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Potential use of carnauba wax oleogel to replace saturated fat in ice cream

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Abstract

The objective of this study was to develop a wax-oleogel (soybean oil—SBO, peanut oil—PNO, and carnauba wax—CBW) to be used as a fat replacer for ice cream formulations. The oleogels were structured with 6%, 8%, and 10% of CBW and were characterized by oil binding capacity (OBC), visual evaluation, thermal properties (DSC), and microstructure by polarized light microscopy (PLM). All oleogels resulted in a firm and stable gel for 60 days, regardless of the concentrations (6%, 8%, and 10%) and temperatures (5 and 25°C). The OBC of oleogels at 8% and 10% addition were significantly higher ($p < 0.05$) than the oleogels with 6% of CBW. However, the oleogel formed with 6% of CBW showed more than 84% of oil retention after 60 days, indicating that the 6% of CBW was sufficient to develop a network that could hold the liquid oil into a gel-like structure. Larger crystals (μm) were observed with 10% CBW addition (2.15 ± 0.16 μm SBO and 2.08 ± 0.22 mm PNO). After preliminary sensory studies, the SBO oleogel with 6% of CBW was chosen to be applied in the ice-cream formulation. The ice cream preparations were analyzed by overrun, melting rate, fat composition, and sensory acceptance. The oleogel fat replacement (50% and 100%) reduced the melting rate of ice creams; however, it negatively affected the ice cream overrun. These results show that 50% replacement of the traditional lipid phase by CBW oleogel can be performed without causing sensory impairment.

KEYWORDS
carnauba wax, fat substitute, ice cream, oil structuring, oleogel

INTRODUCTION

Ice cream contains fat as partially destabilized droplets, the air in tiny bubbles, casein micelles in colloidal suspension, water in the form of ice crystals, and a concentrated unfrozen aqueous solution (Goff & Hartel, 2013). Ice cream fat plays a vital role as a structural agent, stabilizing the air phase (Goff & Hartel, 2013) and creating the expected sensory qualities characteristics (Méndez-Velasco & Goff, 2011). In addition, fat contributes to the release of hydrophobic flavor molecules (McClements, 2015). Therefore, it is a suitable carrier and synergist for added flavor compounds. Fat also helps stabilize the foam, which is mainly responsible for the creamy texture, contributes to good melting properties, and aids in lubricating the freezer barrel while frozen (, 2006b; Goff, 2006a).

The standard of identity in the United States of ice cream requires 10% minimum milk fat content and 20% total milk solids (FDA, 2016). Warren and Hartel (2014)

evaluated the total fat of 11 ice cream products in the U.S. market, and the range of total fat was 9–14.3 ± 0.05. The current Dietary Guidelines for Americans (USDA, 2015) recommends the consumption of low-fat dairy products. These recommendations guide consumer choice and put pressure on manufacturers to reduce saturated fats in dairy products in response to consumer demand. At the same time, new requirements were imposed by the U.S. Food and Drug Administration (FDA) to ban partially hydrogenated vegetable fats, remove trans-fatty acids from food products, and reduce saturated fats in foods (FDA, 2018). These new constraints pose a significant challenge to developing nutritious lipid bases with compatible technological characteristics and similar sensory properties with the fats to be replaced.

Although natural unsaturated oils are considered "healthier," they do not have suitable physicochemical properties for direct use in food formulations; thus, developing fat with low saturated and/or zero trans-fatty acids requires lipid modification techniques to maintain the technological properties of interest. Among the existing lipid modification methods, oleogelation is one of the most promising techniques (Patel & Dewettinck, 2015; Scharfe & Flöter, 2020).

The development of an oleogel involves the addition of structuring agents in oils with limited solubilities. For instance, phytosterols, lecithins, mono and diacylglycerols, fatty acids, fatty alcohols, waxes, and wax esters are structuring agents (Hughes et al., 2009; Pernetti et al., 2007; Rogers, 2009; Zhao et al., 2020). As a result, oleogels exhibit viscoelastic behavior, consisting of a lipid base composed of oil in the liquid state, structured by a three-dimensional network formed by structuring agents (Valoppi et al., 2020).

Oleogels can be applied in several segments in the food industry, such as margarine, dairy, meat, bakery products, and ice creams. For example, in the formulations of ice cream, oleogel replaces the fat initially from the milk (Moriano & Alamprese, 2017; Munk et al., 2018; Silva-Avellaneda et al., 2021; Zulim Botega et al., 2013a, 2013b). The use of oleogels in ice cream is exciting because it allows manufacturers to replace the milk fat with mono/polyunsaturated oils in the formulations instead of using palm oil and its fractions and coconut oil that are currently in use and do not solve the saturated fat reduction (Pellegrini et al., 2012).

A low-fat ice cream developed by Moriano and Alamprese (2017) used a phytosterol:γoryzanol-oleogel to substitute milk fat and presented high-quality characteristics comparable to those of the milk cream samples with better overrun and melting starting time. Although previous studies have found interesting technological properties required to replace milk fat in ice cream, only a few have evaluated the sensorial properties of the final product. Nowadays, it is well known that removing one ingredient may affect the physical properties in the product and multiple sensory characteristics that may or may not be necessary to consumers. For example, fat contributes to texture, mouthfeel, and flavor and functions as a structural element (Rogers et al., 2014).

Based on the satisfactory oleogelation properties promoted by CBW (Yi et al., 2017) and the potential of oleogels for food application (Zhao et al., 2021), the objectives of this research were to investigate the application of CBW oleogel in PNO and SBO in formulations of ice cream, explore the practical applications of trans-free fats in the physical–chemical properties and also focusing on sensory analysis. Sensory evaluation of formulated ice cream samples, although important, is a point that is not so much addressed in studies with oleogels, and it is a vital characteristic to be studied since the waxy mouthfeel might be an obstacle limiting the development of wax-based products in food markets (Doan, Tavernier, Danthine, et al., 2018).

EXPERIMENTAL PROCEDURES

Materials

Refined soybean oil (SBO) and refined peanut oil (PNO) were kindly donated by Cargill Incorporated, (Minneapolis, MN, USA). Carnauba wax (CBW) was purchased from Better Shea Butter (Austin, TX, USA). The SBO and PNO exhibited the following fatty acids composition: SBO (C16:0 11.1%, C18:0 3.9%, C18:1 18.9%, C18:2 58.2%, C18:3 7.1%, C20:0 0.3%, C20:1 0.1%, C22 0.3%), PNO (C16:0 9.9%, C18:0 2.6%, C18:1 56.8%, C18:2 24.7.2%, C18:3 0.1%, C20:0 1.3%, C20:1 1.4%, C22 3.1%).

Preparation of oleogels

Oleogels were prepared by heating the oil to 100°C under magnetic stirring (350 rpm). Once the oil temperature was reached, the structuring agents (CBW) were slowly added (6%, 8%, or 10% w/w) and mixed up to their complete dissolution (5 min).

After the complete incorporation of the wax, the samples, still in the liquid state, were transferred to test tubes and kept in a static condition at room temperature (20 ± 4°C) for 24 h for structuring and stabilization.

Oleogel characterization

Visual evaluation

The oleogels were disposed of in 15 ml falcon tubes and maintained at room temperature (20 ± 4°C) for 24 h. After this stabilization period, the tubes were inverted and then transferred to a temperature-

controlled chamber at 5 and 25°C and evaluated on days 1, 7, 30, and 60 to observe the stability, including phase separation and liquid oil exudation on the surface. The tubes were prepared in triplicate. For the standardization of the evaluations, a classification of Godoi et al. (2019) was used to analyze the oleogels: Totally firm (5), Firm (4), Medium (3), Weak (2), and liquid (1).

