Structure, Rheological and Sensory Properties of Some Animal Wax Based Oleogels

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Abstract: This study was aimed to prepare oleogels of whale spermaceti wax (WsWO) and lanolin wax (LnWO), and to compare them with well-known animal wax oleogels of shellac (ShWO) and beeswax (BsWO). WsWO, ShWO and BsWO were prepared at 5% (w/w) organogelator addition level, while LnWO was necessarily prepared at its minimum gelling concentration (C*) of 30% (w/w) addition level. All oleogels were posed high oil binding capacity and thermal reversibility. Melting peak temperatures were ordered as ShWO > BsWO > WsWO > LnWO by calorimetry. X-ray diffraction patterns indicated the presence of both β’ and β type polymorphs, together with needle-like crystal morphology. Rheological analyses indicated that the stiffness of the gels were ordered as BsWO > WsWO > LnWO > ShWO. All showed good thixotropy, and thermal stability until 40°C (ShWO until 80°C). Finally, the sensory descriptive analysis indicated that LnWO had distinct negative sheep odor, but WsWO was quite similar to BsWO. Overall, LnWO determined to be not proper for food applications, but WsWO was shown to be a suitable oleogel for food applications.

Key words: animal-based wax, whale spermaceti wax, oleogel, rheology, sensory, food

1 Introduction

Waxes refer to the mixture of long-chain apolar compounds forming a protective layer on the surface of some plant and animal tissues, algae, fungi and bacteria. Also, there are some mineral waxes originating from fossil sources. Although chemically, a wax is the ester of a long-chain acid and a long-chain alcohol, almost all waxes are a heterogeneous mixture of various compounds including hydrocarbons, wax esters, sterol esters, aldehydes, ketones, alcohols, alkanoic acids, terpenes and others. Typically waxes are soluble in oils, non-polar solvents, ethers, esters and ketones. They are kneadable at room temperature, have low viscosity above their melting point, generally having no stinginess, coarse or fine crystalline materials, being emulsion forming, combustible, lubricant, having a high flash point and high dielectric constant compounds. They could be produced with various colors and odors depending on the level of refining and/or processing conditions.

Waxes are usually classified according to their origins (plant waxes, animal waxes, microbial waxes, mineral waxes, synthetic waxes, etc.), and named after their source materials. In this study, selected animal waxes (beeswax, shellac wax, whale spermaceti wax, and lanolin wax) were used in the preparation of oleogels. Hence, a brief description of each wax is provided. Whale spermaceti wax is the material extracted from the head sonar organ of the sperm whale (Physeter macrocephalus). Basically the secretion contains fatty esters (65-95%), triglycerides (5-30%), free alcohols (1-5%), and acids (0-3%). Its melting range is around 42-50°C, and has a light mass of white crystals. It is used in cosmetics, pharmacy and candles manufacture. Shellac wax is produced from the secretion of insects (Tachardia lacca). It is a natural thermoplastic bioadhesive polymer, mainly composed of fatty esters (70-82%), free fatty alcohols (8-14%), acids (1-4%) and hydrocarbons (1-6%). Its color ranges from light blonde to dark brown, and it has a melting range of around 43-84°C. It is usually used in varnishes, wood finish and food glaze. Lanolin is a by-product of the wool industry, and get from refined wool grease. Pharmaceutical grade lanolin has a melting range of 35-42°C. It contains fatty esters (14-24%), sterols and triterpene alcohol esters (45-65%), free alcohols (6-20%), sterols (cholesterol, lanosterol) and terpenes (4-5%). It is used in cosmetics, dermatology, ink, fabrics and lubricants. Beeswax is the amorphous solid abdominal secretion of bees (Apis mellifera), usually in light yellow to amber colors. Pure beeswax contains around...
70-80% of long-chain esters, 12-15% of free acids, 10-15% of hydrocarbons, and some diols and sterol esters. Its melting range is around 60-67°C and soluble in aromatic and polar organic solvents. World production is around 7,000 tons annually and mostly used in cosmetic and pharmaceutical sectors1-2. The shellac and beeswax used in this study were food grade, and the other two (whale spermaceti wax and lanolin wax) were pharmaceutical grade. There was none food grade available from the world markets for the whale spermaceti and lanolin waxes. Since pharmaceutical grade is safe by definition, we preferred to use them in this study. In fact, if a pharmaceutical grade wax could be very successful in oleogel formation, it would then initiate producers to produce food grade forms.

One of the new application areas of waxes is the preparation of oleogels. An oleogel is defined as the oil with phase in the macroscopic dimension permanent within the networks of assembled molecules called organogelators. That is the gelled oil having behavior of a semi-solid or soft solid state. These new products, oleogels, are advantageous with unchanged fatty acid and oil minor components composition to replace the partially hydrogenated fats which contain the unhealthy trans and saturated fatty acids. The key material in oleogel technology is the organogelators used. There are diverse organogelators, but they were mostly classified as low molecular weight gelators (LMWG) and polymer gelators. Various plant waxes including sunflower wax, rice bran wax, carnauba wax, candellilla wax, sugarcane wax, berry wax, fruit wax, and some animal waxes like beeswax and shellac wax as some of the LMWG have been used in oleogel preparation. Waxes as organogelators pose thermo reversible, viscoelastic, shear thinning oleogels with thixotropic recovery abilities. Further, most are GRAS and have none or little off-flavors.

