Structuring of Edible Oils by Mixtures of γ-Oryzanol with β-Sitosterol or Related Phytosterols

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ABSTRACT: The relation between molecular structure of oil-structuring agents and their gel-forming capability was investigated for mixtures of the phytosterol ester γ-oryzanol with a series of phytosterols. Dihydrocholesterol, cholesterol, β-sitosterol, and stigmasterol were found to form firm transparent gels with γ-oryzanol in sunflower oil under the conditions used in this work. The mixture of β-sitosterol with γ-oryzanol in sunflower oil does not gel immediately on cooling, but mechanical agitation such as shear promotes gelling. Gels that are formed immediately after cooling show a higher modulus than gels for which there is a time delay between cooling and agitation (150 vs. 100 kPa). The effect of oscillatory shear parameters (amplitude, frequency) is small, as long as the yield stress of the gel is not exceeded. The gels withstand compression very well (up to deformations of 10%), but yield at very small deformations. The enthalpy of melting of the solid phase is estimated to be 26 ± 4 kJ/mol, putting it in the same range as for certain fibrillar steroid-derived organogels.

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KEY WORDS: 5α-Cholestane, [481-21-0]; cholesterol, [57-88-5]; dihydrocholesterol, [80-97-7]; ergosterol, [57-87-4]; organogel; γ-oryzanol, [11042-64-1]; rice bran oil component; β-sitosterol, stigmast-5-en-3β-ol, [83-46-5]; stigmasterol, [83-48-7]; TAG; triacylglycerols.

The firmness of a food product depends in many cases on the gelling capability of its water and oil phases (1,2). However, far more ingredients are known that are capable of structuring water phases (3,4) than of structuring oil phases (other than crystallizing TAG). In this latter category, even fewer ingredients can be considered more or less food grade (5–9). This is regrettable, since such systems could provide an alternative to the common structuring agent for edible oil phases: saturated FA-based crystallizing TAG. Replacement of these hardstocks by ingredients that can structure edible oil could lead to the development of more healthful food products, based on healthful commodity oils such as sunflower, rapeseed, or soybean oil (10).

The use of mixtures of β-sitosterol and γ-oryzanol as structuring agents could be a first step in this direction, as these ingredients are reported to form transparent gels in sunflower oil (6). The system is rather similar to certain (nonedible) low-M.W. organic molecules that are able to structure nonaqueous phases (11–20). Such systems are known as organogels or oil gels. The latter term will be used in the present paper. Both of these structuring agents have a history of consumption: γ-Oryzanol occurs naturally in rice bran oil and β-sitosterol in many vegetable oils. This characteristic sets this type of organogel apart from most alternative systems, which are based on nonedible components.

Systems that gel oil phases usually represent serendipitous findings, as there is insufficient understanding of these systems to allow a prediction of the structuring potential beforehand. The common element is that the structuring agent should form small building blocks to be able to create an oil gel at a low concentration of the structuring agent. Two types of building blocks are encountered often in practice: (i) crystallites sticking together. By this mechanism, crystallizing TAG form a network in a liquid oil (1,2). Such systems tend to be turbid, provided the crystals are large enough. (ii) Fibrillar structures. These can form through either some intermediate mesophase or direct molecular incorporation (20). Such systems can be transparent, provided that the fibrils are very thin compared with the wavelength of visible light and that no other inhomogeneities occur that impart turbidity to the system. To provide firmness to the oil gel, the fibrils also need to be crystalline. Whether oil gels can be formed along either route depends strongly on the molecular structure of the system.

Because detailed information on the oil-structuring agents β-sitosterol and γ-oryzanol mixtures is lacking, it is of interest to study these systems in terms of small changes in the molecular structure of the structuring agents. To do this, a number of phytosterol + γ-oryzanol mixtures were screened in this work for their oil-structuring potential (for fixed gelling conditions), and it was found that β-sitosterol in the mixture can be replaced by a number of other phytosterols.

Since the properties of these alternative systems appear to be quite similar to the β-sitosterol + γ-oryzanol system, the mechanism of gel formation was investigated in more detail for the latter gels only, with the emphasis being on the rheological properties of the oil gels. The time required to form a gel under quiescent and shear conditions was studied, as well as the effects of gelling temperature and phytosterol + γ-oryzanol concentration and ratio on gelling kinetics. For the final gels, the melting and large deformation behavior under compression and shear were also determined.

Combined, the results in this paper provide a systematic empirical rheological characterization of the mechanical

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properties of these systems and identify some possible parameters in non-TAG structuring of edible oils. These properties provide some indicators of the structuring mechanism in these transparent TAG oil gels. Unfortunately, the similarity of structuring agents and TAG oil implies that microscopy cannot be used readily as a technique to provide these clues in the present systems.