Oil binding capacity (OBC)

The OBC method was adapted from Da Pieve et al. (2010). During the preparation, 1 ml of the melted oleogel sample was deposited into the previously weighed Eppendorf tube (weight "a"). The triplicates of the Eppendorf filled with samples were then transferred to a temperature-controlled chamber at 5 and 25 °C and the OBC were evaluated on days 1, 7, 30, and 60. In the day of the analysis, the samples from both temperatures (5 and 25°C) were refrigerated (2–4°C) for 1 h, and the samples were weighed (weight "b") and centrifuged (Z216-MK Refrigerated Microcentrifuge; HERMLE Benchmark). The tubes were centrifuged at 10070*g* for 15 min at room temperature (20 ± 2°C) and turned over onto a filter paper for drainage of released oil. Finally, the tubes were weighed (weight "c") again, and OBC was calculated with the following equation:

$$\mathrm{OBC}\,(\%) = 100 - \frac{[(b-a)-(c-a)]}{(b-a)} \times 100.$$

Thermal behavior

The thermal analysis was performed in a differential scanning calorimeter (DSC TA Instruments, New Castle, DE). 10–15 mg of sample was hermetically sealed in aluminum pans and placed in the DSC. Samples were kept at 25°C for 1 min for stabilization in the DSC and then melted at 5°C/min to 90°C, kept at 90°C for 15 min to erase crystalline memory, and cooled to −60°C at 5°C/min to allow for complete crystallization. Samples were held at −60°C for 30 min and melted to 90°C at 5°C/min. The peak temperature obtained from the last melting step was used to quantify the melting point (Nassu & Gonçalves, 1999).

Microstructure of oleogels

The polarized transmitted light microscope used to obtain the crystallized microstructure of the oleogels was an Olympus BX51 (Japan), attached to a QImaging digital camera (Media Cybernetics, USA), which transmitted live images to a computer using the software Image-Pro Plus 7.0 (Media Cybernetics, USA). The crystal images were magnified 10×. For sample preparation, at 25°C, an aliquot of crystallized sample was taken from the cylinders tubes and placed between a slide and cover slide. The sample was then covered with a coverslip, resulting in a thin film of oleogel. The evaluation parameter selected for quantitative image analysis was the mean diameter of crystals (Dm). Five images of each sample were measured using Image Pro-Plus software version 7.0 for Windows.

Ice cream preparation and evaluation

The oleogels were tested in the ice cream formulations as preliminary studies. Based on an input of a sensorial panel, the soybean oleogel with 6% was chosen to be applied in the final ice cream formulations. A control vanilla ice cream was prepared with 10% milkfat (prepared from melted butter in the laboratory), 11% skim milk solids, 10% sucrose, 5% corn syrup solids, 0.15% guar gum, 0.02% carrageenan (Local grocery stores), 0.15% Grindsted® Mono-Di HV 52 K-A (Danisco Inc., Scarborough, ON, Canada), 0.2% vanilla aroma (Badia Spices Inc., Doral, FL, United States) and the balance with water.

The mix was prepared by heating the water and premixed with skim milk solids, sucrose, corn syrup, and the gums (carrageenan and guar). The Grindsted® Mono-Di HV 52 K-A and the fat source (milk fat or oleogels) were incorporated at 70°C and allowed to melt completely before pasteurization. The mixes were pasteurized at 155°F (68.3°C) for 30 min. The mixes were pasteurized in a VAT (batch container) system. In the VAT the mix was heated and held throughout the holding period while being agitated allowing the dissolution and blending of ingredients during the heating stage. Then the mixes were cooled in the VAT and homogenized using a Fisherbrand™ 850 homogenizer (Fisher Scientific International, Inc.) at 10,000 rpm per 5 min. The mixes were cooled and aged for 24 h at 5°C in the refrigerator (Kenmore 79432, IL, USA). The freezing protocol used was the batch freezing process. 1.5 L of the mixes were frozen in a Cuisinart® Supreme® Commercial Quality Ice Cream Maker (Cuisinart, Stamford, CT, USA). The mixes were churned in the ice cream maker bowl to frozen for 10–12 min. Ice cream was collected in 180-ml foamed plastic containers for meltdown measurements, in a 250-ml beaker for the overrun analysis and 2-L plastic containers for other analyses and storage in a freezer under −20°C (Kenmore Elite 27,003, IL, USA). Twenty four hours before analysis, the ice creams were transferred to a freezer (Kenmore 22202, IL, USA) at −10°C.

All mixes were prepared in triplicate. The ice cream with oleogel was produced with the same formulation except for the fat component. A 50% fat substitution sample was used 50% of the oleogel (SBO + 6% CBW) + 50% of milk fat were used. For a sample with

100% of fat substitution, 100% oleogel (SBO + 6% CBW) was used in the formulation to replace milk fat.

Total solids

Total solids were determined after 2 weeks of storage by the forced-draft oven method (15.10C) in Standard Methods for the Examination of Dairy.

Oil content

The oil content was determined after 2 weeks of storage by the Soxhlet method (AOAC, 2007) by extraction using hexane.

Overrun

Overrun was measured immediately after batch freezing a beaker (250 ml) carefully with the ice cream, and comparisons of the weight of the original ice cream mix allow calculation of the overrun. Overrun (in %) was analyzed in triplicate and calculated by the following formula:

$$\mathrm{OR}\% = \frac{(\text{weight of ice cream mix} - \text{the weight of frozen ice cream})}{(\text{weight of frozen ice cream})} \times 100.$$

Melting rate test

After freezing the ice cream, the samples were placed in foamed plastic cups (180 ml) in triplicate. The melting rate was determined by carefully cutting the foamed plastic cups from the ice cream samples after 48 h in the freezer, placing the ice cream onto a wire mesh over a beaker, and weighing the amount of ice cream drained into the beaker at 21 ± 0.5°C every 10 min. Melting profiles were plotted as the ratio of all drained ice cream weight to the weight of the original sample versus time. The data collected during a period of relatively constant draining was regressed to determine the overall rate of drainage for each ice cream sample (Prindiville et al., 2000).

Sensory evaluation

The analyses were carried out in the Laboratory of Sensory Evaluation of Foods in the Department of Family and Consumer Sciences of North Carolina A&T State University (NCAT). The panel was formed by students and staff of the University, recruited through e-mails, flyers, and notices in classes. A total of 51 panelists evaluated the overall acceptability (OAA), the acceptability regarding appearance, texture, and flavor of three samples of vanilla ice cream (control, 50%, and 100% fat substitution with carnauba wax oleogel). The formulations of ice cream were evaluated through a structured 9-point hedonic scale, ranging from "extremely disliked" to "extremely liked" (Stone & Sidel, 2004) in a single section, in a monadic form. On the day before (24 h) the sensory analysis, the ice cream samples were tempered in the common freezer at −10 ± 2°C. To minimize differences between the sample preparation, ice cream were scooped with the panelist in the booth. The panelists received the samples in a temperature range between 5 and 10°C. All the samples were presented using a balanced complete block design (Wakeling & Macfie, 1995). The subjects were instructed to rinse their mouths out with filtered water between samples to avoid the "cold" carry-over effects (Guinard et al., 1997). No information about the samples was given to the consumers to prevent bias (Thompson et al., 2009). The sensory evaluation was performed in individual cabins under red artificial lighting, temperature control (between 22 and 24°C), and air circulation. Ice cream samples were presented to consumers on a disposable cup containing around 20 g of the product (one scoop). "Compusense software version 5.0 (Compusense Inc, Canada) was used for sensory evaluation, data collection, and statistical analysis.

Statistical analysis

The analysis of variance ANOVA and Tukey's test were applied in the physical properties analysis and for the sensory data. The ANOVA was conducted to determine the differences between means and the level of confidence was at least 95% for all. Statistical analyses were done with Graph Prism software (San Diego, CA, USA).

