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Thermal and rheological properties of organogels formed by sugarcane or candelilla wax in soybean oil

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ABSTRACT

Sugarcane wax (SCW) was used to produce organogels and their properties were compared to candelilla wax (CLW) organogels a well known material for this purpose at concentrations of 1 to 4% (w/w). An empirical phase diagram showed that both waxes can form organogels with soybean oil as immobilized phase, SCW organogels were formed at higher concentration and at lower temperatures. The thermal behavior for SCW and CLW was similar, especially during crystallization (T_{Onset} and T_{Peak} of 42 °C and 41 °C respectively) differing only on enthalpy (1.957 and 4.829 J/g respectively), meaning that SCW organogels need less energy to form a network; the melting behavior showed that SCW organogels also need less energy to melt and that both materials presented two melting peaks one of the break of the network and melting of waxes. Rheological behavior presented similar behavior, but with higher values of complex modulus for CLW organogels. In the same way CLW gels showed larger mechanical resistance on compression/extrusion. Micrographs of organogels showed a more organized network present on CLW organogel than SCW organogels that showed larger crystals comparing to CLW organogels. The difference on the microstructure observed explains the difference on the mechanical behavior of organogels formed with both materials.

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1. Introduction

Organogels can be defined as an organic liquid entrapped within a thermo-reversible, three-dimensional gel network. The self-assembly gel network is formed by a component at a relatively low concentration and organic liquid solvents (as benzene, hexane, etc) or liquid oils (Hughes, Marangoni, Wright, Rogers, & Rush, 2009).

Among the components that have the ability to form organogels only a few of them are interesting for food applications. These components can be separated as Low Mass Organogelators (LMOG) and Polymeric Organogelators. The most important LMOG are triacylglycerols, diacylglycerols, monoacylglycerols, fatty acids, fatty alcohols, waxy esters, sorbitan monostearate, fatty acid + fatty alcohols, lecithin + sorbitan tristearate and phytosterols + γ -oryzanol (Pernetti, van Malssen, Flöter, & Bot, 2007).

Wax is a common term used as a reference for mixtures of long chain nonpolar compounds, including hydrocarbons, waxy esters, sterol esters, ketones, aldehydes, fatty alcohols, fatty acids and sterols. The waxes can be classified as naturals or synthetics. Waxes are naturally present on the surface of plants and they have the function to protect them from the loss of water and attack from insects (Parish, Boos, & Li, 2002). Natural waxes are approved as food ingredients or additives according to FDA (FDA, 2012).

Candelilla wax (CLW) is a wax derived from the leaves of a small shrub native to northern Mexico and the southwestern United States. It is used in the cosmetic industry as a component of lip balms and lotion bars, and in the paint industry to manufacture varnishes. Additionally, CLW can be used as a substitute for carnauba wax and beeswax in different food systems (Toro-Vazquez et al., 2007). Sugarcane wax (SCW) is a component obtained from the filter cake of sugar and ethanol production from sugarcane (*Saccharum officinarum*). It is extracted using hexane (Vieira, & Barrera-Arellano, 2002) and clarified and purified (Barrera-Arellano & Botega, 2007).

Sugarcane wax presents a high potential for industrial use due to its high production potential in Brazil, that leads to a low cost and physical properties as shown in Table 1, that are very similar to some commercial waxes such as candelilla.

There are some studies using waxes as organogelators with candelilla wax (Toro-Vazquez et al., 2007), rice bran wax and carnauba wax (Dassanayake, Kodali, Ueno, & Sato, 2009), but to our knowledge there are no studies using sugarcane wax to produce organogels. The main objective of this study was to evaluate the ability of sugarcane wax to form organogels at different concentrations and temperatures, and also compare its rheological and thermal properties with the already studied organogel of candelilla wax.

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Table 1

Properties of candelilla wax (CLW) and sugar cane wax (SCW).

