Obtention and characterization of soybean oil organogels structured with sugarcane wax and its hot ethanol soluble fractions

Obtención y caracterización de organogелеs producidos con cera de caña de azúcar y sus fracciones solubles en etanol caliente

Resumo
A cera de cana (SCW) foi fracionada usando etanol quente e um sistema de lavagem simples, as frações obtidas solúveis (SSCW) e insólúveis (ISCW) foram usadas para produzir organogéis cristalizados em duas temperaturas diferentes (5 e 25°C) nas concentrações de 1, 2, 3 e 4% (m/m). A pesquisa avaliou o comparamento dos organogéis obtidos da cera de cana-de-açúcar (e suas frações), todos os organogéis foram avaliados quanto à sua microestrutura, comportamento térmico, comportamento reológico e resistência mecânica. Todas as amostras de organogel foram avaliadas quanto à estabilidade em uma estufa BOD com temperatura controlada (a 5, 25 e 35°C), e o comportamento térmico para os organogéis de SCW, SSCW e ISCW foi diferente. A variação de entalpia passou de 118,87 para 129,63 e 85,65J/g para as frações. Os organogéis obtidos a partir desses materiais foram algo semelhantes durante a cristalização (TPeak de 42,83, 37,19 e 36,23°C, respectivamente), a variação da cristalização e da entalpia de fusão apresentou histerese, como observado para outros organogéis de ceras. Os organogéis de SSCW foram significativamente mais difíceis do que os obtidos com SCW e ISCW. As micrografias dos organogéis mostraram uma rede mais organizada presente no organogel da SSCW quando comparada com a SCW que foi mais organizada que os
organogéis da ISCW. A diferença na microestrutura observada explica a diferença no comportamento mecânico dos organogéis formados com frações solúveis e insolúveis em etanol quente da cera de cana de açúcar.

**Palavras-chave:** Organogéis; Cera de cana; Morfologia de cristais; Reologia; Dureza.

**Abstract**

Sugarcane wax (SCW) was fractionated using hot ethanol and a simple washout system, the obtained fractions soluble (SSCW) and insoluble (ISCW) were used to produce organogels crystallized at two different temperatures (5 and 25°C) at the concentrations of 1, 2, 3 and 4% (w/w). The laboratory research evaluated the behavior of organogels obtained from sugarcane wax (and its fractions), all organogels were assessed due to its microstructure, thermal behavior, rheological behavior and mechanical resistance. Samples were visually assessed for stability at a controlled temperature oven (at 5, 25 and 35°C), and the thermal behavior for SCW, SSCW and ISCW were different. The enthalpy variation changed from 118.87 to 129.63 and 85.65 J/g for the fractions. Organogels obtained from these materials were somewhat similar during crystallization (TPeak of 42.83, 37.19 and 36.23°C respectively), crystallization and melting enthalpy variation presented hysteresis as observed for other waxy organogels. SSCW organogels were significantly harder than the obtained with SCW and ISCW. Micrographs of the organogels showed a more organized network present on SSCW organogel when compared with SCW that was more organized than ISCW organogels. The difference on the microstructure observed explains the difference on the mechanical behavior of organogels formed with sugarcane wax hot ethanol-soluble and insoluble fractions.

**Keywords:** Organogels; Sugarcane wax; Crystal morphology; Rheology; Hardness.

**Resumen**

Se fraccionó la cera de caña de azúcar (SCW) usando etanol caliente y un sistema de lavado simple, las fracciones obtenidas solubles (SSCW) e insolubles (ISCW) se usaron para producir organogeles cristalizados a dos temperaturas diferentes (5 y 25°C) a las concentraciones de 1, 2, 3 y 4% (p/p). Se evaluó la estabilidad de todas las muestras de organogel en una camara a temperatura controlada (a 5, 25 y 35°C), y el comportamiento térmico para SCW, SSCW e ISCW fue diferente. La variación de entalpía cambió de 118.87 a 129.63 y 85.65 J / g para las fracciones. Los organogeles obtenidos de estos materiales fueron algo similares durante la cristalización (TPeak de 42.83, 37.19 y 36.23°C respectivamente), la cristalización y la variación de la entalpía de fusión presentaron histéresis como se observó para otros organogeles de ceras. Los organogeles SSCW fueron significativamente más duros que los obtenidos con SCW e ISCW. Las micrografías de los organogeles mostraron una red más organizada presente en el organogel SSCW en comparación con SCW que estaba más organizada que los organogeles ISCW. La diferencia en la microestructura observada explica la diferencia en el
comportamiento mecánico de los organogelos formados con cera de caña de azúcar fracciones solubles e insolubles en etanol calientes.