MATERIALS AND METHODS

Gel preparation. In the present experiments sunflower oil (Impériale; Hartog Union, Merksem, Belgium) and γ-oryzanol (11042-64-1; Tsuno Rice Fine Chemicals Co., Wakayama, Japan) were used, usually in combination with β-sitosterol (83-46-5; Ultrasitosterol; Kaukas Chemical Mill, Lappeenranta, Finland), but sometimes together with another phytosterol-like component (5α-cholestane, 481-21-0; Research Plus Inc., Manasquan, NJ; dihydrocholesterol, 80-97-7, 96% pure, Acros, Geel, Belgium; cholesterol, 57-88-5, 95% pure, Acros; stigmasterol, 83-48-7, 95% pure, Acros; ergosterol, 57-87-4, 98% pure, Acros). The ingredients were dissolved with stirring in sunflower oil at 85–90°C, and the solution was kept for ~10 min at that temperature. The hot solution was filtered through a 220 nm Millipore microfilter (except in the initial screening experiments) so as to form a completely transparent oil gel upon cooling; this treatment did not affect the rheological properties of the oil gel. The ingredients were used without further purification.

In the initial screening study, mixtures containing relatively high concentrations of oryzanol (default sample 8 wt% sterol mixture in oil, mass ratio phytosterol compound/oryzanol 2:3, molar ratio 1:1) were investigated. In the (small-deformation) gelling study, most of the oil gels investigated contained relatively high concentrations of sitosterol, resulting in somewhat turbid gels (default sample 6 wt% sterol mixture in oil, mass ratio sitosterol/oryzanol 3:2). For large-deformation experiments, transparent oil gels were investigated (6 wt% sterol mixture in oil, mass ratio sitosterol/oryzanol 2:3).

Shear small-deformation rheology. Rheological measurements were performed using a Carrimed CSL 500 stress-controlled rheometer, equipped with a cone and plate geometry (cone: 2° angle and 60 mm diameter, gap 53 mm, cone inertia 22.57 $N{\cdot}m{\cdot}s^2$, machine inertia 26.19 $N{\cdot}m{\cdot}s^2$). The instrument was operated at an oscillatory stress (default 1 Hz), and the deformation was detected (lower detection limit: 10^{-3} s^{-1}). Calibrations were performed in line with the guidelines of the equipment manufacturer. Rheological experiments were done in triplicate, except for the 6 wt% sterol mixture default sample for which five repeats were done.

A freshly prepared phytosterol mixture in oil was poured on the lower rheometer plate. Subsequently, the top plate was put into place, and the gel formation process was monitored. The temperature of the sample was controlled using the Peltier element located in the bottom plate of the rheometer.

For aging experiments, an oscillatory stress with a maximum amplitude of 150 Pa was applied to the system, unless stated differently. The dynamic moduli G' and G'' were monitored as a function of time.

For the melting and concentration-dependent experiments, a plate-plate geometry was used, with the gap between the plates set to 1 mm because slip occurred if the experimental protocol for the cone-plate geometry was applied to melting samples. Both the top and bottom plates were covered with sandpaper to provide enough friction to prevent slippage of the gel during melting. The top plate was completely covered with sand paper; the bottom plate was covered with a ring of sandpaper—having an inner diameter of 42 mm and an outer diameter of 60 mm—to allow good thermal contact between gel and bottom plate. As for the aging experiments, the oil gel was prepared *in situ*, and this method does not lead to absorption of the oil from the oil gel by the sandpaper. An oscillatory strain with a maximum amplitude of 0.003 was applied to the system.

The absolute numbers obtained for the moduli in a plate-plate geometry are lower than for a cone-plate geometry because the applied strain decreases toward the center of the sample, although this is mostly compensated by the narrower gap as a result of the presence of the sandpaper. We estimate the cumulative effect as ~10%. All samples within one series are affected in the same way.

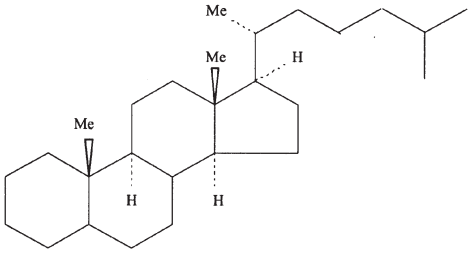
Compressive large-deformation rheology. Oil gels were prepared in a cylindrical Teflon container, which could be split into two parts after gel formation. In this way a cylindrical gel was obtained of 17 mm height and 26 mm diameter. The cylindrical gel was placed on a paraffin oil-covered stationary bottom plate of an Instron 4502 tensile testing machine. The paraffin oil allows the top (and bottom) surface of the gel to expand during compression. Diffusion of the paraffin in the oil gel during the experiment can be calculated to be negligible. The stresses that are presented in this paper have been corrected for this increase in surface area during compression ("true stresses"). Samples were tested at 20°C, using a compression speed of 10 mm/min. Measurements, were done in triplicate.

Shear large-deformation rheology. A Carrimed Weissenberg Rheogoniometer was equipped with a controlled temperature stainless steel concentric cylinder system (outer cylinder 46 mm in diameter, inner cylinder 40 mm in diameter; height, 25 mm). Both cylinders were ribbed to prevent slippage. After pouring approximately 35 mL of freshly prepared solution into the outer cylinder, the inner cylinder was placed in position, and the solution was allowed to gel. Measurements were performed at a shear rate of $1.0{\cdot}10^{-4}$ s^{-1}; deformation experiments typically lasted 400–2000 s. Measurements were done in triplicate.