Property	CLW ^a	SCW ^b
Melting point (°C)	67-79	77.6-80.0
Acidity value (mg KOH/g)	12-22	17.9-18.5
Iodine value	14-27	19.9-20.1
Saponification value (mg KOH/g)	35-87	140.9-141.1

^a Tada et al. (2007) and Tulloch (1973).

^b Lopes (2010).

2. Material and Methods

2.1. Material and sample preparation

Refined, bleached and deodorized soybean oil (Cargill Alimentos S.A., Mairinque, SP, Brazil) was purchased from the local market and candelilla wax (CLW) was obtained from Frank B. Ross Co. Inc. (Jersey City, NJ, USA). Clarified and purified sugarcane wax (SCW) was kindly donated by Usina São Francisco (Sertãozinho, SP, Brazil). Chemical composition and physico-chemical properties of CLW and SCW are presented at Tables 1 and 2.

Samples were prepared firstly by heating soybean oil up to 80 °C under stirring and sugarcane or candelilla solid wax was slowly added (0.5, 1, 2, 3 and 4% w/w) and mixed up to its complete dissolution. After complete dissolution, the mixture was kept under agitation without heating for 3 min. The samples were stored at 20 °C for 24 h to form gel and kept at this temperature up to perform the analyses.

2.2. Qualitative phase diagrams

Phase diagrams were constructed using organogels prepared with both waxes at different concentrations, 0.5, 1, 2, 3 and 4% (w/w) as described on previous item on 12×2 cm tubes containing 10 mL and after crystallization for 24 h at 20 °C, stored at temperatures of 5, 10, 15, 20, 25, 30 and 35 °C for more 24 h. After that, the tubes were tilted and the self-standing ability of the samples was assessed visually and depending on the appearance of the samples was described as gel or liquid.

Materials that did not flow were named as *gel*, materials that slowly flowed were named as *thickened liquid* and the materials that immediately flowed were named as *liquid*.

2.3. Crystallization and melting behavior of organogels

The crystallization and melting thermograms were determined by differential scanning calorimetry (DSC) using a TA Instruments model

Table 2

Chemical composition of candelilla wax (CLW) and sugar cane wax (SCW).

Acids and alcohols	Fatty acid (wt.%)		Fatty alcohol (wt.%)	
	CLW ^a	SCW ^b	CLW ^a	SCW ^b
16:0	2.0	22.5	-	-
18:3	-	3.3	-	-
18:1 e 18:2	-	7.1	-	-
18:0	1.0	3.9	-	-
20:0	12.0	1.6	-	-
22:0	4.0	2.1	5.0	-
24:0	1.0	4.1	-	3.2
26:0	1.0	2.9	3.0	10.4
28:0	7.0	24.8	9.0	58.5
29:0	-	2.0		0.8
30:0	32.0	10.1	65.0	15.6
32:0	33.0	5.4	15.0	7.6
34:0	7.0	5.3	3.0	1.0

^a Tada et al. (2007) and Tulloch (1973).

^b Lopes (2010).

Q2000 (TA Instruments, New Castle, DE, USA). Samples of organogels (10 mg) with 4% (w/w) of wax were placed in aluminum hermetic pans and weighted. The samples were heated at 100 °C and maintained at this temperature for 15 min before the samples were cooled to -40 °C at 10 °C/min. Samples were kept at this temperature for 30 min and then again heated to 100 °C at 10 °C/min.

The thermal parameters (T_{Onset} , T_{Endset} , T_{Peak} , ΔH) were calculated for the cooling (crystallization) and heating (melting) sweeps using Universal Analysis software (TA Instruments, New Castle, DE, USA).

3. Experimental

3.1. Rheological properties

The rheological analyses were performed using a Physica MCR 301 (Anton Paar, Graz, Austria) rheometer. A sandblasted rough plate geometry of 5 cm wide, with a roughness of 5–7 μ m, with a gap of 200 μ m was used for organogels. Temperature was controlled at 5 and 25 °C for flow curves and mechanical spectrum measurements. All determinations were done in triplicate.