RESULTS AND DISCUSSION

Oleogel characterization

Visual evaluation

In order to acknowledge a qualitative perception of the crystal network formation, Figure 1 shows the images taken of the inverted sample tubes containing the

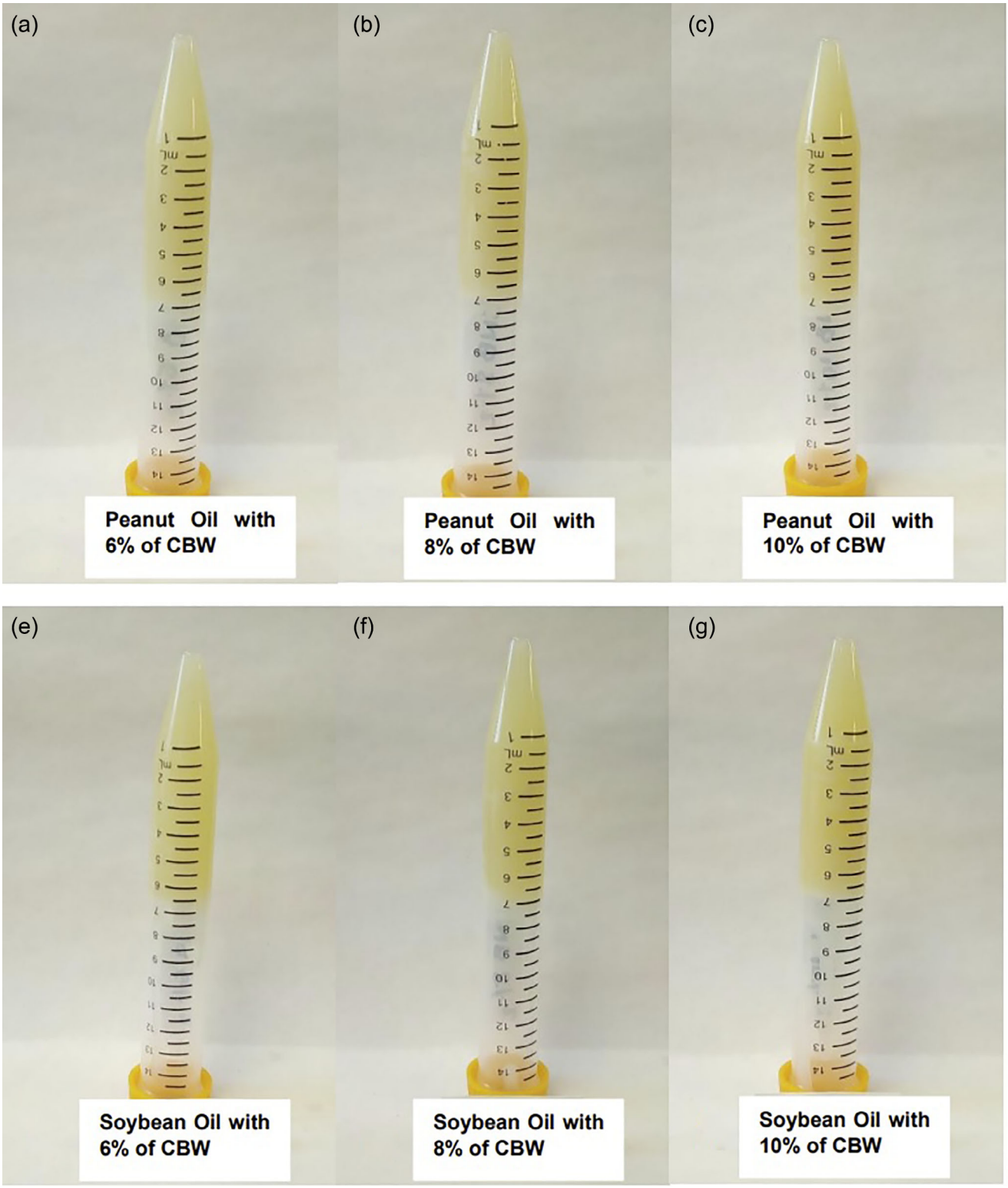

FIGURE 1 Visual analysis performed on day 60 at temperatures of 25°C—(a) peanut oil and 6% carnauba wax, (b) peanut oil and 8% carnauba wax, (c) peanut oil and 10% carnauba wax, (d) soybean oil and 6% carnauba wax, (e) soybean oil and 8% carnauba wax, and (f) soybean oil and 10% carnauba wax

oleogels of SBO and PNO made with CBW (6%, 8%, and 10%) at 25°C after 60 days. The SBO and PNO oleogels studied resulted in a firm and stable gel throughout the analysis period, regardless of the concentration of CBW and temperature studied. Furthermore, based on the Godoi et al. (2019) classification for

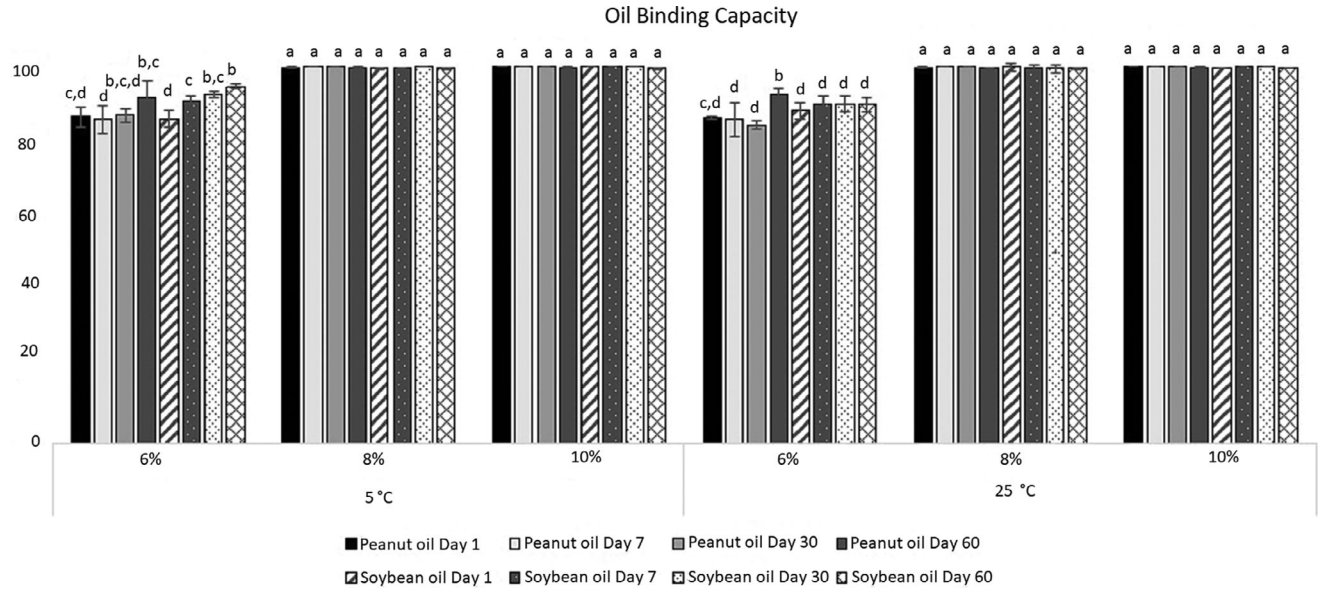

FIGURE 2 Oil binding capacity of SBO and PNO oleogels structured with 6%, 8%, and 10% of CBW stored by 1, 7, 30, and 60 days under 25°C of temperature

oleogels, all the studied samples were classified as firm (category 5). Therefore, the CBW in all percentages was able to solidify the SBO and PNO, producing a material that does not flow when the recipient content is poured or subject to gravity force. Moreover, the oleogels were extremely stable under different temperatures (5 and 25°C) and along the time of storage (60 days). Buitimea-Cantúa et al. (2021) observed the same behavior using different types of CBW to structure SBO in concentrations above 4.5% w/w, which formed solid oleogels also classified as firm (category 5). CBW is known to contain long-chain fatty alcohols (Doan et al., 2017), which may justify the visual results in PNO and SBO oleogels. The study by Valoppi et al. (2016) examined the ability of fatty alcohols with chain lengths from C_{14}OH to C_{22}OH to gel peanut oil. As the fatty alcohol chain length increased, the systems became stronger, exhibiting smaller crystal aggregates.

