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Development of Candelilla Wax Oleogels as a Medium of Controlled Release of Phosphorus in an In Vitro Model

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Abstract: Candelilla wax (CW) oleogels were developed as an alternative bolus material for delivering phosphorus into the rumen of goats. The systems were studied at three CW concentrations (10%, 15% and 20%) in vegetable oil. Each oleogel was added with a specific amount of calcium orthophosphate as a phosphorus source. The thermomechanical properties of the oleogels were characterized by DSC and rheology, and the viability of phosphorus delivery was evaluated using a laboratory model in two mediums (one aqueous and another with ruminal fluid). The results showed that the oleogels had a higher melting point (~52.8 °C) than the temperature of the ruminal environment and greater G′ values (1.6×10^6 Pa in 20% CW oleogel with phosphorus). Such characteristics guaranteed the integrity of the materials during the process in the model systems studied. It was demonstrated that when using the 20% CW oleogel, the phosphorus was slowly released for 84 h, completing a maximum of 83.3–98% of the total amount of phosphorus added to the bolus (in the aqueous and ruminal fluid models, respectively). These results enable us to propose this material as a delivery system for phosphorus supplementation in goats. Its effectivity in goats will be evaluated in future in vivo investigations.

Keywords: oleogels; candelilla wax; phosphorus; controlled release; goats; DSC; rheology

1. Introduction

Goats are ideal domesticated animals for farming in arid and semiarid areas because of their adaptability and eating habits. The Altiplano region of San Luis Potosi, Mexico produces 620,000 head of livestock, which amounts to 7.1% of national production and provides 3212 tons of meat per year [1,2]. In this region, goat production is carried out extensively under traditional management, in which goats graze on native vegetation and receive little to no supplementary feeding. In most cases, food is complemented with farm produce (mainly corn stubble and bean straw), and some herds receive common salt as their only mineral supplement [3]. On the other hand, in highly developed countries that supplement animal feed with high amounts of minerals, pollution increases because excess minerals are secreted [4]. For example, US dairy producers use feed that exceeds the *Nutrient Requirements of Dairy Cattle* dietary phosphorus recommendations by 45–50% [5,6]. For this reason, controlled mineral supplementation alternatives should be sought to avoid both mineral deficiency and overload. An alternative for mineral supplementation in

adequate amounts is the use of boluses (e.g., intraruminal bowel iron matrix or dental cement). The bolus's role is to support the targeted physiological functions of animals over a specific period. They are designed to release over periods where animals are at risk or to fulfill specific needs, such as to correct selenium (Se) deficiencies in sheep [7,8] or to supply copper oxide (AOCu) wire [9]. However, few studies have been conducted on the supplementation of macro elements such as phosphorus. Deficiencies of this mineral in goats have been associated with decreased rates of conception, increased abortions, low birth weight, lower feed consumption and higher offspring mortality [5,10]. Therefore, there is a need to efficiently supplement phosphorus to grazing livestock and thus cover their requirements (i.e., 11.6 mg/kg body weight/day) [11] in the most critical physiological stages: at the beginning of breeding season and during lactation [10]. Mineral supplementation is adequate if the animal's diet is marginal in a mineral but inadequate if that mineral is severely deficient. Unless a documented deficiency exists, it is best not to provide 100% of a mineral, because an excess may depress the absorption of another [12]. Solutions to this problem thus far, such as intraruminal boluses like iron matrices or dental cement, have been invasive to animals. Meanwhile, other non-invasive commercial supplements have proven ineffective and have been shown in some countries to not contain the phosphorus concentration indicated on the label, in addition to the fact that there is no control of the amount of supplement consumed by animals. Therefore, the problems associated with phosphorus deficiencies persist even when mineral supplements are provided to goats [13]. In the present work, a new alternative of boluses to slowly release minerals is proposed. It consists of an oleogel, a type of material that could be non-aggressive to phosphorus supplementation. An oleogel is a material made by the gelation of low molecular weight gelators (molecular weight < 3000 Da) with ambiphilic characteristics. Oleogelation can be achieved by initially dissolving a gelator by heating it in the organic solvent and then cooling the solution to a temperature below the solubility limit of the gelator. Under this condition, gelators such as n-alkanes [14], monoglycerides [15], 12-hydroxystearic acid [16,17], some amides [18], lecithin [19,20] and some plant waxes (e.g., candelilla wax, rice bran wax and carnauba wax) [21–24] go through spontaneous molecular assembly in vegetable oils and mineral oil [20,25–28]. Different studies have shown that oleogels are a viable alternative for saturated and trans fat substitution in food products [23,29–32] and cosmetic uses [33,34], and recently, they have been used for the controlled supply of substances such as minerals and pharmaceutical products, thus achieving a fluid exchange capacity with the environment [35–37]. For this reason, our hypothesis is that the physical and chemical characteristics of oleogels could enable the delivery of controlled amounts of phosphorus in the ruminal matrix. To validate this, we proposed to engineer slow-release phosphorus boluses made from oleogels using biological materials such as vegetable oil and candelilla wax (CW) for use as a phosphorus supplement for goats. In Mexico and the US, candelilla wax (from the *Euphorbia antisyphilitica* plant) is a generally recognized as safe (GRAS) material as described in the Code of Federal Regulations [38]. This wax is composed of a mixture of 7–9% free acids, 12–14% alcohols and sterols, 20–29% esters of acids and alcohols with even-numbered carbon chains (C28–C34) and 49–50% n-alkanes with 29–33 carbons [39]. Such molecular characteristics enable CW to self-assemble in vegetable oils and make self-standing structures, gelling liquid vegetable oil at concentrations as low as 1% or 2%. CW oleogels have well-characterized thermomechanical properties that report a melting temperature close to 42 °C [40–42] (the highest temperature at which it is present in ruminal liquid) and an average value of the elastic modulus close to 600,000 Pa (i.e., higher hardness than other recently evaluated bolus materials) [43]. For the above reasons, the objectives of the present work are to develop CW oleogels and evaluate their functionality as a medium of controlled phosphorus release in an in vitro model.