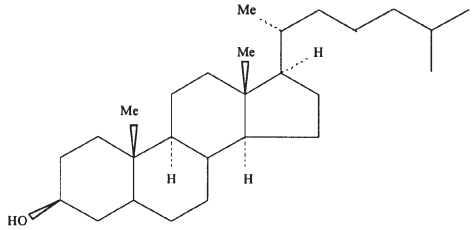
RESULTS AND DISCUSSION

Screening of various phytosterols in relation to gel formation. First a series of experiments was performed in which mixtures of γ-oryzanol with phytosterol or a physterol-related component (5α-cholestane, dihydrocholesterol, cholesterol, β-sitosterol, stigmasterol, or ergosterol) were screened for

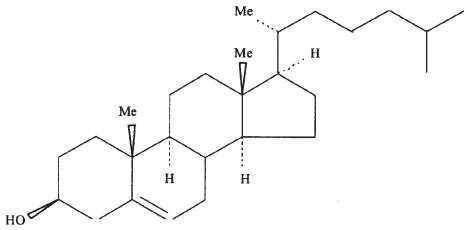
5α-cholestane, [481-21-0]



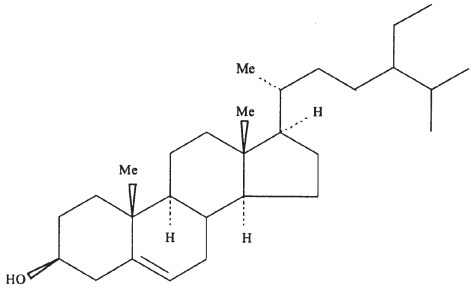
dihydrocholesterol, [80-97-7]



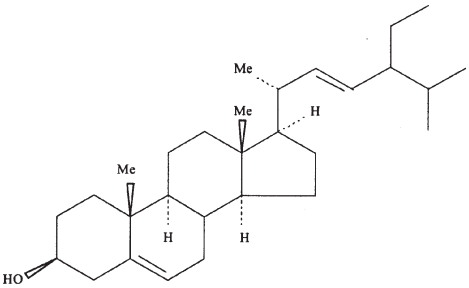
cholesterol, [57-88-5]



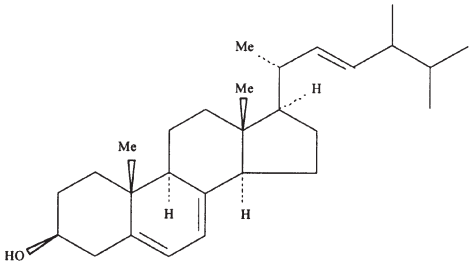
β-sitosterol, [83-46-5]



stigmasterol, [83-48-7]



ergosterol, [57-87-4]



SCHEME 1

their capacity to form an oil gel for the default sample preparation conditions. The default composition of the samples was 8 wt% ingredients in sunflower oil, mass ratio phytosterol/γ-oryzanol 3:2. The phytosterols used in these measurements formed a progression with respect to their molecular bonding and substituents. Cholestane is a "stripped" cholesterol molecule, lacking both the –OH group and the double bond. In dihydrocholesterol the –OH group is present, but the double bond is lacking. β-Sitosterol has a slightly modified alkyl chain relative to cholesterol. Stigmasterol has an additional double bond in the modified alkyl chain. Finally, ergosterol has an extra double bond in the cholesterol ring structure and a small modification in the alkyl chain relative to stigmasterol. The chemical structure of each component is presented in Scheme 1.

Gel formation in sunflower oil in the presence of γ-oryzanol was studied qualitatively under three conditions: Quiescent gelling at 5 or 20°C (after 70 h), and gelling in the presence of (oscillatory) shear at 10°C in a rheometer. The observations among the three methods agreed very well. The visual observations on gelling under quiescent conditions are summarized in Table 1. The rheological results are presented

TABLE 1
Visual Observations on Quiescent Gelling Properties at 20°C of Various Phytosterol + γ-Oryzanol Mixtures

Phytosterol compound	Gel (yes/no)	Appearance	Remarks
5α-Cholestane	No	—	Precipitate of small white particles (diameter typically 0.5–1.0 mm)
Dihydrocholesterol	Yes	Transparent, firm gel	Becomes slightly hazy during prolonged storage
Cholesterol	Yes	Transparent, firm gel	Tiny inclusions (diameter typically 0.3 mm)
β-Sitosterol	Yes	Transparent, slightly hazy, firm gel	Haziness can be removed by filtration without affecting the mechanical properties of the gel
Stigmasterol	Yes	Inhomogeneous gel	Large spherulites (diameter typically 5 mm), slowly growing to become space-filling
Ergosterol	No	—	Fine precipitate

in Figure 1, where the development of the storage modulus for these systems over time is shown. Four components (dihydrocholesterol, cholesterol, β-sitosterol, stigmasterol) form a gel under these experimental conditions and two do not.