3.1.1. Flow curves

Flow curves were obtained using shear rate ranging from 0 to 300 s^{-1} at 5 or 25 °C. The organogels were submitted to three shear rate sweeps (up-down-up) in order to eliminate thixotropy and rheological models were fitted to data obtained in steady state. In addition, apparent viscosity was evaluated at different temperatures between 5 and 85 °C, using a fixed shear rate of 100 s^{-1} , which is commonly associated with food process conditions.

The Power-law model used to fit the behavior of non-Newtonian fluids is defined by the equation $\sigma = k (\dot{\gamma})^n$, σ is shear stress (Pa) and $(\dot{\gamma})$ is shear rate (1/s).

3.1.2. Oscillatory rheometry

Heating–cooling sweeps were performed between 5 and 90 °C at 1 °C/min with a fixed frequency of 1 Hz to evaluate the gel formation process. Changes in the slope of complex modulus (G^*) as a function of temperature were evaluated from the derivation of the data using the Savitzky and Golay filter (Savitzky & Golay, 1964) in order to better visualize the thermal transitions. After gel formation frequency sweeps were done from 0.01 to 10 Hz with 1% deformation at 5 and 25 °C. All measurements were performed within the linear viscoelasticity domain.

3.1.3. Hardness (compression/extrusion)

Hardness of SCW and CLW organogels (2 and 4%w/w) were evaluated by compression/extrusion measurements using a texture analyzer Stable Micro Systems model TA-XT2i (Godalming – UK) using a head cross speed of 1.0 mm/s. The gels were conditioned in glass recipients (35 mm internal diameter and 22 mm high) and compressed using an acrylic cylinder (25 mm diameter and 35 mm high) up to 15 mm height at the temperature of 20 °C.

3.2. Polarized light microscopy

Micrographs of organogels (4%w/w) were obtained under polarized light microscope Olympus System Microscope model BX 50 (Olympus America Inc., Center Valley, PA, USA) equipped with a digital camera model Olympus EX300 (Olympus America Inc., Center Valley, PA, USA). The samples were conditioned at 20 °C and observed using the same temperature. The pictures obtained were evaluated using the software Image Pro-Plus 7.0.1 for Windows by Media Cybernetics (Bethesda, MD, USA).

3.3. Statistical analysis

The results were evaluated by analysis of variance (ANOVA) and significant differences (p<0.05) between the treatments were determined by Tukey test. Statistical analysis was performed using the software Statistica (Data Analysis Software system, StatSoft, Inc, Tulsa – USA) version 7.0.

4. Results and discussion

4.1. Qualitative phase diagram

Phase diagram (Fig. 1) shows that CLW can form gels at lower concentration and at higher temperatures (up to 35 °C), when compared to SCW. Both waxes formed organogels at 4% but only CLW could form self sustained gels at 35 °C; the results for CLW gels were similar to those presented on literature (Morales-Rueda, Dibildox-Alvarado, Charó-Alonso, Weiss, & Toro-Vazquez, 2009).

This behavior could be explained by chemical composition of waxes as shown in Table 2. CLW presented 65% of C30:0 and C32:0 fatty acids while SCW showed nearly 50% of C16:0 and C28:0. Fatty alcohol composition presented the same behavior once CLW showed prevailing C30:0 and SCW mostly C28:0.

These results indicate that a higher concentration of SCW than CLW is needed to produce at same temperature an organogel with similar visual characteristics. Thus the systems with a wax concentration of 4%w/w were evaluated, once after crystallization at 20 °C both samples presented self supported organogels at 25 °C.