**Palabras clave:** Organogelos; Cera de caña de azúcar; Morfología cristalina; Reología; Dureza.

1. **Introducción**

Organogelos son descritos como un sistema donde dos fases continuas coexisten, una fase sólida organogeladora y una fase continua inmovilizada que es un solvente orgánico (Abdallah et al., 1999), se llaman organogelos porque de su comportamiento geliforme y difieren de los hidrogelos en las fases inmovilizadas son un componente orgánico (Rogers, 2009).

Debido a su microestructura como una red tridimensional (Abdallah & Weiss, 2000), son considerados como auto-sustentables, lo que los hace un material interesante para estudios, pero también muestran su potencial para aplicaciones alimentarias.

Organogelos han sido presentados como “el graso para el futuro” (Hughes et al., 2009; Pernetti, van Malssen, Flöter, et al., 2007; Rogers et al., 2009a), a pesar de que tienen un largo camino que recorrer para poder ser aplicados de manera industrial.

Muchos de los materiales que tienen la capacidad de formar organogelos, también conocidos como organogeladores, no son comestibles, como la ácido 12-hidroxistearico (Rogers et al., 2008, 2009b), o implican en costos más altos para futuras posibles aplicaciones, por ejemplo, el colesterol (Almeida & Bahia, 2006) o γ-oryzanol a alta concentración (Bot et al., 2008).

Por el contrario, el uso de materiales comestibles y aprobados como organogeladores es importante para producir organogelos que sean más fáciles de usar como aplicaciones alimentarias. Los organogeladores más comunes son los acilglicerolos (Lupi et al., 2012), ácidos grasos, alcoholes grasos (Rogers et al., 2009b), mezclas de γ-oryzanol y colesterol (Bot et al., 2008), mezclas de lecitina y sorbitano tristearat (Pernetti, van Malssen, Kalnin, et al., 2007), mezclas de ácidos grasos y alcoholes grasos (Pernetti, van Malssen, Flöter, et al., 2007).

Los parafinas que son mezclas de ácidos grasos, alcoholes grasos y componentes menores como alcanes (Parish et al., 2002), son materiales potenciales para ser usados como organogeladores para organogelos comestibles (también conocidos como oleogelos), algunas estudios habían sido realizados utilizando parafinas como organogeladores, como la candelilla (CLW), carnauba (CRW), bran de arroz (RBW), caña de azúcar (SCW) y girasol (SFW) (Alvarez-Mitre et al., 2012; Daniel & Rajasekharan, 2003; Dassanayake et al., 2009; Rocha et al., 2013; Toro-Vazquez et al., 2007) todos ellos presentaron la capacidad de formar organogelos a diferentes concentraciones y condiciones.

El proceso para producir organogelos es simple y usualmente el mismo para cualquier...
organogelators passing through the heating of the continuous phase followed by the addition of the organogelator and posterior crystallization the process has been reported various times at the literature (Morales-Rueda, Dibildox-Alvarado, Charó-Alonso, & Toro-Vazquez, 2009; Patel & Dewettinck, 2016; Rocha et al., 2013).

Also organogels present an important role at the future of fat structure, once called “the fat the future” (Rogers et al., 2009a), even with so many years at the moment there are no products that are structured using organogels, but the feasibility had been evaluated several times for icecream (Zulim Botega et al., 2013), margarine (Chaves et al., 2020; Hwang et al., 2014), cookies (Jang et al., 2015; Mert & Demirkesen, 2016; Yılmaz & Öğütcü, 2015) and some other products such as meat emulsions (Lupi et al., 2014).