The molecular structure of the phytosterol(-related) compound in a mixture with γ-oryzanol and sunflower oil was found to affect the gelling properties of the mixture. In the series 5α-cholestane, dihydrocholesterol, cholesterol, β-sitosterol, stigmasterol, and ergosterol, only the first and the last compound did not form a gel in combination with γ-oryzanol under the present experimental conditions.

In the experiments presented here, relatively small changes in chemical structure affected the gel-forming capability of certain phytosterol mixtures dramatically. In considering the tested compounds as consisting of a hydroxyl group, a ring system, and an alkyl chain, the following conclusions are drawn:

(i) The presence of a hydroxyl group is essential. The compound without an –OH group (cholestane) is incapable of gel formation. The absence of the hydroxyl group results in a much higher solubility of the compound in sunflower oil. Increasing the concentration of this more soluble compound does not result in gel formation.

(ii) The details of the ring system are also important, although their impact is less than the presence of the hydroxyl group. Gel formation appears to occur fastest in mixtures containing compounds with a ring system that does not contain any double bonds (dihydrocholesterol). It is slower for compounds with a single double bond in the ring system (e.g., β-sitosterol and cholesterol). Gel formation does not occur for the compound containing two double bonds in the ring system (ergosterol). This variation in gel-forming capability might originate from differences in the planarity of the ring structure.

(iii) The gel-forming capability of the system is not extremely sensitive to the chemical structure of the alkyl residue.

Because the properties of firm gels of γ-oryzanol with either dihydrocholesterol or appeared to be quite similar to those of the β-sitosterol + γ-oryzanol system, the mechanism of gel formation was investigated in more detail by means of rheology for the latter gels only.

The effect of shear on gel formation. Phytosterol + γ-oryzanol + oil systems showed erratic gelling under quiescent conditions. The system can remain fluid for a long period, but gelling can be induced by a small mechanical disturbance. Without such a disturbance, the moment of gelling is unpredictable. The importance of shear to oil gel formation for a mixture of β-sitosterol and γ-oryzanol is illustrated by leaving a phytosterol solution under quiescent conditions for a specified delay time Δt, after which an oscillating shear stress is applied to the system. The amount of shear depends on the frequency and the amplitude of oscillation. The results are plotted in Figure 2, which shows the storage and loss moduli G' and G'' as a function of time. The storage modulus G' can be taken as a qualitative measure for the firmness of a gel as measured in a penetration test. An apparent plateau value, G'_{max}, is attained if the modulus is plotted as a linear function of time t. For what will be considered the default sample for the remainder of this paper (6 wt% total sterol, mass ratio sitosterol/oryzanol 3:2, gelling at 10°C), the apparent modu-

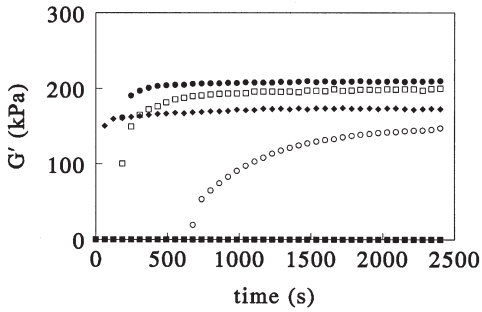


FIG. 1. Rheological data for mixtures of 4.8% γ-oryzanol + 3.2% sterol in sunflower oil at 10°C under oscillatory shear (amplitude 175 Pa, frequency 1 Hz). Gelling is observed for (◆) dihydrocholesterol, (○) cholesterol, (●) β-sitosterol, (□) stigmasterol. No structure is formed in mixtures with (◇) cholestane or (■) ergosterol under the present experimental conditions.

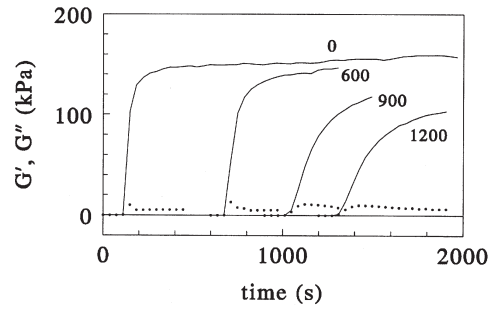


FIG. 2. Increasing shear modulus G' as a measure for gel formation. After a specified delay time Δt, an oscillating shear stress profile (amplitude 175 Pa, frequency 1 Hz) is applied to a sterol + oil system (6 wt% total sterol, mass ratio sitosterol/oryzanol 3:2) gelling at 10°C. The delay time is varied: Δt = 0, 600, 900, 1200 s from left to right. Dots indicate loss modulus G''.

lus G'_{max} was found to be 149 ± 12 kPa based on five repetitions.

Figure 2 shows that the gel strength is near zero after rapid quiescent pre-cooling to 10°C (in ~30 s). The delay time Δt before turning on the oscillatory shear is the main factor affecting the length of the initial period preceding the sudden increase in modulus. Apparently, gel formation is difficult under completely quiescent conditions, whereas gel formation occurs almost instantaneously under shear (after a small lag time τ of only 100–200 s). This is in line with the erratic gelling behavior under quiescent conditions. Once gelling starts, G' increases less steeply and the attained plateau modulus G'_{max} tends to be lower for longer delay times Δt. For the default sample (6 wt% total sterol, mass ratio sitosterol/oryzanol 3:2, gelling at 10°C), the rate of increase of the modulus at the point where G' attained $0.5 \cdot G'_{max}$ was 1.5 ± 0.3 kPa/s based on five repetitions. Note that at the point of gelling, there will be a short period in which the deformations exceed the linear viscoelastic regime.