Oil binding capacity (OBC)

Due to the high liquid oil content in oleogels, the control in the reduction of oil release is essential to the stability, texture, and spreadability of the food products. Thus, the OBC is crucial to determining the applicability of the oleogel (Co & Marangoni, 2012), representing both the oil structuring capabilities of the oleogels network for each formulation and the gel stability (Okuro et al., 2018).

As shown in Figure 2 the OBC increased with the increase in the amount of CBW for both oils (SBO and PNO). Using 6%, CBW showed the lowest OBC in both oils and temperature tested. The OBC for those samples were 86.8 ± 2.7% (PNO) and 86.2 ± 2.3% (SBO) under 5°C and 86.6 ± 0.4% (PNO) and 88.5 ± 2.5% (SBO) under 25°C. These results were in agreement with previous studies for CBW oleogels 85.28 ± 3.19% (Aliasl khiabani et al., 2020) and 91 ± 3% (Shi et al., 2021).

It is important to note that, even without differences in the visual evaluations, the oleogels containing 6% CBW, at the two analysis temperatures (below 5 and 25°C), presented significantly ($p < 0.05$) lower OBC in comparison with oleogels with a higher concentration of the structuring agent (8% and 10%). Considering the results of oleogels with 6% CBW under 5°C, the OBC of the PNO oleogel was lower than SBO only on the seventh day of analysis and was equal in the other periods. At 25°C, only on the sixtieth day of analysis, the OBC of SBO oleogel was lower than PNO.

Even though most of the OBC results did not show statistical differences between the samples, the differences mentioned above probably occurred due to the different fatty acid compositions of the oils that are extremely important to determine the crystal structures and physical properties of the oleogels (Patel, 2015; Zhao et al., 2020). According to Patel (2015), gelling is significantly affected by the levels of saturated and unsaturated fatty acids in oils, the concentration of structuring agents, and processing conditions.

The increment of 2% of CBW in oleogels formulations led to a significant increase in the OBC of oleogel samples ($p < 0.05$). The OBC value in day 1 of oleogels with 8% CBW were 99.9 ± 0.1% (PNO) and 99.6 ± 0.2% (SBO) under 5°C and 99.9 ± 0.01% (PNO) and 100.0 ± 0.0% (SBO) under 25°C, with no

TABLE 1 Melting profile of the PNO and SBO oleogels structured with 6%, 8%, and 10% of CBW in two melting cycles

	Melting cycle 1			Melting cycle 2					
Samples	Onset T_{on} (°C)	Peak T_p (°C)	Enthalpy ΔH_m (J/g)	Onset 1 T_{on} (°C)	Peak 1 T_p (°C)	Enthalpy 1 ΔH_m (J/g)	Onset 2 T_{on} (°C)	Peak 2 T_p (°C)	Enthalpy 2 ΔH_m (J/g)
PNO									
CBW 6%	70.3 ± 0.5	79.1 ± 0.2	7.0 ± 0.1	−21.8 ± 0.0	−12.0 ± 4.7	64.3 ± 0.3	65.5 ± 0.1	74.5 ± 0.3	7.8 ± 0.2
CBW 8%	70.5 ± 0.1	79.1 ± 0.2	7.7 ± 0.4	−21.9 ± 0.0	−12.1 ± 0.1	65.5 ± 0.1	65.6 ± 0.2	74.9 ± 0.3	10.1 ± 0.1
CBW 10%	74.5 ± 2.8	81.5 ± 0.2	9.3 ± 1.2	−22.2 ± 0.0	−12.0 ± 0.3	65.1 ± 1.9	66.4 ± 0.1	76.1 ± 0.2	11.8 ± 1.0
SBO									
CBW 6%	71.6 ± 1.9	78.4 ± 0.1	5.6 ± 0.2	−30.3 ± 0.2	−27.3 ± 0.4	54.0 ± 3.0	64.7 ± 0.9	74.2 ± 0.6	9.7 ± 3.2
CBW 8%	72.2 ± 1.8	79.6 ± 1.4	8.0 ± 1.2	−30.1 ± 0.0	−27.1 ± 0.1	51.2 ± 4.3	66.1 ± 0.2	75.4 ± 0.3	10.9 ± 2.0
CBW 10%	70.1 ± 1.4	79.1 ± 0.4	9.6 ± 0.3	−30.2 ± 0.1	−27.3 ± 0.1	47.8 ± 2.9	66.8 ± 0.3	76.1 ± 1.6	12.3 ± 0.2

significant difference ($p < 0.05$) for oleogels containing 10% of CBW. Depending on the amount of structuring agent used, oleogels may have a certain amount of oil weakly bound to the gel. These results indicate that with the rise in CBW above 8%, the crystalline phase was sufficient to develop a strong network enough to hold more than 99% of liquid oil into a gel-like structure compared with oleogel prepared with 6% of CBW. Even though presenting a lower OBC, 6% of CBW was also efficient to entrap more than 84% of the liquid oils in the tridimensional oleogel network. This behavior was similar in both oils used (PNO and SBO), with no statistical differences ($p < 0.05$) between the oils. The high OBC of CBW was previously attributed to the wax's high melting point and chemical composition. The high content of wax esters (around 60%) was positively correlated with the OBC, but fatty alcohols content was not, and normally CBW with 30% or lower showed a better OBC (Doan et al., 2017).

Thermal behavior

Table 1 shows DSC melting curves of PNO and SBO oleogels prepared with different ratios of CBW (6%, 8%, and 10%). The DSC thermograms show the melting behavior of oleogels in two melting cycles. Firstly, the oleogel sample was kept at 25°C for 1 min and then heated at 5°C/min to 90°C (Figure 3a,c). In the second cycle, the oleogels samples were heated from −60 to 90°C at 5°C/min to record the thermal behavior of vegetable oils (Figure 3b,d). The values of onset temperature (T_{on}), melting peak temperature (T_m), and melting enthalpy (ΔH_m) of the endothermic peaks are listed in Table 1. As observed in Figure 3a, the PNO oleogel structured with 6% CBW presented a melting peak at 79.1 ± 0.6°C and ΔH_m of 7.0 ± 0.1 J/g, a similar value was observed for SBO oleogel (Figure 3c), which presented a melting peak of 79.6 ± 1.4°C and ΔH_m of 5.6 ± 0.2 J/g. Similar T_p (80°C) was also observed for a 6% CBW-SBO oleogel (Aliasl khiabani et al., 2020). These endothermic peaks are associated with the saturated long-chain monofunctional waxes esters and fatty alcohols in CBW that melts around 82°C (Doan et al., 2017; Galvão et al., 2020; Talens & Krochta, 2005). Although no change in T_{pm} related to CBW concentration was observed, an increase of 30% in the enthalpy was observed with the rise of the CBW ratio for both oils (Figure 3a,c). The same behavior was noted in Wang et al. (2019) study with oils based on algae oil and monoacylglycerols and Zhao et al. (2020) with oleogels based on corn oil and rice bran wax.