One of the continuing research efforts in oleogel arena is to find more suitable, cheaper, safe, abundant new organogelators. Among many others already studied, the waxes were usually credited as good organogelators in terms of ease of application, safety, full structure and prolonged stability. Hence, testing of unexplored waxes in oleogel preparation could provide new data to initiate potential new applications.

The present study aimed to prepare and characterize oleogels with some selected animal-sourced waxes (whale spermaceti wax, shellac wax, lanolin wax and beeswax). There are studies in the literature with shellac and beeswax, but as far as we reached, no study reported with the other two waxes. We included all to make also some comparisons. Further, a descriptive sensory analysis was accomplished to characterize the oleogels, in which this type of data is also absent in current literature. The aim was to find possible new suitable wax organogelators for food applications.

2 Materials and Methods

2.1 Materials

Refined-winterized sunflower oil (Biryağ, Trakya Birlik, Tekirdağ, Turkey) was purchased from a local store and used as stock oil for oleogel preparation. Pharmaceutical grade whale spermaceti wax was purchased from Doğa İlaç Hammaddeler Co. (İstanbul, Turkey). Shellac wax (78-84°C melting point, 5-15 mg KOH/g acid value, 45-75 mg KOH/g saponification value) and beeswax 8108 (61-65°C melting point, 17-24 mg KOH/g acid value, 87-104 mg KOH/g saponification value) were purchased from Kahlwax Co. (Kahl GmbH & Co., Trittau, Germany). Pharmaceuticals grade unhydrous lanolin (38-40°C melting points) was purchased from Suzhou Fanrong Biotechnology Co. Ltd. (Jiangsu, China). All other chemicals and solvents used in the analyses were of analytical grade and purchased from Sigma Chem. Co. (St. Louis, MO, USA) and Merck (Darmstadt, Germany).

2.2 Preparation of the oleogels

Since the minimum gelation concentration (C*) of whale spermaceti and lanolin wax is not available in the literature, and the C* of the shellac and beeswax depends on the type and purity of the wax used in every study, the C* of the waxes used in this study were determined first. Sunflower oil and each wax were prepared with different organogelator addition levels by one unit increments (from oil: wax = 99:1 to 70:30, w/w ratios), and heated in a water bath at 90°C for half an hour for full melting. During heating, the tubes were mixed vigorously for full mixing and homogeneity. Finally, the tubes were taken out to the ambient temperature (20 ±3°C) and awaited overnight. The next day tubes were examined visually by tilting 90° and observing any flow. If no flow occurred, then the tube with the labeled addition level value was selected as the C* value for that wax. The minimum gelation concentrations (C*, %) of whale spermaceti wax, shellac wax, lanolin wax, and beeswax were determined as 2.0%, 2.0%, 30.0%, and 1.0%, respectively. It was clear that lanolin could form oleogel only at a very high addition level. Therefore, we selected 5% addition level for the other three waxes, and 30% addition level for lanolin to prepare the oleogels for this study. All further analyses were completed in these prepared oleogels. Also, the abbreviated oleogel names given in the parentheses above were used throughout the paper.

2.3 Physico-chemical properties of the oleogels

The gelation time (GT) of the oleogels were determined first by heating the oleogels in a water bath at 90°C for 30 min for full melting and then removing and cooling at ambient temperature (20 ±3°C). Meantime the time was recorded, and the GT was considered as the minutes of time passed until the oleogels solidified. Under given
conditions, this data may help to estimate actual production times during real applications.

Oil binding capacity (OBC) of the oleogel samples were measured by Yılmaz and Ötcü 
After fully melting (90°C for 30 min) the samples, 1 mL of each oleogel was placed into tared Eppendorf tubes and stored at the refrigerator for 1 h. After gel formation, the tubes weighed again, and then centrifuged (10,000 rpm for 15 min). To drain the liquid oil released, the tubes turned over on a paper cloth. Finally, the tubes were weighed again, and OBC values were calculated gravimetrically.

Minispec Bruker NMR Analyzer mq20 (Bruker Optics, Inc.) instrument was used to assess the solid fat content (SFC) at 20°C by official method 16b-93. After melting (90°C for 30 min) the samples, melted oleogels were placed into NMR tubes (3.5 mL), and conditioned in another water bath at 0°C for 1 h, then at 20°C for another h. Finally, the tubes put into the sample holder of the instrument and SFC was recorded. The instrument was previously calibrated with standards including 0, 31, and 73.5% solid fat.

Instrumental color values were measured with Minolta CR-400 (Konica Minolta Sensing, Osaka, Japan) colorimeter according to CIE standards.

The peroxide values (PV) of the oleogels were determined according to official method Cd 8-53.