2. Materials and Methods

2.1. Materials

Candelilla wax (from the *Euphorbia antisyphilitica* plant) was provided by Multiceras (Monterrey, Mexico), safflower vegetable oil (Coral Manufacturer, San Luis Potosí, Mexico) was obtained from a local supermarket, and distilled water (no brand) was used. Reagent-grade calcium orthophosphate (CAS 7758-87-4, Fermont, Monterrey, NL, Mexico) was used as a source of phosphorus. The experiments were carried out in two stages, the first of which consisted of the design of the oleogels, their thermomechanical characterization and the evaluation of their liquid phase release capacity. In the second stage, the phosphorus release rate in an in vitro model in two mediums (one aqueous and another with ruminal fluid) was evaluated.

2.2. Oleogel Design and Preparation

The experimental design consisted of the evaluation of three different concentrations of CW—10%, 15% and 20%—and two levels of phosphorus concentration—0% and 3% (Table S1)—selected due to their thermomechanical properties according to previous experiments (results not shown). The oleogels were developed in 250 mL glass beakers to prepare 30 g of samples. The vegetable oil was heated to 80 °C using a heating plate with constant stirring, and then CW was added to prepare solutions at 10%, 15% or 20%, which continued to be heated until fully dissolved (~30 min). Separately, calcium orthophosphate (3%) was dissolved in the fraction of distilled water at room temperature and subsequently added to the CW solution in vegetable oil (at 80 °C) and stirred constantly for 5 min until a visually homogeneous mixture was reached. It should be mentioned that the amount of calcium orthophosphate solution that was added to the systems corresponded to a lower proportion than the one considered for the formation of w/o emulsions; therefore, the resulting material was considered an oleogel. The final mixture was poured into 50 mL Corning tubes, which were kept at room temperature until solidified (~30 min). Subsequently, they were stored at 5 °C until their analysis.

2.3. Thermomechanical Characterization of the Oleogels

2.3.1. Differential Scanning Calorimetry

The thermal profile of the samples was determined by a DSC (Discovery 2500; TA Instruments, New Castle, DE, USA). Between 15 and 20 mg of the samples were weighed and sealed in Tzero aluminum pans (TA Instruments, New Castle, DE, USA) and then equilibrated at 25 °C for 5 min. Afterward, the thermal setting was as follows. After 5 min at 25 °C, the melting thermogram of the samples was determined at 5 °C/min until it reached 90 °C. Then, the system was cooled at 10 °C/min to 25 °C with an isothermal stage for 10 min, and finally, a heating ramp was performed at 5 °C/min to 90 °C. In each case, equipment software (Trios V 4.4.1.41651; TA Instruments-Waters LLC, New Castle, DE, USA) was used to calculate the temperature at the maximum heat flow of the endothermic transitions (T_M) and the corresponding heat of transition (ΔH_M) using the first derivative of the heat flow.

