Revisiting pure component wax esters as basis of wax-based oleogels

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Abstract

Current research on wax-based oleogels indicates wax esters to be the key component in many natural waxes. This necessitates understanding the properties of pure wax esters to unravel the gelling mechanism in wax-based oleogels. Therefore, wax esters with different carbon numbers and symmetries were studied and characterized regarding their thermal (DSC) and viscoelastic (oscillatory rheology) behavior. Pure wax esters and binary mixtures of wax esters were studied as such and in oleogels formed in combination with medium chained triglyceride oil at WE-inclusion levels of 10 % (w/w). Interpretation of the observations was based on detailed analysis of pre-existing data on crystallographic (SAXS) and thermal properties. It is found that all observations concerning single pure WE's obey a systematic framework linking molecular make up, crystal structure and behavior. The study on the gelling of four different binary mixtures of wax esters revealed that substantial chain length differences do have the expected consequence of separate crystallization. Mixtures of wax esters with only limited chain length difference reconfirmed earlier speculations on mixing and crystal structure. Applying mixtures of wax esters only differing in their position of the ester bond indicated ideal mixing behavior in the solid phase of the gels. Actually, the data revealed that despite these expected observations in both systems, additional thermal events occur at specific mixing ratios. Their supposed relation to compound formation certainly needs further confirmation. Rheological analysis confirmed that sequential crystallization results in highest firmness values for the systems studied.

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Abstract

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Keywords

wax ester; oleogel; wax; molecular composition; mixing behavior

Introduction

The list of potential oil structurants, substituting triglyceride (TAG)-based high-melting hardstock fats to create semi-solid lipid phases, is long . Among those, waxes are promising candidates to find industrial application. They can be applied without chemical modification and are available in sufficient quantities against reasonable procurement costs . Their gelation mechanism is affiliated to the formation of three-dimensional networks of crystals with a high oil-binding capacity . Even though many natural waxes already achieved GRAS status , their current application in the food industry is predominantly as a coating agent. Nonetheless, this prior use may benefit future consumer acceptance as ingredient in other food products.

For this reason, the potential of natural waxes as gelling agent in liquid oils has been extensively studied by several authors, e.g. . Due to many varying internal and external factors and lacking standardization of characterization methods, a comparison of different studies and thus general characterization of wax based oleogels remains difficult. Previous studies focused on the influence of external factors such as cooling rate , shear or ultrasound . Among the internal factors, the effect of the concentration on the gel properties is understood the best. Above the critical gelling concentration, the gel strength and thermal properties show systematic behavior with increasing wax concentration . Further, it was shown that variation of the oil type can induce different crystal morphologies or changes of thermal behavior of wax-based oleogels.

It is, however, generally acknowledged that the composition of the waxes is the most important parameter determining the wax crystal morphologies and, hence, the properties of a wax-based oleogel . The chemical composition of waxes varies strongly, depending on the origin, the growth conditions and the extraction and purification processes.

Waxes considered for application in food products are, among others, are e.g., beeswax (BWX), candelilla wax (CLX), carnauba wax (CRX), rice bran wax (RBX) and sunflower wax (SFX). These are predominantly composed of long-chained alkyl esters, the so-called wax esters (WE), n-alkanes (HC), free fatty acids (FA) and free fatty alcohols (FaOH). In most natural waxes, WEs are the main component and consequently govern the crystallization and gelling behavior . A waxes composition varies in the content of the different molecular species – WE, FA, FaOH, HC – but also within the makeup of each species- Within the WE fraction, different waxes have a specific chain length distribution, covering an overall range from 34 to 64 C-atoms.

The WE content of different natural waxes and the total carbon number (CN) distribution of their WE

fraction are gathered in **Tab. 1** . As the data, compiled from different sources (reveals, RBX and SFX are the most homogeneous waxes with a WE content above 90 % (w/w).

Typically, oleogel formation is based on low quantities of a high melting material. This approach intrinsically has the risk to deliver structures with dissolution characteristics or structural properties with adverse organoleptic perceptions. To overcome this unfavorable sensory and manipulate functional product characteristics, mixtures of different waxes have been studied . The studies basically showed that properties can be manipulated but also revealed that the control of the changes induced is far from trivial. The results indicated that the thermal and viscoelastic properties of blends of two different waxes not necessarily develop as a linear combination of the individual mono-wax gels. This should not surprise as the molecular composition of the high-melting fraction within a lipid phase is decisive for the crystallization characteristics and, hence, the microstructure. DSC data has shown that the formation of mixed crystals and the presence of multiple solid phases is very sensitive to the chain length distribution (quality) of the WE fraction.

To successfully apply wax-based oleogels in foods, the understanding of the effects of external and internal factors is an indispensable prerequisite. Currently, an a priori definition of compositional and process parameters enabling the manipulation of the resulting product properties is not possible. Similar to the design of functional fat phases, also called hardstock fats, unravelling the solidification and structuring behavior of waxes on a molecular level therefore appears to be a meaningful target. To this end, the aforementioned studies regarding internal and external factors are certainly very helpful but do not allow to relate the molecular composition of the waxes and the structure and functionality of their gels. Earlier works describe the thermal and crystallographic behavior of pure WEs. Hitherto, only one publication deals with the properties of pure WEs as oleogelators studying the thermal, viscoelastic and microstructural properties of different symmetric (CN 28; 32; 36; 40) and asymmetric (FaOH_FA: 18_14; 18_16; 18_20; 18_22) WEs in high oleic safflower oil . The results suggest a systematic behavior, depending on the WE composition and structure.