2.4 Thermal properties of the oleogels

Crystallization and melting onset, peak temperatures and enthalpies of the waxes and the oleogels prepared from them were measured with a Perkin-Elmer 4000 Series Differential Scanning Calorimeter (DSC) (Groningen, The Netherlands). Each sample (5-8 mg) were put into the aluminum pan and sealed. The program was heating samples from 20°C to 100°C by 10°C/min; cooling the samples to –30°C by 10°C/min rate and keeping 3 min at that temperature for full crystal development; and finally heating samples again to 100°C by 5°C/min heating rate. Parameters of the analysis were calculated by Pyris 1 Manager Software.

2.5 Structural Properties of the Oleogels

Polarized light microscopy images of the oleogels were taken with an Olympus CX31 polarized light microscope (PLM, Olympus Optical Co., Japan) and an attached CCD color camera (Canon) at room temperature.

To determine the crystalline polymorph type, the oleogel samples were analyzed with a PANalytical Empyrean model (The Netherlands) X-ray diffractometer according to method Cj 2-95. Samples loaded at ambient temperature (23 ± 2°C) to the holder by plastering the previously prepared oleogel sample into the sample holder by a spatula, and then angular scans (2θ) were performed from 2θ = 2° to 50° by 2°/min scan rate. There was a Cu source X-ray tube (λ = 1.54056 Å, 40 kV and 40 mA). Data analysis was completed with X’Pert HighScore Plus software (Malvern Panalytical Ltd., Royston, UK).
trained under the moderation of panel leader to determine, define and scale the sensory descriptive terms. Some margarine, shortening, previously prepared other wax oleogels and oleogels of this study were evaluated together to select the best describing sensory definition terms. The panel selected seven sensory terms to describe the samples. ’Hardness’ was defined as the force required pushing a knife into the oleogel, and butter and cream cheese were selected to mark the maximum and minimum intensity on a 10 cm line scale used. The second sensory term used was ‘spreadability’, and it was defined as the ability to spread the sample on a bread loaf by a knife. Cream cheese and chewing gum were the references for maximum and minimum spreadability. ’Liquefaction’ was defined as the amount of liquid oil when the oleogel was spread on the surface of the bread loaf. Its references were margarine and liquid oil, respectively. To quantify the intensity of aromas associated with oxidized fat, the term ‘rancid’ was used with long term stored oil as the reference. ’Waxy’ was defined as the aromas and flavors associated with paraffin wax, and paraffin wax was used as the reference. The cooling effect felt inside the mouth when a solid fat melted is defined as ’cooling’ and tested with menthol candy as the reference. Since only LnWO sample had a distinct odor, the ’sheep odor’ was used to describe only that sample with sheep wool as the reference. Sensory evaluation ballots with 10 cm line scale anchored from 0 at the left end for minimum intensity to 10 at the right end for maximum intensity was used. Within each evaluation session, 4 samples were served to the panelist with 3-digit code numbers. The samples were analyzed on different days under daylight at room temperature with water, bread slices, apple slice and an expectoration cup provided. Each replicate sample was analyzed twice at different sessions on different days under daylight at room temperature with water, bread slices, apple slice and an expectoration cup provided. Each replicate production sample was analyzed twice, and each replicate production sample was analyzed for at least in duplicate and for some analyses in triplicate. The data were presented as mean values with standard deviations. The Analysis of Variance (ANOVA) with means separation by Tukey’s test was completed. The level of confidence was at least 95%. Statistical analysis was performed with Minitab v.16.1 software.

### 2.8 Statistical analysis

The four animal wax-based oleogels were prepared twice, and each replicate production sample was analyzed for at least in duplicate and for some analyses in triplicate. The data were presented as mean values with standard deviations. The Analysis of Variance (ANOVA) with means separation by Tukey’s test was completed. The level of confidence was at least 95%. Statistical analysis was performed with Minitab v.16.1 software.

### 3 Results and Discussion

#### 3.1 Physico-chemical properties

The oleogels prepared with sunflower oil and four different animal waxes can be observed in Fig. 1. Whale spermaceti wax oleogel (WsWO), shellac wax oleogel (ShWO), and beeswax oleogel (BsWO) were prepared at 5% (w/w) organogelator addition level, while lanolin wax oleogel (LnWO) was prepared at 30% (w/w) addition level since stable oleogel was only possible at its C* value. The wax oleogel literature shows that almost all wax oleogels were prepared at wax addition levels of 5-10%, but not at their C* value, which usually ranges between 0.5 to 4.0% by weight. In this study, the same rationale was accepted to prepare the oleogels at 5% wax addition level to have enough firmness to easily compare the samples. Further, this addition level yielded oleogels with more solid-like textures resembling more to solid fats. Some physico-chemical properties of the oleogels prepared at the given addition levels were presented in Table 1. The minimum gelation concentrations (C*) of the waxes were determined at first by preparing serial addition levels of the gelators (oil: wax ratios of 100:0 to 90:10, w/w by one unit increments). The C*’s for whale spermaceti wax and shellac wax were 2% and was 1% for beeswax. Literature indicated C* values of 1% and 2% for beeswax and shellac wax oleogels, but data for the other oleogels were absent and provided in this study. Another set of preparations was prepared at higher concentrations, and the C* for lanolin wax determined as 30%. In fact, a 30% addition level is quite high for an oleogel, and this finding itself indicated that lanolin wax could not be suitable as organogelator for edible applications. Nevertheless, 30% of lanolin wax oleogel was included in the study for comparison purposes. Also data provided might have value if one would have interest to use lanolin oleogel for some other purposes. Since literature is totally lacking, any data would have value at least for scientific curiosity.