4.2. Differential scanning calorimetry (DSC)

Thermal properties of SCW and CLW organogels, presented in Fig. 2A, B and Table 3, show that both waxes presented similar crystallization



Fig. 1. Phase diagram for different wax concentration organogels at different temperatures (A) candelilla wax (B) sugarcane wax. Gel (i.e., self-standing gel), thick liquid (i.e., liquid was visibly thickened, but self-standing gel was not observed), and liquid (i.e., no thickening or gelation observed).



Fig. 2. Thermograms for SCW and CLW organogels at 4% (w/w) (A) crystallization, (B) melting, full lines, CLW organogel and dashed lines, SCW organogel.

temperatures (T_{Onset} , T_{Peak} and T_{Endset}), which are similar with the thermal behavior observed by (Morales-Rueda et al., 2009) for CLW organogel.

However two peaks were observed on the melting thermograms as shown on Fig. 2B, these results suggest that two different phenomena are occurring. The first peaks on the right side of Fig. 2B, started close to 32 °C (T_{Onset}) and finished between 51 and 53 °C for both waxes (T_{Endset}); the second peak ranged from 58.52 °C (T_{Onset}) to 75.47 °C (T_{Endset}) for SCW while the CLW melting temperature values occurred between 61.98 °C (T_{Onset}) and 77.31 °C (T_{Endset}) (Table 3). The properties were similar for both waxes except T_{Onset} for CLW organogel, but it was similar to what is observed on literature (Toro-Vazquez et al., 2007).

The crystallization enthalpy of CLW was considerably higher than SCW organogel as shown in Table 3. The sum of melting enthalpy of both melting peaks for each organogel was considerably higher for CLW than SCW (2.843 and 1.625 J/g respectively) showing that the energy needed to melt CLW organogel was higher than needed for SCW organogel, evidencing that CLW organogel has higher resistance to temperature changes than SCW organogel.

The enthalpy needed to crystallize both materials was higher than needed to melt them, 1.957 and 1.625 J/g for SCW and 4.829 and 2.843 J/g for CLW respectively; this hysteresis should be explained by the heat of dissolution of waxes during the heating process (Abdallah, Lu, & Weiss, 1999), as reported by the author the increase of temperature leads to a breakage of the network and the dissolution of the solid material as an exothermic might suppress the visualization of the endothermic melting event.

Table 3			
Thermal properties of SCW and CLW	organogels	at 4%	(w/w).

Sample		$T_{Onset} \left(^{\circ}C \right)$	$T_{Peak}\left(^{\circ}C\right)$	$T_{Endset} \ (^{\circ}C)$	$\Delta H (J/g)$
SCW 4% (crystallization)		43.70	40.98	34.83	1.96
SCW 4% (melting)	Peak 1	31.92	43.14	51.39	0.37
	Peak 2	58.52	72.57	75.46	1.26
CLW 4% (crystallization)		42.95	41.6	14.51	4.83
CLW 4% (melting)	Peak 1	31.84	43.64	53.30	2.38
	Peak 2	61.98	70.15	77.31	0.46

4.3. Rheological measurements

4.3.1. Temperature sweeps

Fig. 3 shows rheological properties during heating and cooling cycle for CLW or SCW (4% w/w) organogels, which showed thermoreversibility.

During heating G^{*} values slightly decreased with the increase of temperature from 30 °C, but a more pronounced decrease was observed between 35–45 °C and 65–75 °C. The complex modulus values (G^{*}) (Fig. 3A) of CLW were higher than SCW organogels at temperatures below 45 °C. Above 70 °C curves shown in Fig. 3, presented a similar behavior, probably because of the complete melting of the organogel network as also observed on the thermal properties (Table 3). Melting temperatures were estimated from rheological



Fig. 3. Temperature sweep for SCW and CLW organogels at 4% (w/w), at 1 °C/min. \bullet : SCW: \blacksquare CLW; full symbols – heating; empty symbols – cooling. (A) Complex modulus and (B) tan delta.

measurements using Savitzky and Golay filter on inflection point, presenting values of 43.1 °C for SCW and 42.1 °C for CLW organogels. These values were similar to the first peak values observed on DSC (Table 3), showing that the first peak observed for thermal analysis is related to the breakage of the organogel network. Another melting temperature was estimated being 64. 1 °C for both organogels. The fat crystallizations in different polymorphic crystalline forms can be explained by the slope changes in G* curves (Lupi et al., 2012).