Therefore, this manuscript focuses on the WE-fraction of natural waxes and their quality. In particular, the CN distribution and the symmetry of the molecule, respectively the chain length of FA and FaOH moieties, are of concern. For this purpose, a systematic analysis of the information available on WE crystallization is performed and own experimental data on pure WEs and oleogels based on pure WEs are discussed. Further, the behavior of WEs in oleogels composed of a single WE as structurant or a binary mixture of two WEs of different quality is investigated. The gels are formed with medium-chained triglyceride (MCT) oil, a simply composed TAG-oil, not taking chemical reactions at regular treatment. Therefore, it exhibits a low level of polar components, whose impact on oleogel was shown e.g. by Scharfe et al. .

Material and Methods

Material

Sunflower wax (6607L, Lot.nr. F1911020-001) (SFX), rice bran wax (2811, Lot.nr. F1851015-001), carnauba wax (2442L, Lot.nr. F1806007-001), beeswax (8108LM, Lot.nr. F1727017-001) (BWX) and candelilla wax (2039L, Lot.nr. F1915044-001) were kindly supplied by Kahlwax GmbH & Co KG (Trittau, Germany).

All WEs were obtained from Larodan AB (Solna, Sweden) with a purity >99 %. WEs used were Palmityl Myristate (CN30: 16_14), Palmityl Stearate (CN34: 16_18), Stearyl Palmitate (CN34: 18_16), Myristyl Behenate (CN36: 14_22), Stearyl Stearate (CN36: 18_18), Behenyl Myristate (CN36: 22_14), Arachidyl Stearate (CN38: 20_18), Behenyl Arachidate (CN42: 22_20) and Behenyl Lignocerate (CN46: 22_24). Throughout the manuscript, the first number indicates the carbon number of the FaOH residue, the second number the carbon number of the FA residue.

MCT-oil was purchased from Caesar & Lorentz GmbH (Hilden, Germany). The FA composition of the TAGs was determined to be 56 % caprylic acid (10:0) and 44 % capric acid (8:0) – determined by GC analysis of FA methyl esters according to DGF method C-VI 10a (00).

All materials were used without further purification or modification.

Methods

Oleogel preparation

The MCT-oil was structured either by pure WEs (mono-ester gel) or binary mixtures of WEs (mixed oleogel). The concentration of the structurant was 10.0 % (w/w) for all oleogel systems. Besides the nine mono-ester gels, four mixed oleogels (C30+C46; C30+C42; C36(18_18)+C38; C36(14_22)+C36(22_14)) were each prepared at three different mixing ratios (2:1; 1:1; 1:2).

Stock solutions, each with a total mass of 300 mg, were prepared in 1.5 mL screw neck vials. Weighing precision of components was +-0.00003 g. Complete dissolution was ensured by heating the samples under agitation (250 rpm) up to approximately 95 degC on a heating plate. To avoid any changes in the gel composition, the samples for different analytical methods were directly transferred to the respective sample configuration while still liquid at high temperature.

Thermal properties (DSC)

All DSC measurements were performed in aluminum crucibles. An empty aluminum pan was used as reference.

Pure wax ester sample sizes were at 5-6 mg, the oleogel samples had a size of 8-10 mg. Stabilized samples were produced by storing the crucibles for 48 h at ambient temperature after having been melted at a temperature of 105 degC and cooled to 20 degC in the DSC. The stabilized samples were subjected to the following measurement protocol: The melting of the stabilized samples was monitored by heating from 20 to 105 degC. After holding for five minutes, the samples were cooled down to 5 degC and kept at this temperature for 30 min. Subsequently, they were heated to 105 degC again, kept isothermally for another five minutes and cooled to 20 degC. All heating and cooling rates were 5 K/min. Samples were undergoing this protocol fourfold.

The melting point temperature of the pure WE were determined from multiple scans at different heating rates and extrapolation to a zero scan rate . The molar heat of fusion of the pure WE was calculated straightforwardly from the experimental heat of fusion and molecular weight. For the gels, assigning temperatures is less well defined. In this contribution, the onset temperature of the heating thermogram are defined as gel-sol transition temperatures. This is done because at this point, the solubility of WE in the oil is larger than the concentration of the WE in the sample. The heat of fusion for the WE in the gels is derived based on the assumption that the 10 % (w/w) dosage of WE is completely solid in the sample. This is obviously wrong ignoring the solubility of individual WE.

Viscoelastic behavior (Rheology)

The viscoelastic behavior of all oleogel systems was studied on a modular compact rheometer using a steel plate-plate geometry with sandblasted surface (PP25-S; d = 25 mm) to avoid slippage. A top and base Peltier-system of the measurement geometry ensured accurate temperature control.

Approximately 100 mg of the hot stock solution were positioned on the center of the preheated (90 degC) plate. The measuring gap was 0.2 mm. For standardization purposes, the samples were agitated under shear ($\dot{\gamma}$ = 10 s-1; t = 5 min) and then stabilized at quiescent conditions. The samples were cooled down to 5 degC (5 K/min) and kept isothermally for stabilization (t = 30 min), analogously to the DSC procedure. To observe gel-sol transitions, the samples were heated to 90 degC (5 K/min). This was followed by crystallization, cooling to 5 degC and isothermal stabilization as described above. On these samples, an amplitude strain test ranging from 0.005 % to 100 % at an angular viscosity of 10 rad/s was performed isothermally. Next, the sample was heated to 90 degC (5 K/min) and the same procedure repeated. During all measurements, an angular frequency of 10 rad/s was used and for temperature and time tests, a strain of 0.005 % was applied.