The gelation times (GT) of the animal wax oleogels were also measured (Table 1). The shortest time (5.67 min) was for BsWO, and the longest time (64.78 min) was for LnWO.
Animal Wax Oleogels

Except for LnWO, GT values were within the same range measured before for various plant wax oleogels\(^8,9\). Oil binding capacity (OBC) values were all over 99\%, and not statistically different. Clearly, once the oleogels formed, they have successfully immobilized the liquid oil. This result is also similar to previously reported plant wax oleogels. Generally, wax oleogels could bind liquid oil very effectively by entrapping the oil within the gel network pores and adsorbing on wax crystals, as discussed in the literature\(^8\).

Solid fat content (% SFC) defines the amount of total solids in an oil at a given temperature\(^18\). Higher SFC values (3.45 and 3.02\%) were measured in WsWO and BsWO at 20\(^\circ\)C, while the lowest value (1.70\%) was in the LnWO sample. Since the same stock oil (sunflower oil) was used in all oleogels, the difference of SFC should be due to the added waxes. It was stated that typical margarines include around 7-16\% SFC at 21.1\(^\circ\)C\(^18\). In natural triglyceride crystals, SFC resembles the phase state of the fat, but in oleogels SFC is not necessarily related linearly to the plasticity of the product. It is well known that oleogels are formed mostly by the junction zones created gel networks, but not directly by the solid triglycerides. Hence, it is quite possible to get plastic consistency with a low level of SFC in an oleogel. Of course, the presence of solid lipids could help to stabilize the oleogel structure\(^6,6\).

Naturally, the color of an oleogel is the sum result of oil and organogelator used to prepare it. As could be observed from Fig. 1, the colors of the samples were significantly different (Table 1). The luminosity (L value) of the ShWO was the highest (59.20\%), while LnWO had the lowest value (35.46\%). All samples had some green color tones (negative a\(^*\) values), and greenness was highest in the ShWO sample (-4.97). Similarly, the b\(^*\) values were significantly different, and the level of yellowness was the highest in the LnWO sample (14.62). Color of an oleogel could be important during food applications, where the occurrence of significant color differences by added oleogels is usually not preferred. Generally, creamy-yellow colored samples would not create a problem in any food formulations.

Table 1  Some physico-chemical properties of the prepared animal-wax oleogels.

|                 | Minimum Gelation Concentration (C*, %) | Gelation Time (min) | Oil Binding Capacity (%) | Solid Fat Content (% (20\(^\circ\)C)) | L       | a*       | b*       | Peroxide value (meqO\(_2\)/kg) |
|-----------------|--------------------------------------|--------------------|--------------------------|------------------------------------|---------|----------|----------|---------------------------------|
| WsWO            | 2.0 ± 0.0\(^a\)                      | 12.33 ± 0.6\(^a\)  | 99.99 ± 0.00\(^a\)       | 3.45 ± 1.2\(^a\)                   | 41.35 ± 0.66\(^a\) | -12.3 ± 0.05\(^a\) | 6.62 ± 0.15\(^a\) | 11.23 ± 0.39\(^a\) |
| ShWO            | 2.0 ± 0.0\(^b\)                      | 7.67 ± 0.5\(^b\)   | 99.94 ± 0.01\(^b\)       | 2.13 ± 1.5\(^b\)                   | 59.20 ± 0.85\(^b\) | -4.97 ± 0.11\(^b\) | 7.08 ± 0.44\(^b\) | 10.58 ± 0.18\(^b\) |
| LnWO            | 30.0 ± 0.0\(^c\)                     | 64.78 ± 11.2\(^c\) | 99.60 ± 0.05\(^c\)       | 1.70 ± 0.8\(^c\)                   | 35.46 ± 4.56\(^c\) | -3.44 ± 0.41\(^c\) | 14.62 ± 0.56\(^c\) | 18.82 ± 0.21\(^c\) |
| BsWO            | 1.0 ± 0.0\(^d\)                      | 5.67 ± 0.5\(^d\)   | 99.99 ± 0.00\(^d\)       | 3.02 ± 0.5\(^d\)                   | 40.99 ± 0.10\(^d\) | -2.00 ± 0.01\(^d\) | 1.74 ± 0.01\(^d\) | 15.48 ± 2.62\(^d\) |

BsWO: beeswax oleogel (5\%), ShWO: shellac wax oleogel (5\%), WsWO: whale sperm wax oleogel (5\%), LnWO: lanolin wax oleogel (30\%)

*Small letters within each column indicate significant differences among the oleogel samples for the mean ± SD values calculated from four determinations by one-way analysis of variance and Tukey's test (p ≤ 0.05).