Fig. 3B shows loss tangent curves for both samples. At low temperatures (<40 °C), the tangent was lower than 1, indicating that the values of G' higher than G". At even higher temperatures, with crystals melting, the tangent values returned to be smaller than 1.

During cooling G* values increased with temperature decrease, a result of the crystallization and reorganization of the organogel network. Crystallization temperatures were also estimated as 48.5 °C and 52.9 °C for CLW and SCW, respectively, being 5–10 °C higher than the ones determined for melting (Fig. 3A). In this range was also observed the G'–G" crossover (tan = 1), which could be also used as a simple criterion for gel point. Gelation temperature relates to the crystallization phenomena and the crystal aggregation in clusters (Lupi et al., 2012). When molten system is cooled, fat crystals in the α format are obtained and their size and number increase with decreasing temperature. Furthermore, during the cooling process, potential transitions ($\alpha \rightarrow \beta'$ polymorphic transformation) and aggregations occur forming a three-dimensional crystalline network (Wastra et al., 2001).

The similarity among DSC and temperature sweep values, specially T_{Peak} during melting for both wax organogels and melting temperatures measured using rheology was almost the same, indicating that the thermal phenomena are in fact correlated with rheological behavior.

4.3.2. Isothermal rheological measurements: mechanical spectra and flow curves

Fig. 4 shows the average results for mechanical spectra of organogels formed with SCW and CLW at 5 or 25 °C. The values of G ' were higher than G" and considered to be independent of frequency, which are characteristic of gels. However G' and G" values for CLW organogel were significantly higher than the observed for SCW organogel. According to Fig. 4, organogels structured with the same wax showed a small difference between the observed values of G' and G" at 5 and 25 °C, but this difference could not be considered significant (Tukey HSD p<0.05).



Fig. 4. Mechanical spectra of SCW and CLW organogels at 4% (w/w) at different temperatures. \bullet : SCW T5, \blacktriangle : SCW T25; \blacksquare : CLW T5, \bullet : CLW T25; T5 - 5 °C and T25 - 25 °C; full symbols - G'; empty symbols - G".



Fig. 5. Flow curves of organogels 4% (w/w) developed with (A) SCW and (B) CLW. Temperatures of 5 °C (\blacklozenge) and 25 °C (\diamondsuit).

Fig. 5A and B shows the flow curves of the SCW (A) and CLW (B) organogels at 5 or 25 °C, which showed that both materials presented thixotropy. Thixotropy can be estimated from the difference between the areas below the curves (hysteresis) during increase (S1 transient) and decrease (S2 – steady state) (Sato & Cunha, 2007; Steffe, 1996). This measurement can be used as a qualitative comparison among the organogels. Table 4 shows that CLW organogel presented a higher hysteresis when compared to SCW organogels. Besides the time dependent behavior, it is possible to observe that both organogels presented stress overshoot (Fig. 5A and B). The fast increase and decrease of shear stress at low shear rates is a consequence of a structure network breaking (Perrechil, Santana, Fasolin, Silva, & Cunha, 2010; Riscardo, Moros, Franco, & Gallegos, 2005). The overshoot for CLW organogels at 5 and 25 °C was 479 Pa at 9.1 s⁻¹ and 196 Pa at 3 s⁻¹ respectively. For SCW organogels the values were considerably lower (144 Pa and 62.2 at 3 s^{-1} at 5 and 25 °C, respectively).

Power-law model for S2 curves (steady state) was fitted to both organogels and the rheological parameters (flow index ${\bm n}$ and

Table 4

Thixotropy estimation for candelilla wax and sugarcane wax organogels.