The gap width was allowed to compensate changes in the sample density during the test (FN = 0 N) and reset to 0.2 mm after each heating step. The crystallization was performed in quadruple, the melting and the amplitude sweep were performed in duplicate.

Microstructure

The samples for the bright field microscopic (BFM) images were prepared by pouring a droplet of the hot stock solution on a pre-heated objective slide and covering it with a cover slip. The slides were cooled down carefully from 70 degC to 5 degC (0.8 K/min) in a constant climate chamber. Before image analysis, the samples were stabilized (T = 5 degC; t = 12 h). The micrographs were captured at ambient temperature.

Results and Discussion

Link between wax esters and natural waxes

Thermal behavior

In **Fig. 1** , the DSC heating thermograms of different natural waxes are given. Below each thermogram, the relative frequency of different CNs in the respective WE-fraction is depicted. They are stacked in order of decreasing WE content from top to bottom. For comparison, the thermogram of a pure WE (42 (22_20)) is given on top of the figure.

The melting thermograms show different behavior for different waxes in agreement with many other publications. The pure WE exhibits a single, narrow endothermic peak smeared out primarily due to thermal lag as a function of the heating rate (5 K/min). For the two most homogeneous waxes, SFX and RBX, a similar peak shape is observed. However, the peaks are less symmetric. The small shoulders, more pronounced in RBX, do not only indicate less purity than for the WE C42 (22_20) but also the presence of multiple solid phases. In contrast, the thermograms of CRX and CLX contain broader, or rather multiple peaks with more pronounced shoulders while BWX displays two distinct peaks. Therefrom, a detailed interpretation with respect to the number of coexisting solid phases and mixing behavior is practically impossible. All thermograms gathered are in line with former observations .

For the computation of the relative CN-frequencies of WEs, analytical data on the presence of FaOH and FA moieties in hydrolyzed WE fractions obtained by Doan et al. by means of GC-MS were used. The data itself provides no information on how these moieties were recombined to constitute the WEs. Only little is known about selectivity in the biological synthesis of WEs, combining FA and FaOH moieties. It is known that triacylglycerol profiles of natural vegetable fats can be predicted quite reliably based on the respective fatty acid profile and the Coleman-assumption . Essentially, a preference for unsaturated FAs on the sn-2 position is combined with a random distribution of FAs over the three possible glycerol positions. Based on this, a similar statistical approach was chosen for the recombination of the profiles of FaOH and FA moieties given by Doan into WEs. Cumulating all symmetries at equal CN, this resulted in relative frequencies of the WEs in a range between 34 and 56 carbon atoms. A publication by Vali et al., reports the WE composition of five different rice bran waxes as well as the chain lengths of the FaOH and FA residues of the saponified WEs. Computation of the WE frequencies as outlined above resulted in good agreement between the experimental CN-profile of the WEs and the prediction suggested here. Thus, the WE profiles in **Fig. 1** are computed this way.

This utter arbitrary juxtaposition of the melting behavior of natural waxes and their respective WE CN distribution depicted in **Fig. 1** suggests anecdotal evidence that the WE composition relates to the thermal properties of natural waxes. Without any doubt, the crystallization behavior is a function of the CN-distribution of the WEs. However, this relation rather is of thermodynamical nature and not simply geometrical. However, the distributional width - mono- or polydispersity or bimodality – gives indications for mixed crystal formation and the presence of multiple solid phases. **Fig. 1**suggests that this even holds

for waxes with low WE content such as CLX. The fact that high melting temperatures correspond in the first place with higher amounts of long-chained WEs is well established. This is illustrated by comparing the thermograms of SFX and RBX and, particularly, of CRX. For this wax, the computed WE profile is in line with the information given elsewhere that even higher WEs are present in the wax than possible with the data given by Doan. The fact that the computed data underestimate the high-melting fraction is most likely due to the challenge to quantify the long FA and FaOH moieties. Overall, this analysis reveals that there is a need to better understand the crystallization behavior of WEs and their mixtures in order to control wax crystallization.

Microstructure

Micrographs of oleogels based on 10 % (w/w) of either a pure WE (C34 (16_18) or a natural wax (SFX) are shown in **Fig. 2** . Herein, the respective microstructure of the oleogels crystallized at different cooling rates after stabilization is depicted.

The image of SFX at a cooling rate of 1 K/min (a) indicates needle-like structures, though these are stated to be platelet-like crystals arranged orthogonally to the objective slide . This could either be attributed to phenomena relating to the hydrophilic glass surface and the hydrophobic wax crystals or just be a result of heterogenous nucleation on the glass surface. Those platelets are sintered and form a three-dimensional network, just as repeatedly reported in the literature . After being cooled at the same rate, the pure WE (b) forms almost two-dimensional platelets which are oriented parallelly to the cover of the objective slides and without discernible junctions. Hence, at equal cooling rates of 1 K/min, the natural wax and the pure WE exhibit quite different microstructures. As reported earlier from our lab , when crystallized at very low cooling rates of 0.05 K/min in purified canola oil, SFX crystallizes into well-defined, flat crystal fragments (c). When, in contrast, the pure WE is crystallized at significantly increased cooling rates of 30 K/min, morphologies typical for SFX, related to upright platelets are observed (d). This dependence of the crystal morphology on the cooling rate underlines that the crystal structures occurring in natural waxes and pure WEs are congeneric. Furthermore, the importance of understanding the role of the cooling rate and the presence of polar minor components on the crystallization is emphasized.