The second melting cycle (Figure 3b,d, Table 1) enables the observation of two prominent endothermic peaks corresponding to the melting peaks of CBW and the liquid oils. An intense melting peak was evident in temperatures ranging from −40 to −10°C for both oils in all CBW ratios. In Figure 3b, the PNO oleogels presented a major endothermic transition at about −12°C and a smaller peak around 75°C. The first peak is characteristic of a PNO oil (Fasina et al., 2008), and the second peak has similar behavior to the first melting cycle, suggesting the melting of the CBW. Interesting, the parameters in Table 1 for the melting of the second peak were significantly different in melting cycle 2. T_p and T_{ons} were dislocated to a lower temperature ($\Delta T \sim 5$°C).

On the other hand, the enthalpy was slightly higher, mainly in higher amounts of CBW. The enthalpy ($\triangle H_m$) can be used as a parameter to estimate the amount of crystalline mass in oleogels and can be influenced by the concentration of the structuring agent (Sun et al., 2021; Zhao et al., 2020).

This suggests that when the oil crystallizes (negative temperatures), it might form a stronger bond with the CBW, resulting in a more crystalline material being formed and a more connected network of oleogel that melts at a lower temperature. The dislocation of the melting peak to lower temperatures was previously described as a more co-crystallized network for many

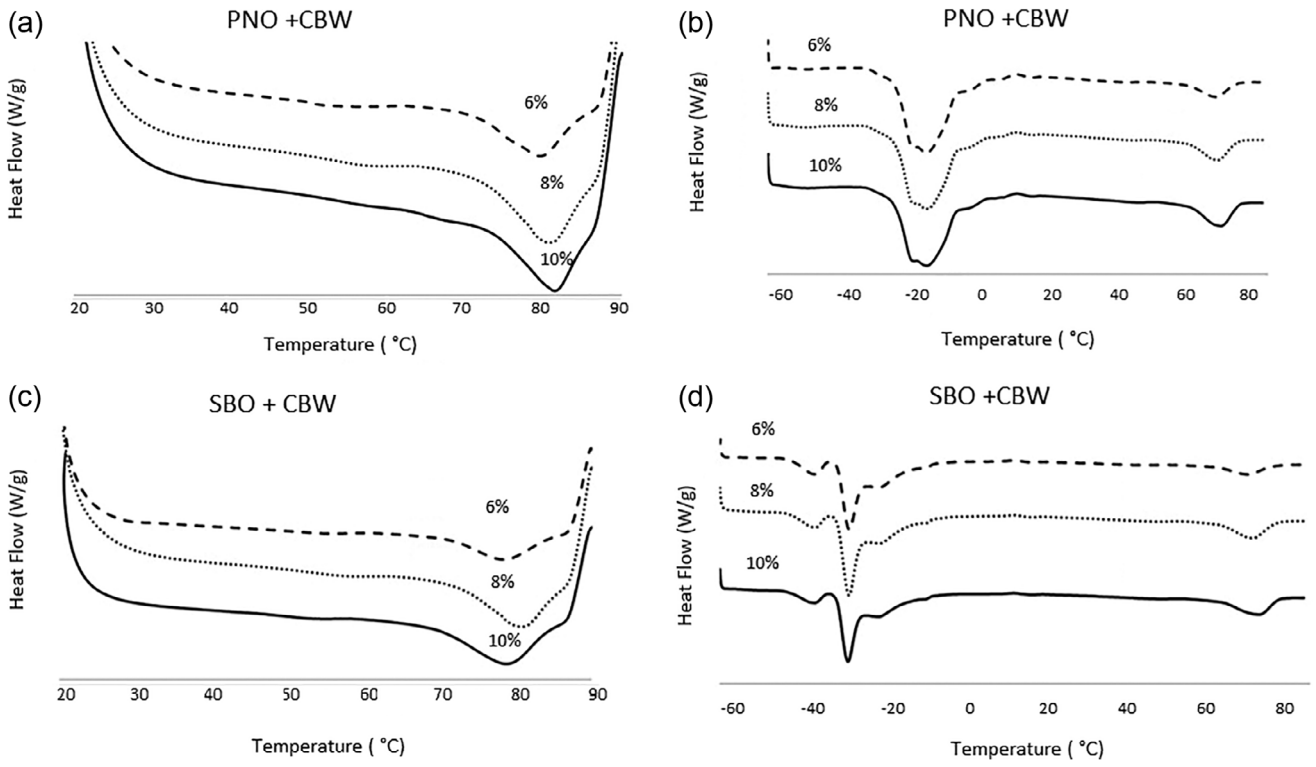

FIGURE 3 DSC melting curves for SBO and PNO structured with 6%, 8%, and 10% of CBW

wax-oleogel systems (da Silva et al., 2019; Doan, Tavernier, Okuro, & Dewettinck, 2018; Ramírez-Gómez et al., 2016) and was attributed to the nucleation promoted by the wax and further interaction with the components of the oil. This well-entrapped network formed is exciting since the final application of the material would be a frozen product (ice cream), and the lower melting point would be very beneficial for mouthfeel and sensory acceptance.

The SBO oleogel presented a prominent endothermic peak at −27°C, a lower temperature than in PNO-oleogel, which was expected due to the high amount of polyunsaturated fatty acids in the SBO compared to the PNO (Fasina et al., 2008). Besides the lower T_{pm}, this peak presented a small shoulder at a lower temperature (−36°C), which can be attributed to the melting of triunsaturated TAG, namely LLL, LLLn, and LLnLn (Lerma-García et al., 2011; Tan & Man, 2002). The main peak of SBO oleogel, around −27°C, can be associated with the melting of OLL, PLL, PLL, OOL, and POL/SLL triacylglycerols in SBO composition (Tan & Man, 2002). Likewise, PNO's last endothermic peak for the SBO thermogram is about 75°C, can be associated with the CBW melting peak, and no differences due to the type of oil were found in this peak. A similar profile was observed for other waxes oleogels in different oils (Holey et al., 2021). The similarity of the dislocation of the second melting peak to lower temperature among both oils suggests that the change in the network formed in the second cycle might be attributed to the saturated fatty acids of both oils that are present in similar proportions (∼15%, topic 2.1). A decrease in unsaturation in oil decreased the melting temperatures (T_{ons} and T_{pm}) values, which indicate that oil saturation assisted the faster gel formation with an increase in the homogeneity (Holey et al., 2021).

Microstructure of oleogels

Figure 4 presents the images of crystallized PNO and SBO oleogels under polarized light microscopy (PLM) at 25°C. In these PLM images, wax crystals generally appear as white, bright scenes, while liquid oil is observed in dark surroundings (Yilmaz et al., 2021). Previous studies have shown that the microstructure of wax-based oleogels consisted of platelet-like spherulites and clustered crystal aggregates along with one-dimensional needle-like crystals. The immobilization of the liquid oil into a three-dimensional structure is due to the van der Waals interactions of crystals and crystalline aggregates (Fayaz et al., 2017). The CBW 6% oleogel sample (Figure 4a) exhibited platelet-like crystals in tiny aggregation forms with a diameter of 1.92 ± 0.15 μm for SBO and 1.73 ± 0.12 μm for PNO oleogels. These results align with a previous study of CBW wax-based oleogels (Doan et al., 2016). The rise of the CBW ratio (8% and 10%) forms an aggregation of the needle-like crystals from a nucleation center with several clusters that form large crystal structures (Bin Sintang