2.3.2. Rheological Measurements

The rheological profile of the samples was determined using a Discovery HR3 rheometer (TA Instruments; New Castle, DE, USA) equipped with 40 mm parallel plate geometries (model 998203), with the top surface sandblasted to avoid slippage of the sample during measurements. The temperature of the sample was controlled with a Peltier system located at the base of the geometry and an upper heat plate. For rheological characterization, the samples were treated using the same thermal profiles for DSC analyses with some variations. The samples were applied to the base of the geometry preset at 40 °C (to simulate ruminal temperature conditions), and the upper plate was set to a gap of 1000 μm and an imposed normal force of 0.1. Strain sweeps, frequency sweeps and time sweeps were performed using the following test parameters. The rheological parameters were obtained

during the isothermal stage at 40 °C. The strain sweeps were performed using a frequency of 1 Hz in an interval ranging from 0.01% to 100%, collecting 5 points per decade; the yield point of the materials was calculated using the results of this measurement. A constant strain percent was applied in the frequency sweeps within the linear viscoelastic region (LVR) of the sample in an interval of frequencies from 100 to 0.01 Hz for measuring the storage modulus (G′) and loss modulus (G″). The time sweeps were performed at 40 °C from 0 to 1800 s, using the respective values of the strain and frequency within the LVR of the sample. This measurement was performed to identify the G′ and G″ behavior of the materials over time. The equipment was controlled using TRIOS V 4.4.1.41651 software (TA Instruments-Waters LLC, New Castle, DE, USA).

2.4. Determination of the Liquid Phase Release Capacity in Oleogels

The liquid phase release of oleogels made with CW and stored at room temperature (approximately 25 °C) for 10 days was evaluated using a technique similar to the one described by Dibildox-Alvarado et al. (2004). The oleogels were placed on previously weighed filter paper (Whatman no. 5, 125 mm in diameter), and once the analysis time had elapsed, the gel was carefully removed, and the paper was weighed again. For each sample, four repetitions were carried out. The percentage of liquid phase release was calculated according to Equation (1), with the liquid phase release (*LL*), filter paper weight (*fpw*), weight of filter paper plus oleogel (*fpw* + *g*) and weight of the oleogel after storage (*gwf*):

$$\%LL = ((fpw + g - fpw) - gwf)100 \quad (1)$$

2.5. Evaluation of the Release of Phosphorus from Oleogels in an Aqueous Medium

To evaluate the release of phosphorus from oleogels in an aqueous medium, a model system was designed to simulate the conditions of the rumen, which consisted of a 1000 mL beaker placed on a stirring hot plate (Corning). Distilled water previously conditioned at 40 °C at a pH of 6.5 (adjusted with concentrated hydrochloric acid) was added to the beaker. In the aqueous medium, the oleogel sample of approximately 3 cm × 3 cm × 6 cm was incubated and kept at a constant temperature (40 °C) and stirred (25 rpm) for 84 h. During the incubation period, 50 mL samples of the medium were taken in Corning glass tubes at zero hours and then every 12 h until completing 84 h. Subsequently, the samples were stored in refrigeration until their analysis. The phosphorus content was determined by spectrophotometry based on the method established in the NMX-AA-029-SCFI-2001 standard, using a MAPADA UV-1600/UV-1800 spectrometer at a wavelength of 470 nm. The results were obtained by comparison with a calibration curve of 0, 10, 20, 50, 100, 200 and 300 ppm of phosphorus made from a standard solution of 1000 ppm of phosphorus.

2.6. Evaluation of the Phosphorus Release Rate in Ruminal Fluid

The phosphorus release rates of the oleogels made with 10%, 15% and 20% CW in ruminal fluid were determined using the same design as the model in an aqueous medium. The ruminal liquid was obtained from a sacrificed goat one hour before its extraction, filtered through a straining cloth (textile filter) and then deposited in thermal flasks. CO_2 was added to keep them under anaerobic conditions. They were transported to the laboratory and placed in a water bath at 40 °C until use. Subsequently, the experimental models were filled with ruminal fluid, and the respective oleogels with 10%, 15% or 20% of CW with phosphorous were added to perform the bolus function. They were maintained at 40 °C and constantly stirred at 25 rpm. The flasks were monitored and opened every 2 h to release gas. Aliquots of 50 mL of the samples were extracted from each experimental unit at 0 h and every 12 h over the course of 84 h, then kept in refrigeration at 5 °C until analysis.