Pure component properties

Wax esters are composed of a long-chained FA and a long-chained FaOH moiety. Commonly, the alkyl chain length of the FA residue ranges between 16 and 24 C-atoms, while the FaOH residues tend to be longer, between 18 and 32 carbon atoms .

In the solid state, aliphatic esters crystallize in unimolecular layers with the alkyl chains oriented lengthwise, either orthogonally or tilted with respect to the methyl end plane . The length of a saturated WE molecule in orthogonal chain arrangement is calculated here, using eq. (1), based on simple geometric considerations. The layer thickness d_{ortho} is build up from three contributions: The alkyl chains (number of carbons in FA (n) and FaOH (m) residue) multiplied by the carbon-carbon-distance in chain direction (1.26 A , the ester-oxygen atom chain contribution ($b = 1.09$ A and the distance between two terminal methyl end groups and thus between two planes (c). The latter being specific for aliphatic esters (2.05 A) .

$d_{\mathrm{ortho}} = (m + n) * a + b + c$ (Eq. 1)

For the tilted chain orientation, the thickness of a layer is conveniently considered as the product of the sine of the inclination angle (62.5 deg) and the orthogonal layer thickness (eq. 1) , represented by eq. 2.

$d_{\mathrm{tilted}} = d_{\mathrm{ortho}} * \sin(\alpha_{\mathrm{tilt}})$ (Eq. 2)

There is different rotational freedom for either the FA or FaOH residue due to the orientation of the ester bond. Consequently, the position of the ester bond in the WE chain molecules matters. The WE properties depend thus on the symmetry of the molecule. The latter is expressed as CN, the difference between the CN of the FaOH and the FA residue (eq. 3).

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$\text{CN} = CN_{\text{FaOH}} - CN_{\text{FA}}$ (Eq. 3)

Fig. 3 shows the evolution of the calculated layer thicknesses (lines) as a function of CN. According to eq. 1 and 2, the layer thickness increases linearly with increasing carbon number. The tilted orientation always results in a smaller layer thickness at equal CN. The markers represent averaged values computed from published experimental long spacing data . The small angle XRD (SAXS) data are derived for neat WEs of different CN. The experimental long spacing data corresponds quite well with the simply calculated layer thickness data. This holds for both, the orthogonal and the tilted chain arrangement. A careful look at the data reveals that only WEs with CN of +2 or −4 appear to crystallize in orthogonal configuration. All other WEs crystallize with an inclination angle to the methyl end plane. At this point, it has to be pointed out, that Kreger and Schamhart found two long spacings for tetradecyl octadecenoate (14_18; $CN − 4$) and docosanyl octadecenoate (22_18; $CN + 4$), indicating that both configurations can be assumed by this WE . This observation hints at possible monotropic polymorphism in WEs as known for many molecules containing aliphatic chains.

The data gathered in **Fig. 4** show a clear relation between the caloric properties and the CN of the WEs. The data on the melting temperature and molar heat of fusion of pure WEs are averaged data per CN and CN, accumulated after critical review of the different sources . Most temperature data was obtained by methods like FT-IR or a hot-plate. However, only few literature data regarding the heat of fusion exists. Black crosses indicate onset temperatures and molar heat of fusion data determined experimentally in this work.

Fig. 4 shows that for a single CN, distinct different data points are found. The WEs experimentally studied for this manuscript are selected such that at equal CN either low values of CN $\in [−2; 0; +2]$ or higher values of CN $\in [−8; +8]$ are studied. This data clearly shows that substantial asymmetry, $CN \in [−8; +8]$, causes lower caloric values compared to more symmetric references. Because of the limited possibility to study more complete rows of symmetry, the data does not suggest other insights. The experimental data gathered are, however, in general in agreement with the literature values (grey circles). The broad spreading at equal CN displayed is due to either different symmetry and also the difference in data acquisition, method and purity of the WEs. Overall, the melting temperature shows a non-linear increase with increasing CN. For n-alkanes and monoacid TAGs, Chickos and Nichols successfully modeled the melting temperatures using a hyperbolic equation . In contrast, the increase of the molar heat of fusion with CN could be considered linear. Such a behavior of the thermal properties has been previously reported for other aliphatic long-chained molecules like n-alkanes , FA or FaOH .

In **Fig. 5** , the same averaged data on the melting temperatures of pure WEs of different CNs and symmetries is depicted as a function of CN . Again, black crosses indicate the onset temperatures of the WEs investigated in this manuscript by means of DSC.

Symmetric WEs (CN = 0) exhibit the highest melting temperatures for a given CN. With increasing asymmetry, thus increasing absolute value of CN, the melting temperatures decrease. This effect is almost independent from the type of asymmetry, being caused by either a longer FA or FaOH residue. Astonishingly, WEs with a CN of +2 systematically yield higher melting temperatures (supported by bold lines) than their isomers with a CN of −2. This may correspond to the data shown in **Fig. 3** , revealing that WEs with a CN of +2 crystallize in orthogonal chain arrangement. Esters with a value of $CN = −4$ were found to crystallize in the orthogonal configuration as well (see **Fig. 3**). However, their melting temperatures do not differ considerably from the smooth evolution of melting temperatures over CN as for +2.

Considering the crystallographic and thermal properties data of pure WEs, it is underlined that next to the CN also the symmetry, thus the position of the ester bond in the alkyl chain is an important characteristic that needs to be taken into account. In a recent publication , it was already established that symmetric and asymmetric WEs crystallize differently and that these differences also propagate into WE-based oleogels.