The potential of sugarcane wax had been already been studied (Rocha et al., 2013), the idea that some of the components of the material should have a better ability to form organogels lead to the idea of wax fractioning to isolate some of the components, once soybean oil is soluble in hot ethanol the use of the same solvent aimed to collect a fraction with similar polarity that should be compatible (Terech, 1992) and possibly could change the amount needed to gelation (Dassanayake et al., 2012) and also improve the quality of the organogels, specially hardness as reported previously at literature the polarity of organogelators and their continuous phases is relevant to such quality (Hwang et al., 2015).

The objective of this laboratory research was to determine the behavior of organgels obtained from sugarcane wax and organogels obtained at the same conditions using fractions of a simple washout fractionating method using hot ethanol. Organogels were compared due to its microstructure, thermal behavior, rheological behavior and mechanical resistance.

2. Material and Methods

An experimental design were elaborated and the research separated at four parts the first was to acquire and prepare the raw materials, after the acquisition the second step was to prepare the wax fractions at a third step the organogels samples were prepared and finally the samples were evaluated at the final fourth step. The research was based on acquisition of qualitative and also quantitative data from several analytical methods (Pereira et al., 2018; Severino, 1996).
2.1. Material and Sample Preparation

Refined soybean oil (SO) (Cargill Alimentos S.A., Mairinque, SP, Brazil) was purchased from the local market, clarified and purified sugarcane wax (SCW) was kindly donated by Usina São Francisco (Sertãozinho, SP, Brazil) and anhydrous ethanol (Merck, Germany), was purchased at the local market.

2.2. Fractioning of Waxes

An amount of 1 l of anhydrous ethanol was added to 500 g of SCW and boiled under reflux for 30 minutes and a hot ethanol soluble fraction (SSCW) diluted at the ethanol was separated as a supernatant, the residue was washed with another 1l of anhydrous ethanol and the ethanol supernatant separated and added to the first fraction the fractioning was performed according to Gandra (2006). The fractionating process presented a yield of 55% for the soluble and 43% for the insoluble fractions (ISCW). Both fractions were dried under vacuum and milled.

The thermal properties of SCW and the fractions were measured and are presented in Table 1 (the obtained results were similar to those obtained by Gandra (2006), fatty acids and fatty alcohols compositions are described in Table 2 (Gandra, 2006; Lopes, 2010).

Table 1. Physical Properties of sugar cane wax (SCW) and its fractions.

<table>
<thead>
<tr>
<th>Property</th>
<th>SCW</th>
<th>SSCW</th>
<th>HSCW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melting point (°C)</td>
<td>78.3</td>
<td>75.8</td>
<td>79.4</td>
</tr>
<tr>
<td>Acidity value (mg KOH/g)</td>
<td>23.2</td>
<td>34.19</td>
<td>18.2</td>
</tr>
<tr>
<td>Iodine value (g/100g)</td>
<td>22.3</td>
<td>42.3</td>
<td>17.2</td>
</tr>
<tr>
<td>Saponification value (mg KOH/g)</td>
<td>79.1</td>
<td>113.3</td>
<td>93.6</td>
</tr>
</tbody>
</table>

(Gandra, 2006)
Table 2. Fatty acids and fatty alcohols Composition of sugar cane wax (SCW) and its fractions.