The rheological properties of the resulting gel can be interpreted in terms of a crystallization model involving the following stages: (i) formation of primary building blocks (possibly involving an intermediate mesophase such as a rod-like micelle, but in a crystalline state at the end of the process); and (ii) aggregation of primary building blocks (collision of crystallites, adhesion, further consolidation). These stages may overlap to some extent in time. The first stage will hardly be sensitive to shear, because it occurs on a molecular scale. The second stage will be sensitive to shear, because aggregation involves mesoscopic objects. Under quiescent conditions, aggregation will hardly occur, whereas primary building block formation will continue in the normal fashion. The application of shear, mainly its rotational component, to the system would be very effective in increasing the chance that primary building blocks collide and aggregate to form a network in oil if these particles are nonspherical (e.g., fibrils, as in Refs. 11,12). Note that thin fibrillar building blocks would be consistent with the transparent character of the oil gel, but obtaining direct proof by means of microscopy is very difficult as the samples are transparent (optical microscopy), the TAG phase cannot be removed easily without affecting the building blocks too (sample preparation for scanning electron microscopy), and the contrast between structuring agents and TAG oil is very limited (transmission electron microscopy).

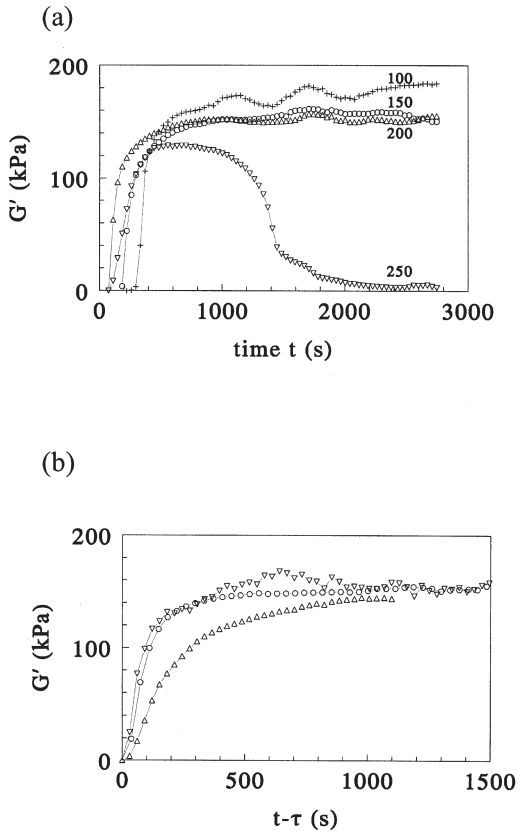


FIG. 3. Effect of oscillating applied stress amplitude and frequency on gel formation for a sterol + oil system (6 wt% total sterol, mass ratio sitosterol/oryzanol 3:2) gelling at 10°C. (a) Oscillating shear stress (fixed frequency 1 Hz) applied at amplitude (+ + +) σ = 100 Pa, (○ ○ ○) σ = 150 Pa, (△ △ △) σ = 200 Pa, (▽ ▽ ▽) σ = 250 Pa; (b) oscillating shear stress (fixed stress amplitude 175 Pa) applied at frequency (▽ ▽ ▽) ω = 0.1 Hz, (○ ○ ○) ω = 1 Hz, (△ △ △) ω = 10 Hz.

Optimizing amplitude and frequency of shear for reproducible gel formation. If the presence of a shear flow field helps oil gel formation, it is clear that the amplitude and frequency of the applied oscillation are important parameters. It is expected that large amplitudes will have a negative effect on gel strength, since they will cause damage to the gel in a relatively early stage of gel formation. The effect of the applied shear stress amplitude was studied on a 6% sterol mixture in sunflower oil. The results are plotted in Figure 3a. There, a higher stress results in marginally lower final G'_{max} for shear stresses up to 200 Pa. Thus, the data indicate that the linear regime for these systems extends to strains of about 10^{-3}. For the curve obtained at a shear stress of 250 Pa, however, the applied stress results in extensive damage to the gel structure and a large decrease in the elastic modulus of the gel.

In contrast, a change in the frequency of the oscillating stress field is not expected to affect the gelling process very much, since the prime function of the stress field is to increase the effective collision cross section of the crystallites. Only if

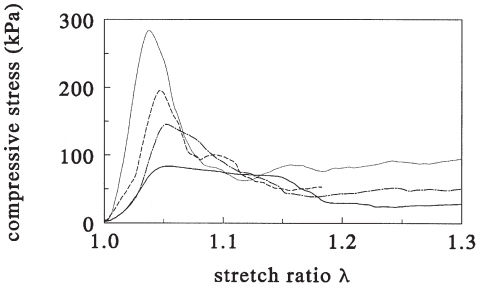


FIG. 4. Uniaxial compression curves for sterol + oil gels, formed during storage for 40 h at 5°C under quiescent conditions (mass ratio sitosterol/oryzanol 2:3). From top to bottom: 12, 10, 8, and 6% total sterol concentration.