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Mono-Ester oleogels

Thermal properties

The DSC analysis was performed for the oleogels composed of MCT-oil and 10 % (w/w) of a single WE (mono-ester gels). In line with the data displayed in **Fig. 1** , the thermograms for oleogels formed with MCT-oil and either SFX (gray) or pure WE C42 (22_20) (black), dosed at 10 % (w/w) respectively, are shown in **Fig. 6** . The data reveals a more accentuated peak of the gel-sol transition for the WE-gel. The WE-gel undergoes this transition at lower temperatures, being attributed to the longer average CN of the WEs in SFX. The fact that the WE-gel has a more accentuated peak is accounted for by the single crystalline phase in this gel compared to multiple solid phases in the SFX-based oleogel. The latter is indicated by the shape of the peak, showing a more pronounced hump. In line with the thermogram shown in **Fig. 6** , all mono-ester gel systems revealed a single melting peak without indicating any polymorphic transitions under the present conditions. This was independent of the CN and $\overline{CN}$. With increasing CN, the gel-sol transitions occur at higher temperatures.

In **Fig. 7** , the dissolution temperatures and the molar heat of fusion as a function of CN are gathered for pure WEs (gray) and the oleogels consisting of MCT-oil and a single WE (10 % (w/w)) (black). Also for the gels, a systematic increase of the caloric properties with increasing CN was found. Obviously, the gel-sol transition temperatures of the mono-ester gels are lower than the melting temperatures of the respective neat structurants.

The display of data reconfirms that the symmetry of the WE alkyl chain is of significance to define both, pure component (**Fig. 4** and **Fig. 5**) and gel properties, see **Fig. 7** . The effect on the gel properties, thus molar heat of fusion (right) and gel-sol transition temperatures (left), is less clearly documented than for the pure components (see **Fig. 5** for comparison). However, the higher stability of WE C34(18_16) over WE C34 (16_18) is found in the molar heat of fusion as much as the effect of asymmetry for the WE with a CN of 36. Considering the right graph showing the molar heat of fusion, the pure WE data (gray symbols) are shown for comparison. The data reveal that the differences between different WE are magnified in the gels, see CN 34 and CN 36. It needs to be pointed out here that a proper accounting for the solubility of WEs in MCT oil should have let to identical values of the heat of fusion for the WEs either neat or in the gel. Data on the solubility are however currently not available. Actually, the differences in enthalpy of fusion illustrated in **Fig. 7** allow to calculate the solubilities of the WE in MCT-oil. With that in mind, the fact that the distance between the curves drawn in **Fig. 7** becomes smaller with increasing CN properly illustrates the reduced solubility of larger molecules within a homologous series.

Overall, **Fig. 7** illustrates that the behavior of the pure WEs propagates to their solidification in oleogels.

Viscoelastic behavior

The amplitude test revealed that the amplitude of 0.005 % was within the linear-viscoelastic range (LVR) for all oleogels formed by 10 % (w/w) of a single WE in MCT-oil. Further, as all samples exhibited storage moduli (G') larger than the loss moduli (G") in the LVR, they behaved as gels at 5 degC. This is not surprising since the chosen concentration is high compared to the critical gelling concentration known for natural waxes and higher than the concentration used by Avendano-Vasquez et al. to investigate the behavior of pure WEs in oil .

Whilst holding the samples at low oscillatory stress for 30 min, no significant effect of hardening due to further sintering of the primary crystals or softening due to a weakening influence of small oscillatory shear stress was detected.

During the temperature tests, the phase transitions (sol-gel or gel-sol) occur within a small temperature range. This is illustrated in **Fig. 8** for the oleogel formed with MCT-oil and 10 % (w/w) of WE C42 (22_-20). In logarithmic scaling of the ordinate, this results in a characteristic, almost sigmoidal shape. The complex shear modulus G* does not change significantly outside the temperature range in which phase

transitions occur (gray highlighting). For both, gel-sol and sol-gel transition, good agreement between DSC and rheological data was found. This indicates that the shear applied at the level used here does not influence the crystallization behavior as such. The data, however, do not offer any insights with respect to the effect of shear on the gel network structure.

The maximum G* values of the mono-ester gels after the holding time, measured at 5 degC within the LVR, are depicted as a function of CN in **Fig. 9** . The complex shear modulus is the length of the resulting vector of the viscous (G") and the elastic (G') component and therefore reflects the viscoelastic behavior of the sample. For predominantly elastic samples, it is considered as a measure for the rigidity of the sample, not to be mistaken for the gel hardness . The highest G* values ($G^*_{C30} = 7.54 \times 10^6$ Pa; $G^*_{C34(18\_16)} = 7.49 \times 10^6$ Pa) are observed for gels formed by the shortest WEs with a CN of 30 and 34. As the CNs increase, the G* of the mono-ester gels decreases ($G^*_{C46} = 2.2 \times 10^6$ Pa). Basically the same observation was made by Avendano-Vasquez for the storage moduli (G') of 3 % (w/w) solutions of pure WEs in safflower oil . Also in the rheological characterization, the two WEs with a CN of 34 differ significantly. WE C34 (18_16) has a significantly higher maximum G* than WE C34 (16_18). Within the group of WEs with a CN of 36, it is remarkable that the WE C36(14_22) yields a higher G* ($G^*_{C36(14\_22)} = 6.2 \times 10^6$ Pa) than both, the other WEs with asymmetric, C36(22_14), or symmetric configuration, C36 (18_18). This is again in line with Avendano-Vasquez as well .