2.7. Quantification of Phosphorus in Oleogels in Ruminal Fluid

The oleogel samples extracted from the ruminal liquid during the previous experiment were digested using a microwave oven (PREEKEM model WX-6000) at (1) 120 °C and 10 atmospheres for three minutes, (2) 150 °C at 20 atmospheres for three minutes, (3) 180 °C

at 30 atmospheres for three minutes and (4) 200 °C at 40 atmospheres for 10 min. This digestion procedure was performed on each of the samples, previously conditioned with 4 mL of nitric acid per mL of sample. After digestion, the samples were allowed to cool to room temperature, measured in a 25 mL flask and stored in tubes under refrigeration until they were read in the spectrophotometer, following the same methodology used to determine the phosphorus content in the model in an aqueous medium. Quantification was obtained by subtracting the concentration of phosphorus in the ruminal fluid from the amount of phosphorus in the control (oleogel samples without phosphorus).

2.8. Statistical Analysis

Two replications of all measurements were made, and the results were analyzed by ANOVA and comparison of means using STATISTICA Version 10 software (Stat Soft, Tulsa, Oklahoma, OK, USA).

3. Results

3.1. Thermal and Mechanical Properties of Oleogels

The results of the thermal characterization of the oleogels are shown in the thermograms in Figure 1, where the melting of these systems is observed to have occurred at temperatures higher than 52.8 °C. The increase of the melting temperature was a function of the CW concentration (10% CW: 52.81 °C ± 0.54 °C; 10% CW + phosphorus [P^+]: 53.32 °C ± 0.52 °C; 15% CW: 55.10 °C ± 0.30 °C; 15% CW + [P^+]: 52.89 °C ± 0.0 °C; 20% CW: 55.98 °C ± 0.60 °C; 20% CW + [P^+]: 56.18 °C ± 0.22 °C), and for each formulation, the presence of phosphorus did not affect this temperature ($p > 0.05$). It should be mentioned that the digestion process in the rumen of goats takes place at 40 °C; therefore, all CW oleogels would have the property of maintaining their entire structure at this temperature [40].

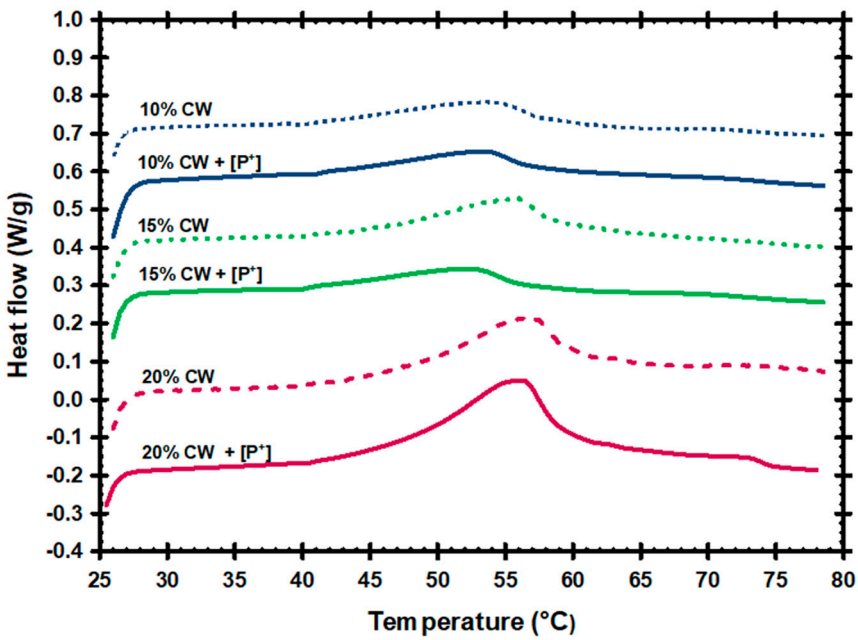

Figure 1. Melting thermograms of oleogels developed with different concentrations of candelilla wax (CW), with and without the addition of phosphorus [P^+].

On the other hand, the results of the rheological studies (i.e., frequency sweeps) that were performed on the oleogels at 40 °C demonstrated the formation of true gels by presenting values of the elastic modulus (G′) greater than those of the viscous modulus (G″) in the entire frequency range (Figure 2), while also having high G′ values to the order of 10^5 and 10^6 Pascals (Table 1). It was observed that with a higher concentration of CW

(10% < 15% < 20%), the G′ values increased, regardless of the presence of phosphorus ($p > 0.05$).