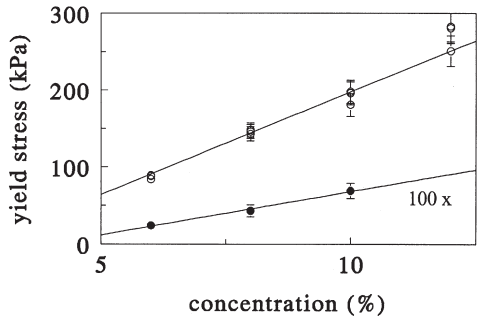


FIG. 6. Yield stress for sterol + oil gels (mass ratio sitosterol/oryzanol 2:3) under compression (○) and shear deformation (●) as a function of concentration. For details on gel preparation, see Figures 4 and 5. Yield stress for shear deformation multiplied by 100. Error bars are SD.

the adhesion of two building blocks (on collision) is a slow process can a significant effect of frequency be expected. Figure 3b shows the effect of oscillation frequency of the applied shear stress on a 6% sterol mixture in sunflower oil. The minor differences between the curves confirm that the effect of variation of frequency is rather small over the experimentally accessible range of frequencies.

The foregoing experiments indicate that shear flow helps to obtain reproducible gel formation of phytosterol mixtures in oil. Application of a constant flow would damage the structure, but an oscillating shear field is an acceptable alternative. A shear amplitude of 150–200 Pa and a frequency of 1 Hz were convenient settings for further experiments.

Large-deformation properties of the gel. A number of large-deformation experiments were done to confirm that the small-deformation experiments were indeed performed under no-slip conditions. This is relevant because these systems are much firmer under compression than under shear deformation, as can be established manually quite easily: Fingers slip along the interface without too much resistance when rubbing the gel.

Therefore, a number of stress-strain curves for the phytosterol–oil gels were obtained, both in (uniaxial) compressive deformation and in shear deformation. The maximum stress is called the yield stress; the deformation at which this maximum stress occurs will be expressed in terms of the stretch ratio $\lambda = \sqrt{(h_0/h)}$ at yield, where h_0 is the initial height of the gel cylinder and h the actual height during deformation (21).

Large-deformation compression experiments were performed on gels formed during storage for 40 h at 5°C under quiescent conditions, and equilibrated to 20°C in 4 h. The total phytosterol concentration of the gels was varied between 6 and 12%. In Figure 4 a number of examples are shown. An increase in the yield stress and a decrease in the yield stretch are observed with increasing total phytosterol concentration. The yield stresses are of the order of 10^5 Pa. The yield stretch is ~1.05, which implies that the gel can be compressed by ~10% in height before breaking. Thus, these gels can be considered to have a relatively short texture.

Similar experiments were performed for shear deformation. In Figure 5 the stress is plotted as a function of stretch ratio λ, which is related to the normal shear strain γ by the relation $\gamma = \lambda - \lambda^{-1}$ [$\lambda = \gamma/2 + (1 + \gamma^2/4)^{1/2}$] (21). The stretch ratio at yield was found to increase slightly with increasing total phytosterol concentration. The yield stresses were found to be of the order of 10^2 Pa. The stretch ratio at yield for a 6% phytosterol oil gel is about 1.002, which corresponds to a yield strain of 0.004, and is much smaller than the value obtained under compression. These numbers are in agreement with results derived from Figure 3a. These results confirm that the yield mechanism under shear in Figure 5 is slip and that firmer gels are less sensitive to slip. They also confirm that the small-deformation experiments were done under conditions in which no slip occurred.

Figure 6 shows a linear relation between yield stress and concentration in both shear and compression ($R^2 = 0.996$ and 0.986, respectively). This indicates that the strength of the gel is proportional to the amount of structuring material in the gel, and not to the number of cross-links (note that the breaking and elastic properties do not need to depend in the same way on concentration). This suggests that the gel structure breaks at the building blocks and not at the cross-links (3). Usually, the cross-links are the weakest spots in an aggregate structure. However, for crystalline long fibrillar building blocks, even modest bending forces occurring with deformation of the gel may result in failure of the crystallite.

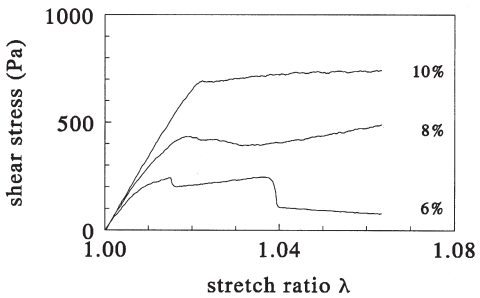


FIG. 5. Shear deformation curves for sterol + oil gels (mass ratio sitosterol/oryzanol 2:3, $\dot{\gamma} = 10^{-4}$ s^{-1}), formed during 20 h at 5°C under continuous application of a shear oscillation (strain amplitude 0.005, frequency 1 Hz) in the Weissenberg rheogoniometer. From top to bottom: 10, 8, and 6% total sterol concentration.