<table>
<thead>
<tr>
<th>Fatty Acids % (w/w)</th>
<th>Fatty Alcohols % (w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SCW</td>
</tr>
<tr>
<td>16:0</td>
<td>22.5</td>
</tr>
<tr>
<td>18:3</td>
<td>3.3</td>
</tr>
<tr>
<td>18:1 e 18:2</td>
<td>7.1</td>
</tr>
<tr>
<td>18:0</td>
<td>3.9</td>
</tr>
<tr>
<td>20:0</td>
<td>1.6</td>
</tr>
<tr>
<td>22:0</td>
<td>2.1</td>
</tr>
<tr>
<td>24:0</td>
<td>4.1</td>
</tr>
<tr>
<td>26:0</td>
<td>2.9</td>
</tr>
<tr>
<td>28:0</td>
<td>24.8</td>
</tr>
<tr>
<td>29:0</td>
<td>2.0</td>
</tr>
<tr>
<td>30:0</td>
<td>10.1</td>
</tr>
<tr>
<td>32:0</td>
<td>5.4</td>
</tr>
<tr>
<td>34:0</td>
<td>5.3</td>
</tr>
</tbody>
</table>

(Gandra, 2006; Lopes, 2010)

2.3. Organogels Preparation

Organogel samples were prepared firstly heating soybean oil up to 80 °C under stirring and SCW, SSCW or ISCW solid wax was slowly added (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 put inside 50 ml glass beakers and stored at 5 or 25 °C for 24 hours to static crystallization and kept at 25 °C temperature up to perform the analysis.

Two temperatures were used for static crystallization aiming to achieve evaluate the effect of different supercooling at the formation of different crystallization polymorphs (Himawan et al., 2006) although it was not possible to evaluate the cooling rates due to processing conditions it is known that with the lower temperature a faster crystallization is expected.
3. Methods

3.1. Visual stability

Organogel liquid samples at (1, 2, 3 and 4% w/w), were put inside glass tubes and conditioned at the studied temperatures of 5 and 25°C for static crystallization for 24 hours. The crystallized samples were kept at 5, 25 and 35°C for 7 days and visually assessed for oil exudation of phase separation at first, third and seventh days. The aimed temperatures ideally represent refrigeration (5°C) and room temperature (25°C) while above 35°C sugarcane organogels showed no stability as reported by Rocha et. al. (2013), meaning that there is no point in evaluating the samples at temperatures above 35°C.

3.2. Crystallization and Melting Behavior of Organogels

The crystallization and melting thermograms were determined by differential scanning calorimetry (DSC) using a TA Instruments model Q2000 (TA Instruments, New Castle, DE, USA). Samples of organogels (10mg) were placed in aluminum hermetic pans and weighted. The samples were heated at 100°C and maintained at this temperature for 15 minutes before the samples were cooled to -40°C at 10°C/min. Samples were kept at this temperature for 30 minutes and then again heated to 100°C at 10°C/min, as evaluated by Rocha et. al. (2013).

The thermal parameters (TONset, TEndset, TPeak, ΔH) were calculated for cooling (crystallization) and heating (melting) sweeps using Universal Analysis software (TA Instruments, New Castle, DE, USA).

The average of 3 measures was used to perform parameters calculation.

3.3. Hardness (Compression/Extrusion)

Hardness of SCW, SSCW and ISCW organogels (1, 2, 3 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, 30 ml of the gels were conditioned in 50 ml glass beakers glass recipients and compressed using an acrylic cylinder probe (25mm diameter and 35 mm high) up to 15 mm height at the temperature of 25°C.

The average of 4 measurements of the maximum force measured at any depth was considered as the result.
3.4. Polarized light microscopy

Micrographs of organogels (4 %w/w) were obtained under polarized light microscope Olympus System Microscope model BX 51 (Olympus America Inc. Center Valley, PA, USA) equipped with a digital camera model Olympus EX300 (Olympus America Inc. Center Valley, PA, USA).

Samples were melted at 80 °C and a small drop put over a heated glass slide and covered with a glass coverslip. The glass slides were crystallized on at 5 and 25°C for 24 hours and analyzed at 25°C. Samples were treated using Image cell Sens Standard 1.7.1. (Olympus, USA).

3.5. Scanning Electronic Microscopy (SEM)

Morphology of 4% (w/w) samples were analyzed by SEM, using a TM3000 Microscope (Hitachi, Japan). All samples were crystallized at 50 μl aluminum pans at controlled temperature, after complete crystallization all samples were washed out by immersion at an Acetone:Chloroform 1:1 (v:v) for 6 hours and dried at low pressure at room temperature using a vacuum chamber.