Peroxide value (PV) is one of the best indicators of the oxidation level in edible oils. The vegetable oil codex\(^19\) stated 10 meqO\(_2\)/kg PV as the upper limit. All oleogel samples exceeded the codex limit (Table 1). This might be due to the added waxes and/or oxidation occurring during the oleogel preparation, where heat and air readily present. Therefore, waxes used in oleogel preparation must be as pure as possible for both chemical impurities and odor-active substances. Further, precautions like vacuum or neutral gas atmosphere, and addition of antioxidants could be taken into action during oleogel preparation.

3.2 Thermal properties

Thermal properties (crystallization and melting onset and peak temperatures and enthalpies) of the animal waxes and the oleogels prepared with the animal waxes were determined and the results are presented in Table 2. While shellac wax had three fractions for thermal phase transitions, the rest of the waxes had only one peak. Whale spermaceti wax (WsW) crystallized at around 44.60\(^\circ\)C, and melted at around 56.25\(^\circ\)C. Literature indicated 42-50\(^\circ\)C as melting point\(^2\). The melting and crystallization peak temperature ranges for the three fractions of shellac wax were 50.77-71.10 and 45.46-69.67\(^\circ\)C, respectively (Table 2). The melting and crystallization peak temperatures for shellac wax were reported as 75.19 and 67.81\(^\circ\)C, respectively\(^4\). Further, neat shellac was showed melting peaks at 55, 66.4 and 79.4\(^\circ\)C, and crystallization peaks at 69.9, 60.8 and 50.2\(^\circ\)C, respectively\(^17\). Lanolin wax used in this study had 35.25\(^\circ\)C crystallization and 37.15\(^\circ\)C melting peak temperatures. Literature only indicated 35-42\(^\circ\)C as the melting point for lanolin\(^6\). Hence, the onset and peak temperatures and enthalpy values provided in this study could add up to the literature. Measured crystallization and melting peak temperatures for the beeswax were 57.85 and 64.55\(^\circ\)C, respectively. Ruguno et al.\(^4\) reported 68.60 and 58.72\(^\circ\)C peak melting and crystallization temperatures for beeswax. Clearly, thermal properties reported in the literature and determined in this study for the animal waxes were usually concurred, and small differences occurred most probably due
to material source and purity differences, as expected.

The thermal properties of the animal wax-based oleogels were also measured (Table 2). The order of melting peak temperatures from highest to lowest was ShWO>BsWO>WsWO>LnWO, respectively. There is no data for WsWO and LnWO in literature, but 5\% shellac oleogels prepared with rapeseed oil showed 49.5\(^\circ\)C onset of crystallization\(^{17}\). Olive oil organogel with 7\% added beeswax showed 49.18\(^\circ\)C of melting peak temperature\(^{11}\). Thermal data of the shellac and beeswax oleogels in this study concur with literature, but thermal data for the WsWO and LnWO are provided for the first time. The difference between melting peak and onset temperatures in oleogel samples was accepted as an important parameter for thermal behavior in mouth space\(^{29}\). Order of this difference was WsWO>ShWO>BsWO>LnWO. Clearly, LnWO melts very quickly at body temperature (around 36\(^\circ\)C), whereas ShWO and BsWO stay a little longer in the mouth for complete melting. Further, melting peak temperature of the WsWO sample (33.41\(^\circ\)C) was the closest to body temperature, while its onset to peak melting temperature range was the largest (12.81\(^\circ\)C) among all. Overall, as long as the human body temperature is considered, it seems that WsWO and BsWO had better thermal profiles. ShWO could stay longer as unmelted in mouth space, while LnWO melts so quickly.

### 3.3 Microstructural properties

The polarized light microscopy images of the oleogels were shown in Fig. 2. In these images, the wax and adhered lipid crystals were observed as white scenes on the dark background, which is the liquid oil\(^{18,19}\). As could be observed BsWO and WsWO seem large needle-like crystals, while ShWO looks spherulitic blossoms. LnWO, on the other hand, seems to have very thin, less orderly, quite small crystals. Patel et al.\(^{17}\) studied the material properties of shellac as organogelator. They have shown shellac crystals at 5\% addition level as large spherulitic aggregates. The image seems similar to the one in this study (Fig. 2b).