At first sight, it seems to be counterintuitive that at same inclusion levels, WEs with higher CN and lower solubilities yield gels with decreased G*. A line of thought to be possibly explored is considering the dosage of WE in the gel. In this work, dosage is done per mass, hence, yielding less WE molecules in the sample at higher CN. Avendano-Vasquez et al. observed larger crystals for WEs with higher CNs, relating this to changes in the crystal-crystal interaction and elasticity of the oleogels . Unravelling the link between crystallization behavior, crystal size and viscoelastic behavior of the gels certainly necessitates more attention.

Mixed oleogels

Thermal properties

Building on the data of the mono-WE gels, also gels using binary mixtures of WEs as structuring agent were investigated. Four binary structurant systems were applied at inclusion levels of 10 % (w/w) in MCT-oil. Within the two WEs, three mixing ratios (1:2; 1:1; 2:1) were used. The constituting WEs differed in terms of CN and CN. The heating thermograms of the resulting mixed oleogels are gathered in **Fig. 10** (black lines). For completeness, the thermograms of the related mono-ester gels are depicted are shown as well (dashed lines).

Prior to discussing the data, it is meaningful to emphasize that these data are on oleogels and not on binary mixtures of pure WEs. This designates the systems, depending on the interpretation of the MCT-oil, to be at least a ternary system. As dictated by the Gibbs phase, this has some consequences regarding the degrees of freedom , e.g. the points relating to three coexisting phases under isobaric conditions do not need to follow an isothermal line.

Mixing WEs with a large difference in CN, 16 (**Fig. 10** (a)) or 12 carbon atoms (**Fig. 10** (b)) leads to two separate peaks with clearly distinguished transition temperatures. It should be noted that the sharp peaks obtained for the pure WEs (see **Fig. 1**) – isothermal as equilibrium thermodynamics dictates – are smeared out due to continuous dissolution of the WEs as the temperature increases. This is in line with the remark made above. This is also valid for the mixtures of WEs. For the mixed structurant systems with a high difference in CN, two thermal events are observed. The evolution of the peaks is very regular. Starting from the mono-WE gel with the higher gel-sol transition temperature, the peak temperatures develop corresponding to the reduced concentration of this WE. Starting from the WE with the lower gel-sol transition temperature, a similar behavior is observed. The thermal distance between the two individual peaks observed in the mixtures increases with higher inclusion levels of the higher-melting WE. This is in line

with the underlying principles of solubility due to differences in the heat of fusion of the WEs. In general, the evolution of the peaks implies individual crystallization of the WEs, suggesting a eutectic behavior in the binary mixtures of WEs. This involves that the peaks at lower temperatures in the mixtures relate to three phase (solid-solid-liquid) equilibria. For the system containing a 1:1 WE mixture of C30 + C46, the high temperature peak is accompanied by another high temperature shoulder. Currently, the data available do not allow to derive any explanation, such as the formation of a compound or alike. One might be tempted to relate this to the discrepancy in the lower transition temperatures comparing the two systems, relating in both systems to C30 (16_14). This is, however, directly related to the fact that a fixed mass fraction C42 (22_20) has a higher molar dilution effect than the one of C46 (22_24).

The systems containing WEs with smaller difference in CN (two or zero carbon atoms) behave quite differently (**Fig. 10** (c) and (d)). In **Fig. 10** (c), the CNs of the oil structuring WEs differ only in two carbon atoms. Considering the information shown in **Fig. 3** , the actual difference between the two constituting WEs is bigger than it appears at first sight. The shorter WE C36(18_18) has aCN of 0 and crystallizes in orthogonal chain arrangement, whereas the longer WE C38(20_18) with a CN of +2crystallizes in the orthogonal chain arrangement. In the first place, the evolution of the peaks – only a single thermal event in the mixtures – suggests that the two WEs mix ideally in the solid phase of the oleogels. Nonetheless, the changes of the peak shape and the relation to the mono-ester gels indicate a different behavior. However, in an early contribution by Kreger and Schamhart, the spacings of binary mixtures of WEs (CN difference of two carbon atoms) have been investigated by means of XRD . They found that independent of the mixing proportion, mixtures of WEs crystallizing individually in tilted chain arrangement, never crystallized in orthogonal chain arrangement. Even if a WE individually crystallizing in orthogonal chain arrangement is added to a WE individually crystallizing in tilted chain arrangement at equal mass ratio (1:1), the mixture still accepts the tilted chain arrangement. Still awaiting further confirmation, it appears likely that all mixtures assume the tilted configuration. Additionally, the different shape of the peak observed in the oleogel formed by the 2:1 mixture recommends further investigations of this system.

The thermograms for oleogels structured by binary mixtures of two WEs with identical CN 36 but reverse symmetry, either with CN of−8 or +8, are shown in **Fig. 10** (d). Except for the thermogram of the 1:1 mixing ratio, the data suggest ideal mixing in the solid phase with almost indistinguishable peaks, just as seen in**Fig. 10** (c). This is also supported by practically invariable heat of fusion data compared to the mono-ester gels. Similar to the data displayed in **Fig. 10** (a), the mixing ratio of 1:1, here also equal mole fractions, behaves differently. Again, a small second dissolution peak at elevated temperatures is discernible. This is accompanied by a heat of fusion (sum of both peaks), lower than for the mono-ester gels. Taking into account that the second peak, particularly the shoulder, has the highest transition temperature, the observation is possibly related to the interplay of equilibrium and kinetics. The system might tend to form a 1:1 compound crystal, highest melting point, which cannot be formed under the given process conditions due to the complication of the growth regime at equal mole fractions. This may yield a suboptimal non-equilibrium solid state. The mixtures of two WEs with identic CN but reverse symmetry and a CN of either −6 or+6 – C36(14_22) and C36(22_14) – showed single dissolution peaks at the mixing ratios of 1:2 and 2:1. Their heat of fusion was comparable to the mono-ester gels. At a mixing ratio of 1:1, thus equal mole fractions of both WEs, a second dissolution peak at higher temperatures is discernible. The heat of fusion of the overall thermal event is lower compared to the mono-ester dissolution peaks. Interestingly, the second peak occurs at higher temperatures than the dissolution of the single components what is quite interesting. Until now, no explanation for this behavior is possible and requires further investigations.