Samples were directly observed using an acceleration of 15 kVA and an increase of 1000x.

3.6. 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. Visual stability

SCW organogel samples crystallized at 25°C were stable for 7 days at all studied conditions (5, 25 and 35°C) at all concentrations, the same material crystallized at 5°C did not present the same stability presenting a small oil exudation after 3 days at 35°C up to the seventh day.

At Figure 1A, it was possible to observe that the ISCW organogel at 1% (w/w) did not form a stable phase after 3 days at 25°C, with the increase of the amount of wax the samples
became just one phase as shown in Figure 1B for 4% (w/w) organogel samples.

**Figure 1.** Organogel samples crystallized at 25°C at (a) SCW, (b) SSCW and (c) ISCW (A) after 3 days at 25°C; (A) 1% (w/w), (B) 4% (w/w) and (C) SSCW organogel at 4% (w/w) crystallized at 5°C after 7 days at 35°C.

The SSCW organogels at 4% (w/w) presented oil exudation after 3 days at 35°C at both crystallization conditions (5 and 25°C) as shown in Figure 1C, the oil exudation was not visible for SSCW samples at lower concentrations. All other samples that formed a visually continuous phase did not show oil separation. The results are similar to those observed by Rocha et al. (2013) for organogel samples produced using SCW and also candelila wax.

Based on polarity it is possible to explain why the ISCW showed lower stability specially compared to the SSCW, with polarity similar to the studied soybean oil. If we observe the polarity table presented by Hwang et al. (2015), it is the expected behavior with similar polarity the organogels are expected to be more stable and organized, and at the present study the same behavior was observed.

4.2. Thermal Analysis

The thermal properties of SCW, SSCW and ISCW were measured, the Figure 2A shows the crystallization of the waxes and it is possible to observe that the waxes presented changes on the crystallization behavior especially the peak temperature, 67.88°C for SCW changed to 54.98 and 66.89°C for SSCW and ISCW respectively. The enthalpy variation changed from 118.87J/g for SCW to 129.63J/g for SSCW and decreased to 85.65J/g for ISCW.
The melting behavior was presented in Figure 2B and allows to observe that organogels from SCW and ISCW presented very similar peaks (although with different intensities) while SSCW was different. The measured peak temperature for melting was 61.91°C for SCW, 60.77 and 65.41°C for SSCW and ISCW respectively. The enthalpy variation changed from 121.70J/g for SCW to 132.27J/g for SSCW and decreased to 87.21J/g.
for ISCW.

The crystallization thermogram for ISCW also indicates that this fraction is, a concentrate of some of the wax components, as expected, presenting a narrower and pronounced peak which for thermal analysis represents pure components (Campos, 2005), allowing to see that this fraction presents a higher uniformity.

At SSCW thermogram a wider peak is presented, but without the presence of the higher peak that is present on the SCW and were pronounced for ISCW, such difference should be due to chemical composition, according to Gandra (2006), the ISCW presented an increase from 22.5 to 39.6% of the amount of palmitic acid when compared with SCW and also presented a decrease of the amount of fatty alcohols from 21 to 18% (w/w), also SSCW presented 59.5% of octacosanol compared with 51.1% of ISCW, but also the SSCW presented an increase of the fatty alcohols content from 21 to 25% compared with SCW (Gandra, 2006; Vieira, 2003).

Table 3. Results of the thermal analysis of organogels developed with SCW, SSCW and ISCW.

