40 mixture of oryzanol:sitosterol) in different oils in emulsions containing 10% pure water. Data taken at 10 °C, after 1 week storage at 10 °C. From top to bottom: eugenol, castor oil, sunflower oil, 50% sunflower oil/50% decane, limonene, decane.

Firmness. Figure 10 shows the firmness of the emulsions as a function of the oil used. The firmness was strongly dependent

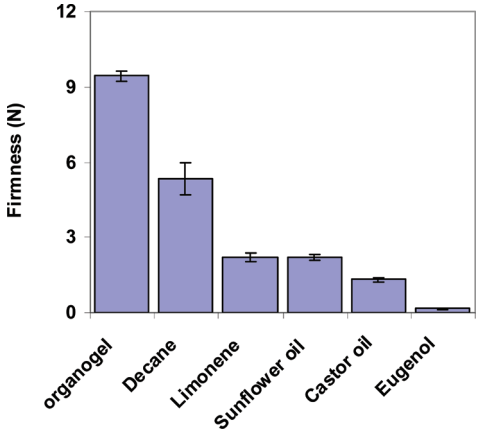

Figure 10. Firmness of emulsions prepared with 90% continuous oil phase, consisting of 32% sterols (60% oryzanol–40% sitosterol) in different oils, and 10% pure water aqueous phase. The organogel was prepared in sunflower oil with a total sterol concentration equivalent to that in the emulsions and with the same oryzanol–sitosterol ratio.

on the type of oil. It was found that the firmness decreases with increasing solubility of the water in the oil phase (see Table 2 for solubility). Emulsions prepared with decane showed the highest firmness, comparable to the firmness of the water/sunflower oil emulsions prepared with high salt concentration (i.e., 20% w/w NaCl). In contrast, the lowest firmness was found for the emulsion prepared with eugenol (i.e., firmness with decane was at least 30 times higher than with eugenol; see Figure 10). This corresponds to a low melting temperature of the emulsion prepared with eugenol (see Figure 7). The variation in the firmness is related to the structures of the emulsions; the self-assembled tubular network as a building block of the emulsion is much stronger than individual hydrated crystals as was demonstrated in this paper for the emulsions with varying amounts of salt in the aqueous phase.

In conclusion, the self-assembly of oryzanol and sitosterol into tubules in an emulsion-gel can be promoted and the firmness can be enhanced by reducing the water activity through the addition of salt to the water phase (i.e., water activity <~0.9, at a salt concentration >10% w/w) and/or by using oils with low polarity (i.e., dielectric constant <2.5).

Aging of the Emulsions. To investigate the stability of the tubules upon storage of the emulsions, SAXS measurements were performed on the emulsion samples at different storage times. Figure 11 shows the effect of storage on a w/o emulsion