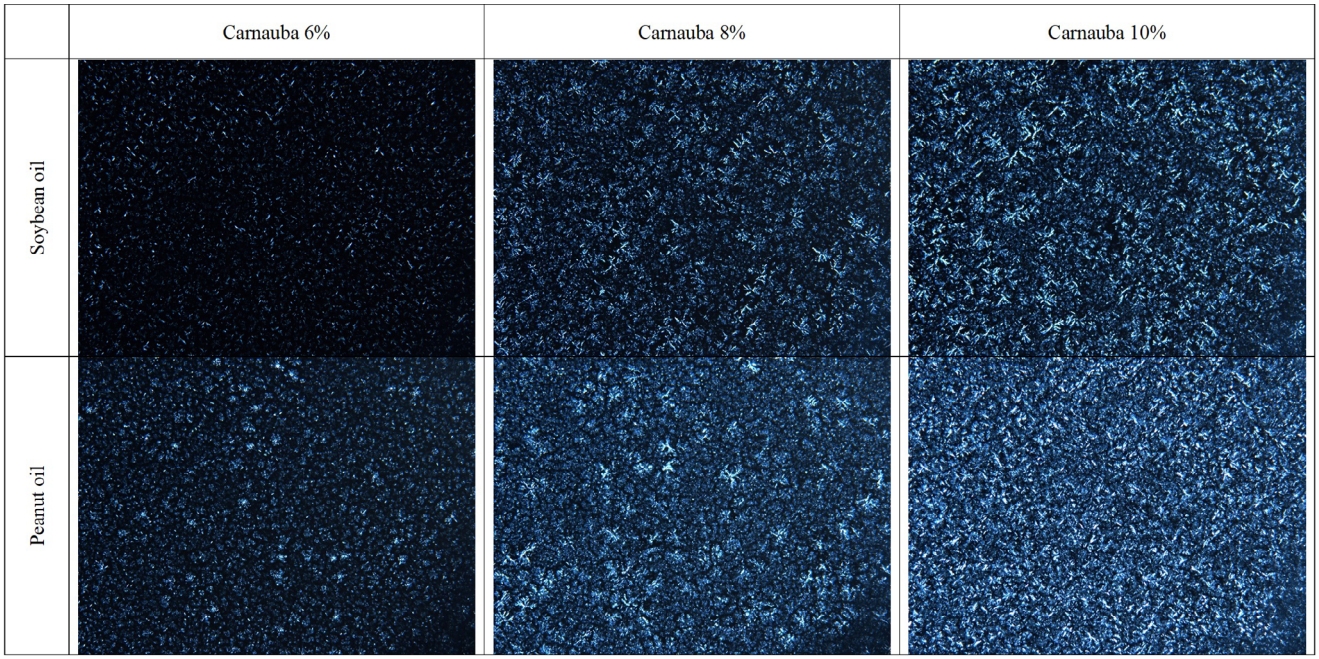

FIGURE 4 Polarized light microscopic images of SBO and PNO structured with 6%, 8%, and 10% of CBW under 25°C

TABLE 2 Diameter (μm) number of crystals and area (%) crystallized of PNO and SBO oleogels structured with 6%, 8%, and 10% of CBW under 25°C

	Diameter mean (μm)	Number of crystals	Area (%)
PNO			
6% CBW	1.73 ± 0.12	5997 ± 781	0.36 ± 0.10
8% CBW	1.82 ± 0.12	7720 ± 1058	0.58 ± 0.19
10% CBW	2.16 ± 0.17	7260 ± 694	0.76 ± 0.23
SBO			
6% CBW	1.92 ± 0.15	4609 ± 699	0.34 ± 0.12
8% CBW	1.79 ± 0.10	6379 ± 819	0.39 ± 0.11
10% CBW	2.08 ± 0.22	5105 ± 1124	0.48 ± 0.24

et al., 2017), demonstrating a growth in the diameter mean and in the crystalized area (Table 2, $p < 0.05$). This increase promotes gelation within the continuous network, achieved by cross-linking crystals and oil trapping. According to Dassanayake et al. (2009), fiber-like tiny needle crystals form stronger, hard gels with higher oil binding capacities, which correspond to the results obtained in this study (Figure 4) for all the concentrations of CBW in the PNO and SBO oleogels. The higher CBW concentration (Figure 4) exhibited a denser crystal network with stronger birefringence.

Ice cream characterization

Total solids, oil content, and overrun

After a preliminary sensory test, the panelists expressed an intense wax mouthfeel in ice creams prepared with oleogels containing 8% and 10% CBW. Since our study's primary purpose and novelty was to evaluate the sensory trial and acceptance of ice cream using oleogel to reduce saturated fat, the soybean oleogel with 6% CBW was chosen as a fat source. Some previous studies (da Silva et al., 2018; Silva-Avellaneda et al., 2021) demonstrated that oleogels with higher OBC, higher hardness, and viscoelasticity formed the worst filling for sandwich cookies (low structuration product) (Silva-Avellaneda et al., 2021). Moreover, softer oleogels with higher OBC formed the better-structured filling. These authors have shown that oleogels behave differently in process or when in a food matrix. Hence, being necessary to test the oleogels even though sometimes the gel is not perfect.

Table 1 shows the total solid, fat content, and overrun of the three ice cream formulations. Although the solid and fat content results are not totally in accordance with the formulation percentages, it shows the

TABLE 3 Total solids and oil content obtained for ice cream samples (control, 50% and 100% oleogel)

	Control	50% CBW	100% CBW
Total solids	33.9 ± 0.02^{c}	34.4 ± 0.13^{b}	35.2 ± 0.23^{a}
Oil content	13.2 ± 0.98^{a}	13.9 ± 0.92^{a}	10.9 ± 1.82^{a}
Overrun	54.8 ± 1.40^{a}	37.7 ± 1.18^{c}	48.2 ± 0.69^{b}

[a,b,c]For all variables with the same letter, the difference between the means is not statistically significant ($p < 0.05$).

composition of the triplicate formulations mixes and reflects the real composition of the ice creams prepared. These discrepancies in the formulation are possibly due the moisture content of the milk fat and skim milk powder that were not taken into consideration. In this study, the total solid content of the formulations falls within the minimum recommended by the FDA of 20% (FDA, 2016). However, the total solids present in the control sample was 33.9 ± 0.02 g/100 g (Table 3) which was significantly lower ($p < 0.05$) than the 50% of fat substitution (34.4 ± 0.13 g/100 g), and 100% of fat substitution (35.3 ± 0.23 g/100 g). These slight differences probably can be best explained by the different fat sources used in the ice cream mix.

Both the control sample and the 50% CBW sample had a slightly higher lipid percentage when compared to the 100% CBW sample. However, there was no statistical difference between the samples. This performance may be related to the use of milk fat since both that used this ingredient had similar percentages, and the sample that presented 10% of lipids used only oleogel in the formulation.

In order to be labeled as "ice cream," the FDA requires that a product has a minimum of 10% dairy milkfat, has no more than 100% overrun, and weighs no less than 4.5 lbs per gallon (FDA, 2016). Overrun is the % increase in the volume of ice cream than the amount of mix used to produce that ice cream. The lower the overrun percentage, the more dense the product. Products that do not meet this standard cannot be labeled as ice cream, and they are called "frozen dairy desserts." Either they do not meet the 10% milkfat requirement, or they are churned and fluffed up with so much air that they exceed the 100% overrun limit. Besides the "regular" ice cream, the industry also classified this dairy product as "premium" ice cream, when the milkfat percentage exceeds the minimum (12%–14%), and generally has a lower overrun (90%–50%) and the "superpremium" ice cream which the milkfat percentage is between 14%–16% and with an overrun under 50%.