<table>
<thead>
<tr>
<th>Sample</th>
<th>T_{Onset} (°C)</th>
<th>T_{Endset} (°C)</th>
<th>T_{Max} (°C)</th>
<th>ΔH (J/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Crystallization</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCW</td>
<td>46.75a</td>
<td>15.82b</td>
<td>42.83a</td>
<td>3.53a</td>
</tr>
<tr>
<td>SSCW</td>
<td>41.89c</td>
<td>14.20c</td>
<td>37.19b</td>
<td>3.44a</td>
</tr>
<tr>
<td>ISCW</td>
<td>44.94b</td>
<td>18.20a</td>
<td>36.23c</td>
<td>2.20b</td>
</tr>
<tr>
<td><strong>Melting</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCW</td>
<td>59.50a</td>
<td>77.29a</td>
<td>72.62b</td>
<td>0.88a</td>
</tr>
<tr>
<td>SSCW</td>
<td>49.22b</td>
<td>78.44a</td>
<td>69.92c</td>
<td>0.42c</td>
</tr>
<tr>
<td>ISCW</td>
<td>58.17b</td>
<td>78.15a</td>
<td>73.88a</td>
<td>0.62b</td>
</tr>
</tbody>
</table>

*Same letters indicate that there is no significant difference between samples at the same column (p>0.05).

The 4% (w/w) organogels samples obtained with each of the waxes were also evaluated and the crystallization and melting thermograms presented in Figure 2A and 2B respectively. The calculated thermal parameters are presented in Table 3, they allow to observe that compared with the SCW organogel, both fractions presented a change on enthalpy variation (ΔH), for melting, this difference should be related to the separation of
components of each fraction. The effect was not observed for the crystallization, SCW and the SSCW organogels presented no statistical difference, only ISCW organogel presented a statistically different enthalpy variation, it was also possible to observe that the enthalpy variation for melting was significantly different between all organogel samples.

Enthalpy variation for crystallization and melting showed a hysteresis for all organogels samples, such effect according to Abdallah, Lu, & Weiss (1999), is due to the presence of an exothermic event of dissolution of the organogelator during the heating of the sample Rocha et al. (2013), observed the same effect for SCW organogels and the present work using the same wax and its fractions observed the same effects.

4.3. Mechanical Properties

**Hardness (compression/extrusion)**

The samples at a concentration of 1, 2, 3 and 4% (w/w) of each of the studied organogelators SCW, SSCW and ISCW were analyzed and the results are shown in Table 2.

As expected the samples obtained with higher amounts of waxes were significantly harder than the organogels with lower concentrations, except for ISCW that did not show significant difference with the increase of wax concentration.

Crystallization temperature showed a significant influence on the hardness of the organogels for SCW and SSCW while it did not influence the hardness of the organogels obtained with the ISCW.

Hardness tests are handy to evaluate changes in physical behaviour of wax organogels (Dassanayake et al., 2009; Rocha et al., 2013; Toro-Vazquez et al., 2007), and for this case it was possible to observe that a higher crystallization temperature resulted at an increased mechanical hardness of the organogels for SCW and SSCW, such increase of hardness that was observed is probably caused by an increase in the organization of the organogel network, that should be the result of a smaller supercooling that allow the crystals to change their polymorphism (Himawan et al., 2006; Morales-Rueda, Dibildox-Alvarado, Charó-Alonso, Weiss, et al., 2009) and achieve a more stable polymorph.

4.4. Polarized light microscopy

Micrographs of the organogels obtained with all three waxes allowed to observe the network formed by the organogelators.
Figure 3. Polarized light micrographs of SCW organogels obtained at (A) 5°C and (B) 25°C, SSCW organogels obtained at (C) 5°C and (D) 25°C and ISCW organogels obtained at (E) 5°C and (F) 25°C.

Figure 3A shows the organogel developed with 4% (w/w) of SCW at 5°C while Figure 3B shows the same material crystallized at 25°C. At both images it is possible to observe the presence of needle-like crystals that should represent a bi-dimensional view of the network that keeps the liquid phase immobilized, we can notice that the crystals on Figure 3A are slightly smaller than the observed on Figure 3B, 3.8 ± 0.7 and 4.2 ± 0.9 μm respectively, but it is possible to observe a higher organization of them at Figure 3B.

The organogels obtained with SSCW at 4% (w/w), at 5°C are shown in Figure 5C and
25°C on Figure 3D. On Figure 3C it is possible to observe a lot of crystals so small that measured around $2.0 \pm 0.3 \mu m$ and at Figure 3D it is possible to observe a large amount of crystals with the average size of $3.5 \pm 0.7 \mu m$.