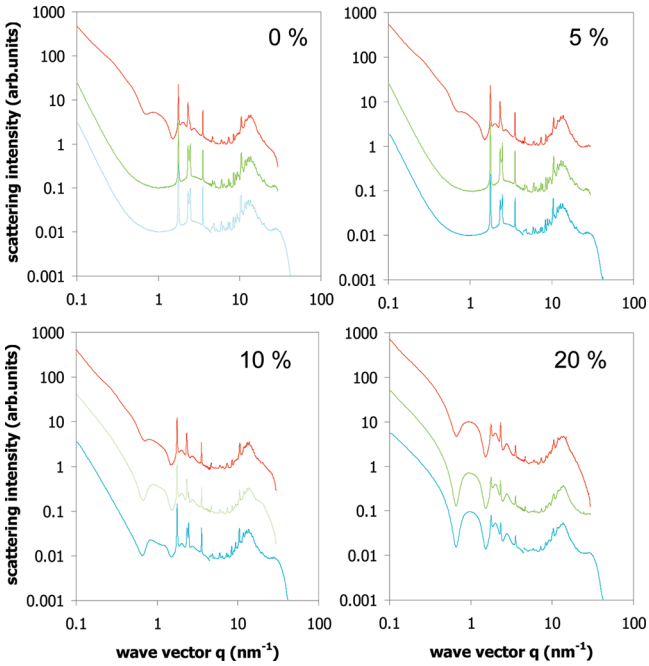

Figure 11. Effect of storage period on w/o emulsions based on a continuous sunflower oil phase, structured with 32% sterols (40:60 sitosterol:oryzanol), and an aqueous phase containing 0%, 5%, 10%, or 20% salt. In each panel from top to bottom: 4, 29, and 50 weeks storage time at 5 °C.

containing 32% total sterols in the oil phase and 10% aqueous phase. The aqueous phase contains 0%, 5%, 10%, or 20% salt. SAXS experiments were performed after 4, 29, or 50 weeks storage at 5 °C. After 4 weeks of storage, all samples still show features reflecting the presence of tubular structures in the emulsion gel. For the emulsions with 0% and 5% salt in the aqueous phase, such features have disappeared after 29 and 50 weeks storage. The sample with 10% salt in the aqueous phase still has similar features at 29 and 50 weeks compared to the sample after 4 weeks. The 20% salt sample has the most pronounced features, which have even become slightly more pronounced after storage over longer periods. The conclusion from this experiment is that the self-assembled tubules decompose in the presence of water–salt mixtures with a water activity above 0.9 (0% and 5% salt in water) on a time scale of a couple of months. Thus, monohydrate formation can be concluded to be a very slow process. In the presence of a water phase with a water activity around 0.9 (10% salt in water), the tubules are marginally stable. The tubule-like features persist for almost one year but never develop into the pronounced pattern that is observed in organogels. Finally, the

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emulsion gels containing a water phase with a water activity below 0.9 are very stable for at least one year, showing a clear fingerprint for the presence of tubules in the emulsion gel.

A similar storage experiment was performed for w/o emulsions using the oils described in the previous section. SAXS curves were recorded after 2 and 23 weeks storage at 5 °C (see Figure 12). The emulsions prepared with decane, limonene, and 50:50 sunflower oil:decane are relatively stable against sitosterol monohydrate formation. Emulsions based on sunflower oil and even more on castor oil show an increase in the amount of monohydrate present in the emulsion over the storage period. In the emulsion based on eugenol, no tubules are present even after two weeks storage.

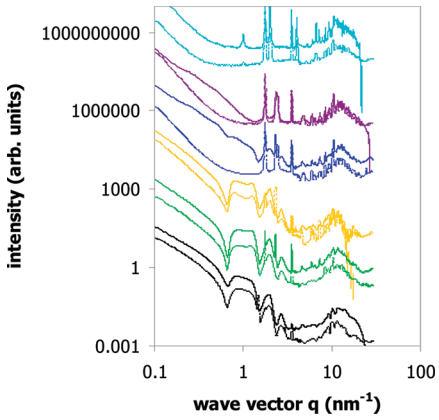

Figure 12. Effect of storage on SAXS curves for w/o emulsions based on pure water and various lipid phases, structured with 32% of a 40:60 sitosterol:oryzanol mixture in the organic phase. For each pair of curves, the top one is after 2 weeks storage and the bottom one is after 23 weeks storage at 5 °C. From top to bottom: eugenol, castor oil, sunflower oil, 50:50 sunflower oil:decane, limonene, decane.

The stability of the tubules in the low polarity oils might be attributed to the low water concentration in the oil phase which reduces the water transport through the oil phase to the sitosterol and consequently delays the formation of sitosterol monohydrates. In addition, the low polarity oils might form a protection layer around the tubules, thereby hindering the formation of monohydrate crystals. It is also possible that the interaction between the sitosterol and the oil increases as the polarity of the oil is decreased, making the formation of the sitosterol monohydrates energetically unfavorable.

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Notes

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