Without further detailed analysis and crystallographic data, the chain arrangement of these mixtures remains unresolved.

Viscoelastic behavior

The mixed oleogels studied were subjected to rheological characterization of the viscoelastic behavior. The amplitude-strain test confirmed that a strain of 0.005 % lies within the LVR. During the temperature

tests, a two-step phase transition behavior was observed for the mixtures with a large CN difference of the constituting WEs, illustrated by **Fig. 11** as an example for the systems of WE C30 (16_14) and C42 (22_20). The DSC data of this system are depicted in**Fig. 10** (b). The systematic development of G* on temperature increase almost perfectly matches the DSC data, confirming a two-step dissolution process for the mixed systems with high CN difference. The temperature test data also confirmed a two-step gel-sol transition process for the 1:1 mixture of C36(14_22) and C36(22_14) (data not shown).

In **Fig. 12** , the maximum G* values of the different mixed oleogel systems are depicted. The mixing ratios of the WEs are again 2:1, 1:1 or 1:2. The values were determined after the samples were stabilized for 30 min at 5 degC.

The two systems containing mixtures of WEs with a large chain length difference, i. e. C30 + C46 (black diamonds) and C30 + C42 (empty circles), show clearly higher G* values than the gel containing solely the long-chained WE. The values for the 1:1 mixture of C30+C46 ($G^*_{30+46(1:1)} = 1.17 \times 10^7$ Pa) are also substantially higher than those for the short-chained WE, e.g., C30(14_16) ($G^*_{C30} = 7.54 \times 10^6$ Pa. For the systems containing WEs C30+C42, the picture follows a similar trend, but less pronounced. The G* level of the mixtures remains in the range of the short chain reference. Thus, if the magnitude of the G* values is taken as a measure for the rigidity of the samples, the mixture of two WEs with a large difference in CN leads to a more rigid oleogel network. The observation of lipid network structures developing higher resistance to deformation if sequential crystallization events occur is well established for fat crystal networks . This mechanism is usually designated as sintering. Accordingly, it is presumable that the lower melting WEs crystallize on the original scaffolding of the higher melting component. This type of observations in the field of non-TAG oil-structuring was already made by other authors .

Regarding the G* values of the systems containing mixtures of WEs with a small chain length difference, i.e., C36 + C38 (black triangles) and C36 + C36 (empty squares) do not show a positive synergy upon mixing. In contrast, at a mixing ratio of 1:1, lowest G* values were yielded ($G^*_{C36+38 (1:1)} = 4.17 \times 10^5$ Pa) and the C36+C36 ($G^*_{C36+38 (1:1)} = 1.13 \times 10^6$ Pa).

Conclusion

In this contribution, the oleogelation of WEs in MCT-oil is discussed. Prefatorily to the experimental work, the relevance of WEs for the subject of oleogelation by natural waxes was established and the knowledge on WE crystallization was reviewed. It was shown that significantly different cooling rates can induce the same crystal habit of either pure WEs or natural waxes in oleogels. Oleogels formed by MCT-oil and 10 % (w/w) of different WEs or binary mixtures of WEs were studied. The combination of literature and newly gathered data allowed to map the crystallization behavior of WEs.

The review of crystallographic data revealed that WEs crystallize in a mono molecular chain packing, either in an orthogonal or a tilted orientation of the alkyl chains within the layer. The preferred polymorph seems to be a feature of the position of the ester bond: Only for WEs with a FaOH moiety two carbon atoms longer ($CN = +2$) or four carbon atoms shorter than the FA moiety ($CN = -4$), the orthogonal chain arrangement seems to occur. The thermal properties of the pure components show systematic behavior. While the melting temperature increases asymptotically with increasing CN, the molar heat of fusion shows a linear increase. The position of the ester bond strongly affects the thermal properties as increasing asymmetry of the WE relates to reduced melting points and molar heats of fusion at constant CN.

Due to solubility effects, the gel-sol transitions of mono-ester gels occur at lower temperatures than the melting of the pure WEs. The overall behavior corresponds to the melting behavior of the pure WEs. Further analysis of more data will show in how far engineering thermodynamics can predict dissolution temperatures and solid wax content. Analysis of the heat of fusion data indicates a systematic development of the WE solubilities, suggesting that larger WEs are less dissolved than shorter ones. The viscoelastic behavior showed that all prepared WE-in-oil solutions were able to form gels (G'>G") under the conditions

studied. These gels at inclusion levels of 10 % (w/w) of structurant exhibited the highest rigidity (G* values) when smaller WEs were used, being in line with a recent study on the gelling behavior of pure WEs .