In our previous study beeswax-pomegranate seed oil oleo-
gels indicated needle-like crystals for beeswax oleogel\textsuperscript{21}. Further, Mattice and Marangoni\textsuperscript{8} classified beeswax oleogel crystals as fibrous or ‘sea urchin-like’, sunflower wax, rice bran wax and sugarcane wax crystals as needle-like, and candelilla and carnauba wax crystals as spherical type. Generally, images of BsWO and ShWO in this study concur with literature images, but WsWO and LnWO are provided the first time. Generally, WsWO crystals seem similar to BsWO crystals. There are some claims in the literature about the relationship between crystal morphology and gelation ability of the waxes. It was stated that needle-like or fibrillar crystals entrap more liquid oil compared to spherulitic or granular morphology\textsuperscript{8}. Further, Dassanayake \textit{et al.}\textsuperscript{22} indicated that fiber-like, smaller crystals form stronger and harder gels. Findings of this study usually agree that C* of BsW, WsW and ShW are much lower than that of the LnW, with much less gelation time to support the idea of stronger gels with needle-like crystal morphology. The rheology of the gels will be discussed later with the same perspective.

The X-ray diffraction patterns of the oleogel samples showing the short spacings corresponding to different wide-angle peaks could be observed from the graphs (\textbf{Fig. 3}). There are 3.72, 4.13 and 4.56 Å peaks for WsWO; 4.05 and 4.42 Å peaks for LnWO; 3.72, 4.12 and 4.45 Å peaks for ShWO; and 3.71, 4.11 and 4.51 Å peaks for BsWO, respectively. These diffraction patterns must be the sum result of all crystalline and amorphous solids found in the samples\textsuperscript{3,8}. There is a classification of crystal polymorphs based on the wide-angle peaks according to AOCS method\textsuperscript{23}. The method state that if a sample presents a peak at 4.2 Å, its polymorphic form is α, if a sample contains 3.8 and 4.2 Å peaks, it must be β polymorph, and if the peak appears at 4.6 Å position, then it should be β polymorphic form. Accordingly, WsWO, ShWO and BsWO contain both β\textsubscript{L} and β polymorph crystals, but LnWO contains only β polymorph type. The difference among the oleogels must be due to the added organogelators, and it was probable that the kinds and proportions of the components found in the waxes and of their chain lengths were the main reason behind different crystalline polymorphs. Crystals with the β\textsubscript{L} polymorph type were reported previously for candelilla, carnauba, rice bran, sunflower and beeswax oleogels\textsuperscript{15,22}. It was stated that for margarine and spreadable type fat products β\textsubscript{L} polymorphic form of fat crystals are preferred for dispersibility, smooth texture, optimum melting and good mouthfeel, while β type polymorph is preferred for chocolate and baking shortening type products for the

\begin{figure}[h]
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\caption{The X-ray diffraction (X-RD) patterns of the oleogels (BsWO: beeswax oleogel, ShWO: shellac wax oleogel, WsWO: whale sperm wax oleogel, LnWO: lanolin wax oleogel).}
\end{figure}
desired textural properties. Clearly, WsW could be used for similar purposes as BsW and ShW have been used due to the very similar crystal morphology if other properties would also satisfy the requisites, but LnW seems not quite proper for food applications.

3.4 Rheological properties

The amplitude sweep test results of the oleogel samples were presented in Fig. 4. The tests were carried out with 0.01-100% strain and 1 Hz frequency at 10°C. This test was aimed to describe the deformation behavior and to determine the upper limit of this range. The linear viscoelastic region (LVR), which indicates the range within which the sample could be stressed without destroying its structure. The LVR could be observed as a plateau value where the LVR, the G′, and G″ values were around 400-800 Pa, 300-400 Pa, and 5,000-10,000 Pa for the WsWO, ShWO, LnWO, and BsWO samples, respectively. In all four samples, within the LVR, the G′ > G" condition was observed, indicating that the samples were more gel-like or solid structured.

Further, the G′ values in the LVR were around 400-800 Pa, 400-500 Pa, 300-400 Pa, and 5,000-10,000 Pa for the WsWO, ShWO, LnWO, and BsWO samples, respectively. It was clear that the BsWO had the strongest gel, while the structure was the weakest in the LnWO sample. All samples also showed flow point values, where the G′ = G" (crossover point). Shear values higher than yield point or crossover point would cause the sample to flow, meaning that the sample lost its viscous portion and behaves like a liquid. The crossover points of the samples as % strain values were 1.183, 2.857, 2.885, and 1.963 for the WsWO, ShWO, LnWO, and BsWO samples, respectively (Fig. 4). Therefore, oscillatory strain values higher than above given values will destroy samples gel structure and will cause them to flow.

Frequency sweep tests were also conducted for the oleogel samples (Fig. 5). Oscillatory frequency sweep tests at the determined strain range within the LVR (0.099-0.11%) for each sample, and angular frequencies from 1-1,000 rad/s were completed at 10°C to observe the long term stability of the oleogels. Throughout the measuring region, the storage (G′) modulus, loss (G″) modulus and complex viscosity (η*) values were determined and shown in Fig. 5. The gelled materials are usually described with the G′ > G" flow behavior. The storage modulus (G′) of a sample indicates the elastic portion and represents the solid-like properties, while the loss modulus (G″) indicates the viscous portion and determines the liquid-like properties. Consequently, a gel, which is more solid-like due to the absence of free flow, must hold the G′ > G" condition. This situation was observed for all oleogel samples (Fig. 5), as the deformation force enhances until the upper limit. Further, the same situation holds true throughout the measuring range, and the complex viscosity (η*) values approached infinity within the lower ranges of the frequencies. As clearly be observed from Fig. 5, the η* values were decreased as applied force gradually enhanced, indicating a shear thinning behavior for the oleogels. The very similar behavior was previously reported for some plant wax oleogels. The loss factor (tanδ) value was calculated as the ratio of G″/G′ to define the strongness of a gel. If a sample holds the G″/G′ < 0.1 conditions, then it would be accepted as a strong gel. The loss factor ranges of the samples within the measured frequency range were 0.380-2.840 for WsWO, 0.319-2.261 for ShWO, ~0.256-2.503 for LnWO, and ~0.155-0.429 for BsWO, respectively. None of the