	SCW		CLW	
	T5	T25	T5	T25
Area S1 (Pa/s)	22.244	9.682	53.328	25.113
Area S2 (Pa/s) Hysteresis	13.960 8.840	5.947 3.735	19.463 33.865	8.985 16.128

Table 5

Estimated Power-law parameters and apparent viscosity at 10, 50 and 100 $\rm s^{-1}$ for candelilla wax and sugarcane wax organogels.

		n	k (Pa s ⁿ)	R ²	η ₁₀ (Pa s)	η ₅₀ (Pa s)	η ₁₀₀ (Pa s)
SCW	T5 T25	0.867 ± 0.000 0.846 ± 0.000	0.633 ± 0.000 0.296 ± 0.001	0.999	0.476 0.206	0.354 0.152	0.332 0.140
CLW	T5 T25	0.711 ± 0.014 0.671 ± 0.014	1.852 ± 0.318 1.124 ± 0.057	0.998 0.999	1.023 0.681	0.554 0.300	0.470 0.241

Table 6

Maximum force of sugarcane (SCW) and candelilla (CLW) waxes organogels.

	Maximum force (N)*
CLW (2%)	$1.75^{\rm b} \pm 0.10$
SCW (2%)	$0.10^{c} \pm 0.05$
CLW (4%)	$14.60^{a} \pm 1.15$
SCW (4%)	$1.65^{\rm b} \pm 0.77$

* Same letters at the same column indicate that there is no statistical difference (p<0.05).

consistency index **k**) were estimated with great adjustment as shown in Table 5. As seen in Table 5 the flow index values for SCW organogel were slightly higher than 0.8 independent of the temperature. However, CLW organogel showed higher pseudoplasticity (lower **n**), consistency index and viscosity than SCW organogel, which is characteristic of a more complex and dense network.



Fig. 6. Polarized light microphotographs, at a magnification of 1000×, (A) SCW and (B) CLW organogels at 4% (w/w).

4.3.3. Hardness

Based on the analysis of compression–extrusion measurements, it can be seen that the organogel formed using 4% w/w of CLW showed a much higher mechanical resistance than organogels obtained with SCW (14.6 and 1.7 N maximum force, respectively). Organogels obtained using SCW at 4% showed almost the same hardness with that of CLW at 2% as seen in Table 6.

The higher maximum force shown for CLW organogels on hardness analysis together with higher values observed on rotational rheological measurements are evidences of a more dense and complex network.

4.4. Polarized light microscopy

Polarized light microphotographs of the sugar cane wax and candelilla wax organogels are shown in Fig. 6A and B. Micrographs show that the crystal network formed in the CLW organogel is tighter (Fig. 6B) and more evenly distributed compared with the crystals of the SCW organogel network (Fig. 6A). The structure presented by CLW organogel (with smaller crystals) is an indication of stronger intermolecular interactions, which might explain the higher hardness and complex modulus, when compared to the SCW organogel at the same concentration.

5. Conclusion

The studied organogelators presented the ability to form organogels at the crystallization conditions (20 °C). The thermal behavior observed using DSC analysis showed that the materials had low thermal resistance. Rheological properties measured using rotational rheology and compression-extrusion analysis showed that CLW organogels were harder and presented higher mechanical resistance under shear. The network observed on the polarized light microscopy was similar for both materials but candelilla wax organogel presented a more organized and tighter network explaining the higher mechanical and thermal resistance of CW organogels. The use of a higher amount of sugarcane wax is needed to obtain an organogel with similar hardness than candelilla wax at the same temperature. The similarities among both organogelators were responsible for both of them produce organogels at the studied conditions, but also the chemical composition that is somewhat different, especially on the chain length of fatty acids and alcohols, affects the interaction of the organogelator and the liquid continuous phase (soybean oil) that causes the changes on the network that leads to the physical differences on organogels.

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