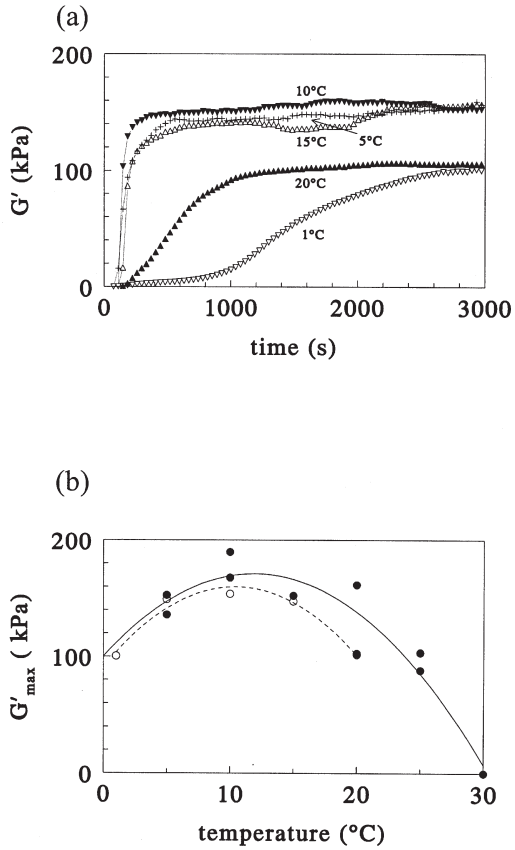


FIG. 7. Effect of temperature on G' and G'_{max} during gel formation for a phytosterol oil gel (total sterol concentration 6 wt%, mass ratio sitosterol/oryzanol 3:2): (a) G' during gelling at (▽ ▽ ▽) 1°C, (+ + +) 5°C, (▼▼▼) 10°C, (△ △ △) 15°C, (▲▲▲) 20°C (stress amplitude 175 Pa, frequency 1 Hz); (b) maximum modulus during gelling G'_{max}, (●) 150 Pa, (○) 175 Pa (frequency 1 Hz). Lines are parabolic fits meant to guide the eye.

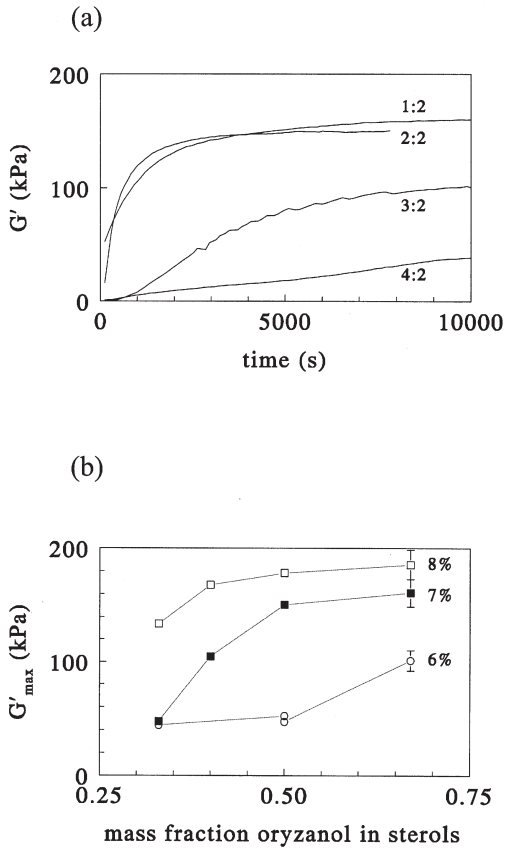


FIG. 8. Effect of relative sterol/sterol ester concentration and total concentration on gel formation. An oscillating shear stress profile (applied deformation $\gamma = 0.003$, frequency 1 Hz) is applied to a phytosterol + oil system gelling at 10°C: (a) Mass ratio sitosterol/oryzanol, from top to bottom: 1:2, 2:2, 3:2, 4:2 (total sterol concentration 7 wt%); (b) total sterol concentration: (○) 6 wt%, (■) 7%, (□) 8 wt%.

Effect of temperature on gel formation. Figure 7a shows the effect of temperature on gel formation. Lag time τ displays a minimum around 10°C, and G'_{max} shows a maximum in that temperature range. Apparently, oil gel formation is limited both at low and at high temperatures. At the high-temperature end, the increased solubility of the structuring agents is the most likely explanation. As a result, fewer building blocks will be available to form the gel, which will form slower and be less firm, as observed. Alternative explanations, such as an increased flexibility of the building blocks themselves, do not seem appropriate for crystalline materials. At the low-temperature end, the situation is more complex, and the explanation must therefore be more speculative. Sufficient structuring material is available, so either the building blocks do not stick very efficiently or else the properties of the building blocks change. An example of the latter could be that many more building blocks are initiated at lower temperatures, leading on average to smaller building blocks, which makes the shear-induced aggregation process less effective. It is clear that the weaker gels are always associated with slower gel formation, in line with the observation in Figure 2.