The mixed oleogels, fabricated with binary mixtures of WEs as structurants, behave at first sight according to the expectations. When two WEs of significantly different CNs are applied in combination, DSC and rheology data clearly indicate a formation of two separate crystal phases, both relating to either of the two constituting WEs. This is in line with a eutectic behavior for binary mixtures of WEs. The fact that the lower transition temperatures do not arrange isothermally, despite representing three-phase equilibria, is owed to the fact that the system studied is at least ternary in nature. In contrast, applying mixtures of WEs with similar CN (two carbon atoms) resulted in a single thermal event for the gel-sol transitions. Interestingly, all of these mixed systems seem to assume a tilted chain arrangement despite different structures (C36(18_18): tilted; C38 (20_18): orthogonal) as single WE systems. This is in line with earlier observations . Mixing two WEs of same CN but different symmetry (WE C36 (14_22) and C36 (22_14)) yields almost identical gel-sol transition signals, except for the 1:1 mixture. For this particular system, a high-temperature shoulder at the peak appears. Even though thus observation may be assigned to compound formation, further research is required. The rheological characterization by temperature tests confirmed the DSC data. Amplitude strain tests revealed that the strongest gels were produced from structurants that crystallize in two steps. This is the case for mixtures of WEs that crystallize separately. This behavior has been observed earlier in other oleogel systems and fat crystal networks and is designated as sintering.

The compilation of own data and literature data presented here illustrates the basic rules of WE crystallization. As the oleogels investigated in this work are of low complexity, especially when comparing to natural waxes, it is obvious that a correlation between natural waxes and these synthetic systems still remains difficult. However, the relevance of WE crystallization for wax-based oleogels could be illustrated by on one hand illustrating that depending on the cooling rate, similar crystal habits can be generated in either WE or wax-based oleogels. Once the structuring of MCT-oil is based on at least two substantially different wax esters, resulting in sequential crystallization, structural characteristics of the gel resembled those of SFX-gels. This illustrates that the study of pure wax esters in oleogels can deliver detailed information necessary to better understand wax-based oleogelation.

Tab. 1 Composition of different natural waxes regarding the content and average CN of the WE fraction

1: ; 2: ; 3: ; 4: ; 5:

	WE [%]	CN
BWX	58-71 [1,2]	40-50 [2, 4]
CLX	16-35 [1, 2]	34-54 [2]
CRX	62-85 [1, 2]	34-64 [2]
RBX	92-97 [1]	44-64 [5]
SFX	96-100 [1, 3]	36-54 [4]

Fig. 1 DSC heating thermograms (lines) and relative quantities of the WE fraction in terms of CN (pins) for waxes; waxes in order of decreasing total WE content (top: highest)

Fig. 2 BFM images of oleogels consisting of MCT-oil and 10 % (w/w) structurant (SFX (a), (c)); WE C34(18_16) (b), (d)) crystallized at different cooling rates. (Image (c): purified canola oil (0 % polar components) as solvent - contrast enhanced)

Fig. 3 Comparison of calculated layer thicknesses (eq. 1, eq. 2) (lines) and experimental SAXS data (marker) as a function of CN; SAXS data:

Fig. 4 Melting temperatures (left) and molar heat of fusion (right) of pure wax esters as a function of CN. Black crosses: experimentally determined for this manuscript, gray circles: reviewed literature data

Fig. 5 Melting temperature of WEs with different symmetries and CN as a function of [?]CN. Lines indicate equal CN. Black crosses: experimentally determined for this manuscript, other markers: reviewed literature data

Fig. 6 DSC heating thermograms for oleogels formed with MCT-oil and 10 % (w/w) of either SFX (gray) or pure WE C42 (22_20) (black)

Fig. 7 Dissolution temperatures (left) and molar heat of fusion (right) as a function of CN for pure WEs (empty gray markers) and mono-ester gels consisting of a single WE (10 % (w/w) and MCT-oil (filled black markers); Circles: [?]CN [?] [-2;0;2]); squares: ([?]CN [?] [-8; +8]); value for pure WE C46 is extrapolated (dashed gray circle)

Fig. 8 Complex shear modulus G* as function of temperature obtained from temperature test (oscillatory rheology) showing the crystallization (left, gray) and the melting (right, black) curves for an oleogel based on MCT-oil and 10% (w/w) of WE C42 (22_20). Between the two temperature tests, an isothermal phase (5 °C) was performed for 30 min

Fig. 9 Maximum G* level (in LVR; T = 5 °C) as a function of CN for mono-ester gels consisting of a single WE (10 % (w/w) and MCT-oil ((circles: [?]CN [?] [-2;0;2]); squares [?]CN [?] [-8; +8])

Fig. 10 Heating thermograms of mixed gels consisting of binary mixtures of WEs (10 % (w/w)) and MCT-oil; (a): C30+C46; (b): C30+C42; (c): C36+C38; (d): C36(14_22)+C36(22_14). Dashed lines: Mono-ester gels for comparison

Fig. 11 Complex shear modulus G* as function of temperature obtained from an oscillatory rheology temperature test for oleogels consisting of MCT-oil and 10 % (w/w) of either pure WE (C30 (16_14) and C42 (22_20) - gray) or binary mixtures of these WEs at three different mixing ratios (2:1; 1:1; 1:2 – black)

Fig. 12 Maximum G* values (in LVR; T=5 degC) for mixed gels consisting of binary mixtures of WEs (10 % (w/w) and MCT-oil as a function of the mass fraction

13

endo →

Heat flow

CN [-]
30 36 42 48 54 60

WE C42

(100 %)

SFX

(96-100 %)

RBX

(92-97 %)

CRX

(62-85 %)

BWX

(58-71 %)

CLX

(16-35 %)

14

35   45   55   65   75   85

Temperature [°C]