The control ice cream met the requirements of the FDA for ice cream, presenting 10% of milk fat and 54.8% overrun (Table 3), and could be classified as premium ice cream. However, the "ice creams" formulated with oleogel are designed as frozen desserts due to the amount of milk fat. The use of 50% of CBW oleogel reduced the incorporation of air in the frozen dessert compared to the control. Thus, it can be concluded that the use of CBW oleogel accounted for a decline in ice cream quality characteristics with respect to the amount of incorporated air. This result can be related to the higher ratio of solid: liquid fat attained during mix aging, as reported by Goff (, 2006a; 2006b), a partially crystalline emulsion is needed for partial fat coalescence in the whipping and freezing steps, leading to air bubble stabilization and, as a consequence, to higher overrun and melting resistance. In the study by Zulim Botega et al. (2013a, 2013b), the overrun of ice cream samples made with oleogels was more significant than the control sample made with liquid oil without structuring; however, it was not as high as that found for the control of milk fat. This fact suggests that aeration and air stability are improved with the use of wax in the oil but was not fully promoted by the gelled oil compared to crystalline fat. The use of 50% of oleogel in the milk fat resulted in an even lower overrun ($37.7 \pm 1.1\%$) than the ice cream containing 100% CBW oleogels ($48.2 \pm 0.6\%$).

Melting rate

The main factors affecting structural collapse during ice cream meltdown are the level of fat agglomeration, ice crystal size, and the size of the fat agglomerates (Muse & Hartel, 2004). The network of partially coalesced fat agglomerates stabilizes the air bubbles. However, it also increases the flow resistance of the serum phase during ice melting, decreasing the meltdown rate. Furthermore, increasing the size of ice crystals increases the melting rate rather than increasing the number of ice crystals (Muse & Hartel, 2004). Melt rate analysis can be found in Figure 5, and the melt resistance capacity is shown in Figure 6.

The melt resistance capacity can confirm this data as shown in Figure 6, where it is possible to observe that both 50% CBW and 100% CBW samples presented higher melt resistance when compared to the control sample formulated with milkfat after 40 min of analysis. In contrast, the control sample showed $89.06 \pm 3.0\%$ melted after 90 min), and the samples with 50 and 100% CBW showed 79.87 (3.6%, and 78.61 (9.8%) melted, respectively, after the same period.

Zulim Botega et al. (2013a, 2013b) reported the same behavior using rice bran wax (RBW) oleogel, which incorporated less air than the control sample made with milk fat, justified by the lack of interaction with the air interfaces or the lack of interaction between the drops, but fat replacement represented a higher melting rate. However, in our study, the samples had a

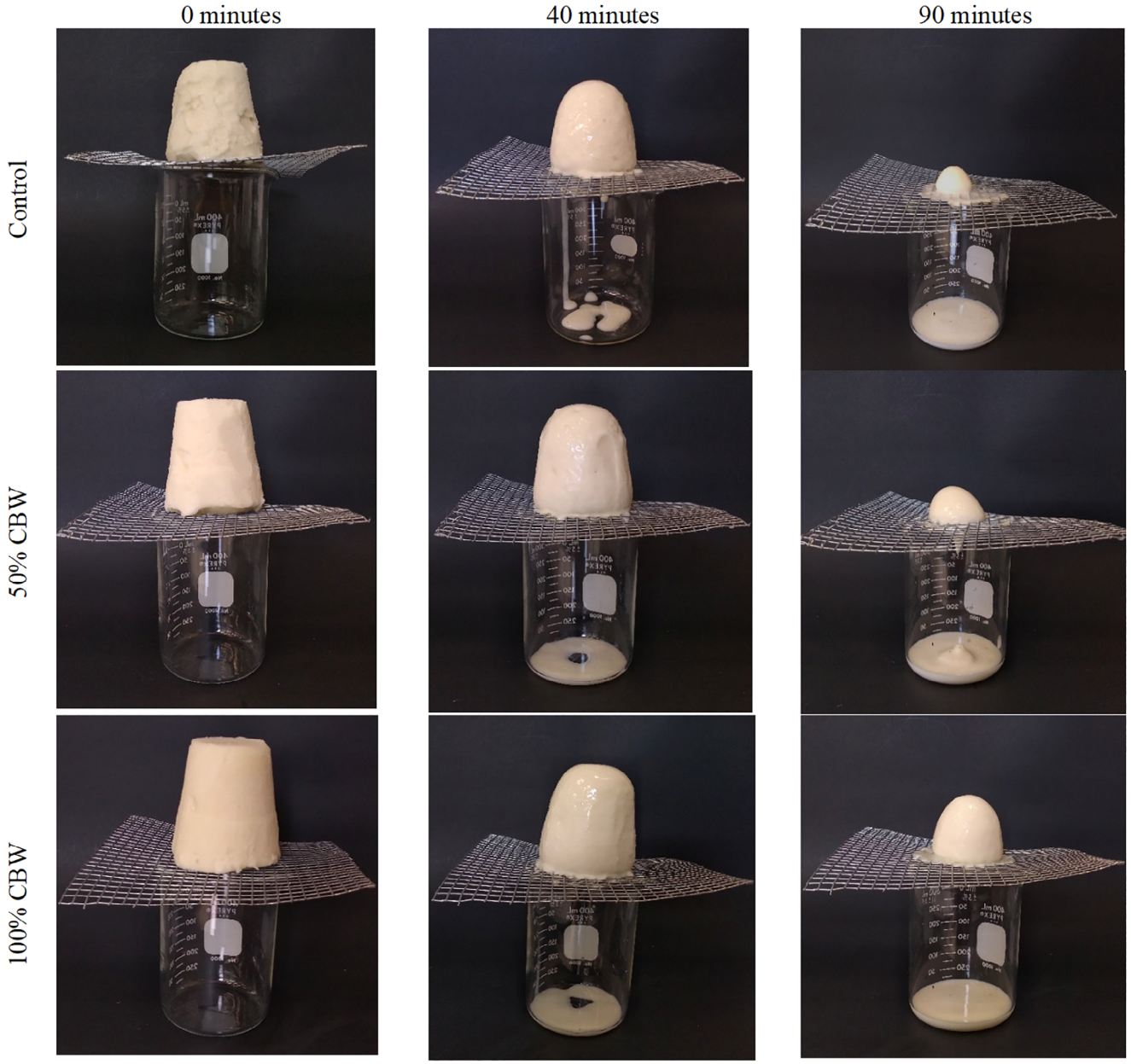

FIGURE 5 Melt rate of the sample of ice cream with milkfat and the samples with the fat substitution for 90 min

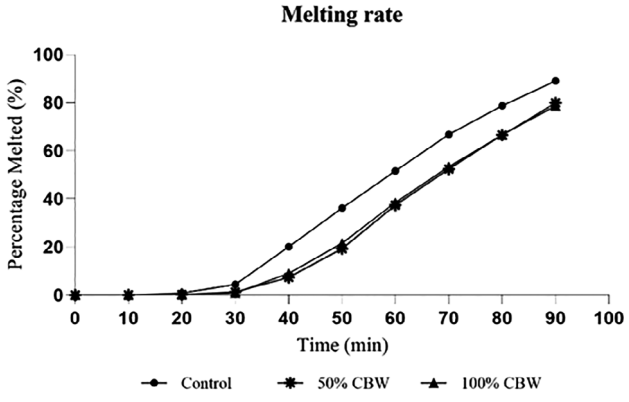

FIGURE 6 Melt rate profile for ice cream samples

slower melting rate. This result may be related to the high melting point of carnauba, around 82°C (Tinto et al., 2017).

Ice cream sensory evaluation

Sensory evaluation is extremely important for analyzing a product that has its formulation modified because the nutritional quality can be improved, but the product may not meet consumer expectations. Table 4 summarizes the sensory analysis results of the ice cream (control) and the samples with 50% and 100% fat substitution. The "flavor liking" and

TABLE 4 The consumer test results of ice cream (control) and the samples with fat substitution (50% and 100%)

	Control	50% fat substitution	100% fat substitution
Overall liking	7.10 ± 1.54^{a}	6.39 ± 1.76^{ab}	5.88 ± 1.82^{b}
Appearance liking	7.55 ± 1.10^{a}	7.24 ± 1.42^{a}	7.16 ± 1.41^{a}
Texture liking	6.69 ± 1.84^{a}	6.12 ± 2.08^{a}	5.98 ± 1.78^{a}
Flavor liking	7.04 ± 1.64^{a}	6.31 ± 1.94^{ab}	5.80 ± 1.95^{b}

Note: Significance level $p < 0.01$. ^{a,b}For all variables with the same letter, the difference between the means is not statistically significant ($p < 0.05$).