Figure 7b illustrates that it is impossible to obtain much stronger gels by changing the temperature at which gelling takes place if all other conditions remain constant. The gel strength is already at its maximum for the present default sample preparation conditions.

Effect of concentration on gel formation. Another parameter that is expected to affect the formation of the primary particles is the composition of the phytosterol mixture in oil. From Figure 8a, one can see that the rate of gel formation is higher for mixtures containing higher levels of oryzanol. Again, fast gel formation results in stronger gels, which are also more transparent. Note that in these and further experiments shear during gelling was applied by means of a constant maximum strain instead of maximum stress.

Figure 8b demonstrates the effect of total phytosterol concentration on G'_{max}. The plateau modulus vanishes at 4% phytosterols and below, but this will be caused partly by the experimental procedure used to characterize these very weak gels. For mixtures rich in oryzanol, G'_{max} depends more or less linearly on phytosterol concentration. For mixtures containing less oryzanol, this trend is less clear. Thus it is not possible to derive any composition-independent scaling laws from these data (22).

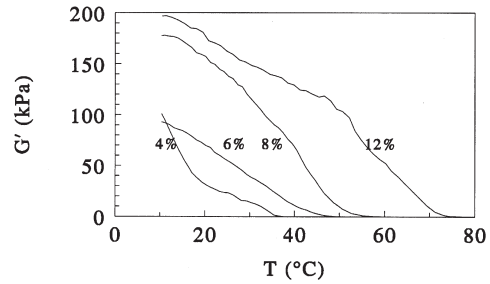


FIG. 9. Melting curves for sterol + oil gels during a temperature sweep from 10 to 70°C. An oscillating shear stress profile (applied stress 150 Pa, frequency 1 Hz) is applied to a sterol + oil system (mass ratio sitosterol/oryzanol 1:1) during gel formation at 10°C. An oscillating strain of 0.003 is applied during melting. Total sterol concentration: 4, 6, 8, and 12 wt%.

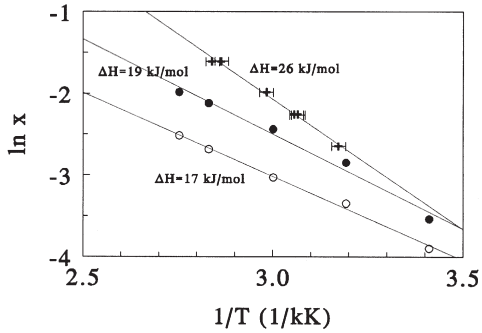


FIG. 10. Concentration dependence of the m.p. for a sterol + oil gel (mass ratio sitosterol/oryzanol 1:1, applied strain of 0.003, gel formation at 10°C), and of the solubility of oryzanol and sitosterol: (+) gel, (●) oryzanol, (○) sitosterol. Solubility of pure components obtained from unpublished data of H. Ritter and R. van de Sande). *x,* mole fraction of the solute; *T,* temperature (Kelvin); Δ*H,* enthalpy of melting for the solid. Error bars are SD.

These observations are in qualitative agreement with results by Ritter *et al.* (6).

Melting behavior of the gel. Figure 9 shows that the melting temperature of a phytosterol gel depends strongly on concentration, with gels containing very little phytosterol melting at lower temperatures than more concentrated gels. Such melting behavior can be described thermodynamically in a semiquantitative way by using the concentration dependence of the temperature at which the gel modulus vanishes as an indication for m.p.

The melting curves in Figure 9 confirm the increase of the m.p. of the gel with increasing total phytosterol concentration. It can be seen that the storage modulus of the gels decreases with temperature as the solubility of the phytosterols in the sunflower oil increases. Slip is prevented by the use of sandpaper and the application of a protocol in which a fixed strain is applied during measurement rather than a fixed shear stress. Treating the phytosterol mixture as a single component and using the Gibbs–Helmholtz equation for an ideal solution of a pure solid in a liquid (see, e.g., Refs. 23,24),

$$\left(\frac{\partial \ln x}{\partial 1/T}\right)_{sat,p} = \frac{-\Delta H_{\text{melting}}}{\text{R}}$$

with x the mole fraction of the solute, T the (absolute) temperature, $\Delta H_{\text{melting}}$ the enthalpy of melting for the solid, and R the gas constant, allows an estimate of the enthalpy of melting for the solid phase of 26 ± 4 kJ/mol (see Figure 10).

The present value of $\Delta H_{\text{melting}}$ is consistent with results on fibrillar nonedible steroid-derivative gels (11,12,20), for which an enthalpy of formation of $\Delta H_{\text{melting}}$= 20–40 kJ/mol was obtained (15,16). This suggests that the same type of molecular interactions are responsible for gel formation in those systems as in the current transparent phytosterol–oil gels, which can be taken as additional support for the hypothesis that mixtures of β-sitosterol and γ-oryzanol form a crystalline fibrillar network in sunflower oil.

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