Fig. 4 The amplitude-sweep test results of the oleogels (BsWO: beeswax oleogel, ShWO: shellac wax oleogel, WsWO: whale sperm wax oleogel, LnWO: lanolin wax oleogel).
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The frequency-sweep test results of the oleogels (BsWO: beeswax oleogel, ShWO: shellac wax oleogel, WsWO: whale sperm wax oleogel, LnWO: lanolin wax oleogel).

For these four animal wax oleogel samples, oscillatory time sweep tests were also completed to evaluate time-dependent viscoelastic behaviour (Fig. 6). The tests were carried out at constant frequency and temperature (1 Hz, 10°C) with strain values determined at the LVR for each (to simulate the resting condition), with strains well above the LVR strains (to simulate structural breakdown) and with strains well below LVR strains (to simulate structural recovery) by applying the selected strains for 180 s for the first and second region, and 900 s for the third region. In the first region (resting condition), the G’ values were higher than the G” values indicating the ready gel structure. In the second time region, the applied force was high enough to destruct the gel structure for all, as evidenced by the decreased G’ values under the values of G” for each sample. At this stage, the gelled consistencies were lost, and the samples became more fluid-like. Finally, the applied strain was lowered below LVR strain values to observe the structural recovery abilities of the samples. As could be observed (Fig. 6), structural reformation or recovery was evident in all samples, as the G’ values enhanced and exceeded the G” values. The same recovery was also evident for the G” values, but in an unordered way (the values were enhanced but not linearly). Clearly, full time-dependent recovery of the initial state upon the reduction of the initial load was present for the samples. This type of behavior is called thixotropic behavior and was observed previously for the sunflower, candelilla, carnauba, rice bran, and fruit wax oleogels. Since during the food processing unit operations, the solid fat stock could be mixed, whipped or forced to flow, and a structural breakdown of the gelled state might occur, it would be praised if it could recover the structure after the applied force ceased. Since in all oleogel samples studied, this behavior was evident, and they could recover during food processing if they would be used as the solid fat source.

It has been very well documented that flow behavior of all gels including oleogels would heavily be affected by
A temperature ramp test was done for each oleogel sample (Fig. 7) to observe the flow behavior at different temperatures from 0°C to 70°C, respectively. The changes in the storage modulus ($G'$), loss modulus ($G''$), and $\tan\delta$ values through the heating ramp under constant amplitude and frequency could be observed in Fig. 7. In all samples, as temperature enhanced, both storage and loss modulus values were decreased gradually. The gelled structure is known to be stable until the crossover ($G' = G''$) point reached. Accordingly, the WsWO sample reached the crossover point at around 40°C. Similar phenomena were occurred at around 80°C for ShWO, at around 48°C for LnWO, and at around 52°C for BsWO sample. Clearly, these oleogel samples showed different thermal stability values. Although the crossover point indicates the flow point, the DSC determined peak melting temperature ($T_m$) indicates a melting point. The $T_m$ values for the oleogel samples given in Table 2 were much lower than those crossover point values measured in the temperature ramp rheological measurements (Fig. 7). Similar situations were observed in previous studies with some plant wax based oleogel samples. These findings may indicate that even after melting, some gelled consistency might be remained until the temperature values of crossover point since the rheometer detected measurable storage and loss modulus values after the DSC determined $T_m$ values until the temperatures reached the crossover points. It would be probable that even after melting the crystalized portions of the oil, some junction zones of the waxes might be remained to yield detectable consistency, which was measured with the rheometer until temperatures up to the DSC determined $T_m$ points. Further, the damping temperature (tan$\delta$ line) of samples (except ShWO sample) cross the storage modulus just before the crossover point ($G' = G''$), indicating that the glass transition temperatures of the oleogels were around the crossover point. It was around 48°C for the ShWO sample, which was lower than the crossover point. These temperature ramp rheology data could be important when oleogels were used in a food product, and some heat treatments are needed. One can know the limit temperature where the gelled structure remains. Further, like all previously studied wax oleogel in the literature the oleogels prepared in this study showed thermo reversibility. During the gelatin time and oil binding capacity measurements (Table 1), after completely melting the previously formed oleogel, the gels reformed once the samples were taken out from the water bath to the ambient temperature. This feature would also
create an advantage during the utilization of wax oleogels in processed food products.