For ISCW organogels the micrograph of the organogels at 4% (w/w) as shown in Figure 3E and 3F, shows a large difference among the materials obtained at 5 and 25°C. On Figure 3E the organogel was obtained at 5°C and it is possible to observe an organized structure with crystals of $5.1 \pm 1.1 \mu m$, while on Figure 3F the observed crystals were larger ($7 \pm 1.8 \mu m$) and also a smaller organization on the network was observed, being possible to observe crystals that were not part of the network.

The observation using polarized light microscopy corroborated the results obtained for mechanical properties, a more organized network as seen in Figure 3B and Figure 3D are correlated with a higher mechanical resistance. The observed structures were similar to those observed to candelilla wax (Blake et al., 2014; Dassanayake et al., 2012; Morales-Rueda, Dibildox-Alvarado, Charó-Alonso, Weiss, et al., 2009), rice bran wax (Dassanayake et al., 2012), carnauba wax (Dassanayake et al., 2009) organogels, meaning that the organogels should also present similar behavior.

4.5. Scanning Electronic Microscopy (SEM)

At Figure 4B, 4D and 4F (where the 25°C organogels are presented), it is possible to observe that the SCW and its fraction organogels are indeed a three-dimensional network at a rupture point (the material suffered a volume reduction due to the removal of the liquid oil).
Figure 4. SEM micrographs of SCW organogels obtained at (A) 5°C and (B) 25°C, SSCW organogels obtained at (C) 5°C and (D) 25°C and ISCW organogels obtained at (E) 5°C and (F) 25°C.

Once the measure of the cells was not possible once the material was modified, the structure of the material can be observed as a really organized structure of a foam-like network, as observed in the literature (Ema et al., 2006), such authors used SEM of polymeric foams and it was possible to observe the resemblance among their material and the studied
organogel samples.

The type of foam is indeed a question to be observed, the literature shows that waxy organogels formed closed cell foams, that immobilize the liquid phase as a physical barrier (Miyazaki et al., 2011), but once we compare the images obtained in this study with the ones from EMA et. al., (2006), the material is more similar to an open cell foam, a less stable structure that keeps the immobilized liquid oil due to chemical interactions (such as steric hindrance), making the material less stable to oil exsudation once it is not physically kept inside a cell.

Although SCW and SSCW organogels presented similar structures the ISCW organogel presented a different structure with the presence of some loose crystals.

Comparing SCW organogel (Figures 4B) with SSCW organogel (Figures 4D), crystallized at 25°C it is possible to observe the presence of open cells at the foam structure.

The same organogels crystallized at 5°C, shown in Figure 4 (A, C and E), it is possible to observe that there are no different among the SCW and SSCW organogels, while ISCW organogel formed a structure with no open cells, just a few pores where visible at the surface. The structure was indeed similar to those observed by (Rogers et al., 2009b).

The observed structure was similar at polarized light microscopy and SEM allows to observe that the higher crystallization temperature of 25°C formed a more organized structure.

5. Final Considerations

The performed analysis allows concluding that SCW presented the ability to act as an organogelator and that the SSCW presented a better potential for this application, while the ISCW fraction did not. SSCW produced harder organogels and better-organized networks as verified using microscopy.

Organogels produced using SSCW were harder than the obtained using SCW, probably due to the chemical composition and its interactions with the immobilized phase (soybean oil).

The thermal behavior observed on DSC, especially the enthalpy variation was similar to the results for hardness and rheology, where the samples that presented higher enthalpy values were also harder and the sample of ISCW organogels that presented a really lower enthalpy variation was not able to form proper organogels.

Although sugarcane wax is not yet a commercial product, its potential as
organogelator has been evaluated and confirmed, for the next steps a better understanding of how organogels behave under shear during food processing should allow the inclusion of organogels structured food at our shelves.

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References


**Percentage of contribution of each author in the manuscript**

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