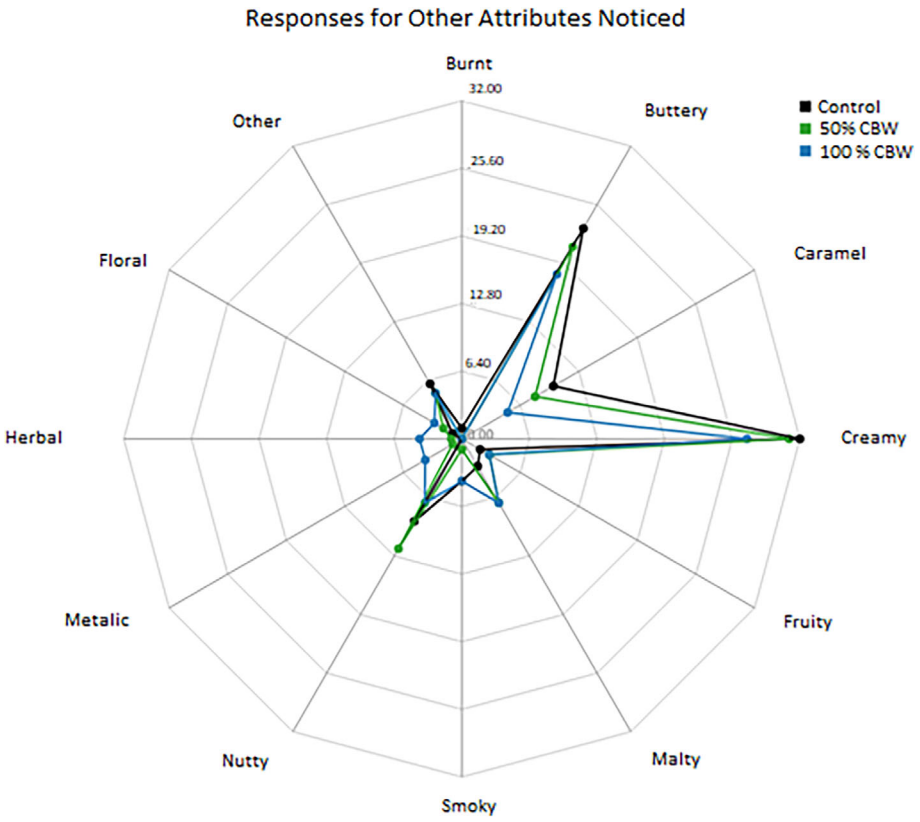

FIGURE 7 Responses for the other attributes noticed in the ice cream sample and the samples with the fat substitution

"overall liking" parameters distinguished the samples, as there was a statistical difference between the 100% CBW sample and the others, which was less accepted. These results show that the flavor is the main influence on the panelists' appreciation.

Twenty-one participants "moderately liked" the 100% CBW sample in the category of "overall liking," followed by participants preferring the 50% CBW sample. The 100% CBW sample obtained the lowest grades of all liking attributes, even though there was no statistical difference in appearance and texture from the other samples.

On the other hand, the 50% CBW sample matched the control sample in all parameters, which shows that the panelists demonstrated the same level of acceptance of these two samples. Da Silva et al. (2019) observed similar acceptability for wax-oleogel margarine, although the panelists could sense the 100% fat replacement for flavor and overall liking, the product's appearance was not significantly different for them (da Silva et al., 2018). It is essential to point out that even though the panelists were able to taste the difference in flavor, the majority of responses to the acceptability questions presented were between "moderate liked" and "very much liked," which shows that panelists liked the samples more than they disliked. In this study, even though no significant difference was observed between the control and the 50% CBW sample, it is important to point out that a decrease in the scores was noted for all attributes with the addition of the oleogel, which could be attributed to the wax-mouthfeel described by the other authors (Zetzl & Marangoni, 2012).

The parameter "appearance liking" was the best voted among the acceptability questions, which did not show any statistical difference between all samples. The 50% CBW sample was chosen most in the question of "liked very much," with 18 participants favoring

the appearance. The appearance of both the control and 50% CBW were pretty similar in "moderately" liked and "liked very much."

Even the three samples showed different results of overrun and total solids, factors that can influence the texture of the product, the "texture liking" parameter also did not present a significant difference ($p < 0.01$) between the samples, even though the control sample received 16 "liked very much" mentions from the participants.

The participants were asked to answer about other attributes they could notice in the samples Figure 7. The attribute "creamy" was the most noticed by panelists who mentioned 63% of responses for the control samples, 61% for 50% of the fat substitution sample, and 53% for the 100% fat substitution sample. Other attributes most mentioned for the samples are: "buttery," "nutty," and "caramel." The samples made with oleogels received fewer mentions of the "creamy" attribute, probably due to the high melting point of the CBW. The creaminess is an attribute of ice cream related to the amount of fat (Roland et al., 1999). The reduction in the amount of fat to produce low-fat ice cream was also followed by a decrease in the creaminess of the product (Roland et al., 1999; Warren & Hartel, 2014).

Emphasizing the attributes that were most referred to in the 100% CBW sample are "floral," "herbal," and "metallic," probably due to the aftertaste of the wax, a known drawback of the use of this structuring agent in food products. The waxy flavor was also observed with 100% wax-oleogel replacement in butter and margarine (Yilmaz & Ogutcu, 2015).

Although the waxes are very promising as structuring agents, they give oleogels an undesirable aftertaste (Kodali, 2014). This fact was also cited by Zetzl and Marangoni (2012) when they said that oleogels formed from waxes could leave a waxy sensation in the mouth, which would make the product unsuitable for the food industry. However, the result of this study shows that to improve the nutritional quality of the ice cream, the partial replacement (50%) of the traditional lipid phase by CBW oleogel can be performed without causing sensory impairment.

CONCLUSION

All oleogels resulted in a firm and stable gel for 60 days, regardless of concentrations (6%, 8%, and 10%) and temperatures (5 and 25°C). The OBC of the oleogels with 8% and 10% addition was significantly higher ($p < 0.05$) than the oleogels with 6% CBW. In addition, larger crystals (μm) were observed with the addition of 10% CBW. The replacement of oleogel fat (50% and 100%) reduced the melting rate of ice cream; however, it negatively affected the overrun of ice cream. In addition, the sensory evaluations showed that the ice cream with 50% fat replacement by CBW oleogel showed the same level of acceptance as the control sample. This study demonstrates that making ice cream using an oleogel as a cream substitute is possible without significant differences in sensory properties. Therefore, oleogels can be essential ingredients in searching for fat substitutes in ice cream production. As a next step, the authors suggest that ice cream mixes with oleogels as a fat source must be tested on a large scale with higher shear equipment.

AUTHOR CONTRIBUTIONS

Roberta Claro da Silva supervised the research, conceived and designed the study and provided the resources (Lipid Research Laboratory, North Carolina A&T State University). Rafaela Airoldi performed the research, analyzed the data and wrote the first draft of the manuscript. Salam A. Ibrahim and Heather L. Colleran analyzed the statistical data. Juliana Neves Rodrigues Ract and Aline Foguel carried out the microscopy analysis. Thais Lomonaco Teodoro da Silva carried out the thermal properties analysis and contribute to the final manuscript. All authors contributed to and approved the final draft of the manuscript.

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ETHICS STATEMENT

The sensory analysis was conducted after the IRB approval (IRB 019/19) and no animal subjects were used in this research.

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