3.5 Sensory properties

The final success of any new oleogel for food applications would definitely be dependent on its sensory properties, despite all mechanical and structural feasibilities. In this respect, a trained panel determined descriptive sensory properties of the oleogel samples were presented in Table 3. Five sensory descriptors (and one more only for LnWO sample) were used to quantify the sensory attributes of the samples. The ‘hardness’ attribute was defined as the force required pushing a knife into the oleogel sample by the panelist, and findings indicated significant differences among the samples. WsWO and BsWO samples had much higher and similar (5.37 and 5.87) hardness values, while ShWO had the lowest (0.87) value. In fact, this result is quite consistent with the rheological finding presented in Fig. 5, showing the storage modulus (G’) values of around 10,000, 200, 1000, and 16,000 Pa for the WsWO, ShWO, LnWO and BsWO, respectively. ‘Spreadability’ describes the easiness level of the oleogel to be spread over a bread loaf by a knife. Statistically there was no significant difference among the samples, and all were quite spreadable (around 7.50-8.50 on 10 max scales). It was stated that spreadability was the most highly regarded attribute for margarine, butter, spreads and similar products perhaps second only to flavor. It was also stated that solid fat index of 10-20 at serving temperature was found optimal for spreadability for consumer satisfaction.

In this study, the solid fat contents (SFC) at 20°C were determined (Table 1) for samples, and ranged between 1.70 and 3.45%, respectively. No direct relationship between sensory spreadability and SFC was determined. In fact, a similar result was reached before in our laboratory. Since oleogels contain gelled liquid oils entrapped within the junction zones of the organogelators used, but not triglyceride crystals, SFC could not directly relate to spreadability. Once an oleogel sample was spread over a surface by mechanical force, the kinetic energy released to cause the gel to melt partly. The amount of melting as the sensory attribute was assessed with the ‘liquefaction’ term. All samples showed little amounts of liquefaction, and there was no significant difference among them. In fact, all partially melted oleogels were immediately solidified once spread. This phenomenon is known as the thermo reversibility, and was present in all samples in this study. ‘Rancid’ defines the level of perceived oxidation compounds from the oleogels. There were some significant differences, and LnWO had the highest (3.00) and BsWO had the lowest (0.75) scores on the 10-point ballot. The sensory rancid scores were not exactly matched with the peroxide values (PV, Table 1) determined. In fact, the LnWO had the highest and the ShWO had the lowest PVs (18.82 and 10.58 meq O₂/kg). The ‘waxy’ attribute measures any aroma and flavor perceived from paraffin or other pure waxes. LnWO had the lowest waxy score, while both WsWO and BsWO had the highest scores. Similar findings were observed in our previous studies with other wax oleogels. When a solid fat melts in mouth space, the body heat absorbed for melting causes a feeling of cooling sensation, as a well-known property for margarine, spread, chocolate and similar products. WsWO and BsWO samples had higher cooling effects than both of the ShWO and LnWO samples. In fact, this property was well correlated with the SFC reported in Table 1. The samples with higher SFC yielded higher cooling sensory attribute quantified. Since only LnWO sample had another perceived attribute, the panel evaluated the ‘sheep odor’ term for it and found 8.00 score on 10 max point. Hence, LnWO sample had a quite high sheep odor. In fact, the panel also orally stated a negative image value against this smell, which quite limits food applications of the LnWO.
4 Conclusions
In this study, oleogels of whale spermaceti wax and lanolin wax were prepared first time for possible food application evaluation, and also they were compared with well-known two other animal-based wax (beeswax and shellac wax) oleogels. The C* of lanolin wax was quite high (30%, w/w), and its oleogel had a distinct negatively perceived sheep odor. Hence, food applications of lanolin wax oleogel seem improper at all. Whale spermaceti wax oleogel (WsWO) was, on the other hand, found quite proper for food applications. The physico-chemical, thermal, and structural properties of the WsWO were very similar to the beeswax oleogel (BsWO). Rheological analyses indicated that the strongness of WsWO was just second to BsWO, and even much higher than that of the shellac wax oleogel (ShWO). It was thermo reversible like others and thixotropic as well. WsWO also had thermal stability of the gelled consistently until around 40°C. Finally, descriptive sensory analysis indicated that WsWO was quite similar to the BsWO. As indicated previously, pharmaceutical grade whale spermaceti wax has been used in cosmetics and pharmacy, but its potential application in oleogel preparation was unexplored. This study concluded that it could be used to prepare oleogels, which are suitable for food applications, but international concern about whale capture must also be considered. Further, within legal limits of whale capture, the spermaceti wax could be prepared as food grade product to be alternatively used in oleogel preparation. This study also suggests further researches for food products applications of the whale spermaceti wax oleogels.

Acknowledgements
This research is funded by the Scientific and Technical Council of Turkey (TÜBİTAK) with the Research Project No: 2170094. We gracefully thank for the support.

Conflict of Interest
The authors have declared no conflict of interest.

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