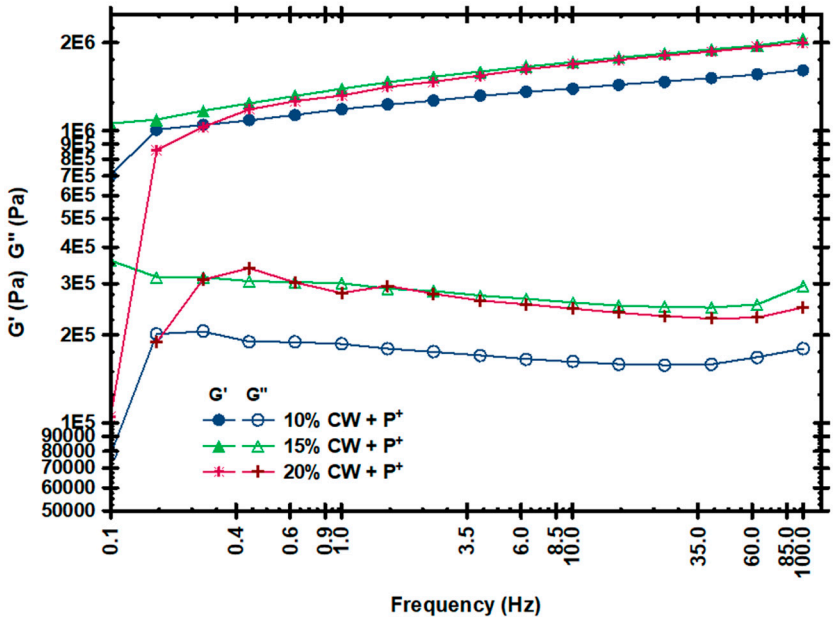

Figure 2. Viscoelastic moduli as a function of the frequency of the oleogels with different concentrations of CW + P^+ at 40 °C.

In Figure 2, it can be seen how the values of G′ tended to augment when the frequency increased, while G″ remained almost constant over the entire frequency range. This phenomenon is common in polymeric materials with high hardnesses that tend to release moisture, oxidize or show changes in their chemical configurations [44]. In the case of our oleogels, this behavior was attributed to the expulsion of the liquid phase from the network formed by the CW molecules. It was important that the oleogels added with phosphorous presented this type of mechanical characteristic, because this provides a first approximation of the effectiveness of the material in releasing the liquid phase in which the phosphorus is contained. Once both the mechanical properties and the thermal properties of the developed oleogels were known, it was possible to propose the use of these systems as controlled release boluses, since it is expected that when they reach their active site, they will remain intact and, in turn, they will expel the liquid phase so that phosphorus is released into the animal's rumen.

Table 1. Elastic modulus (G′) values of the oleogels with different concentrations of candelilla wax (CW) with and without phosphorus [P^+].

CW Concentration	G′ (Pascals, Pa)
10%	$1.9 \times 10^6 \pm 9.2 \times 10^{5\,a}$
10% + [P^+]	$1.8 \times 10^6 \pm 7.8 \times 10^{5\,a}$
15%	$2.4 \times 10^6 \pm 2.5 \times 10^{5\,a}$
15% + [P^+]	$2.1 \times 10^6 \pm 1.8 \times 10^{5\,a}$
20%	$1.1 \times 10^6 \pm 5.2 \times 10^{5\,b}$
20% + [P^+]	$1.6 \times 10^6 \pm 6.0 \times 10^{4\,b}$

Values are shown as the mean ± standard deviation of two replicates. Means represented by different lowercase letters indicate that the samples are statistically different ($p < 0.05$).

To have an approximation of the time in which the developed oleogels released the liquid phase, time sweeps were performed (Figure 3). The results showed that after 3 min at a constant strain and frequency, within the viscoelastic linear region, the G′ values of all oleogels increased as a function of time. It is worth mentioning that this trend was

different in the system with 20% CW with phosphorus, in which G′ began to increase until minute 20 and then reached a maximum value that subsequently remained constant for approximately 8 min until completing the 30 min of the preset sweep. According to these results, it could be assumed that this increase in the elasticity of the oleogels occurred when the molecular network was compacted as a consequence of the release of the liquid phase. This trend was observed for a relatively short period. If the time sweep were to increase, the trend could likely continue for a few more minutes or even hours. To verify the results of the liquid phase release from the functional point of view of the macrostructure of the developed oleogels, their liquid phase release capacities were evaluated and, on the other hand, their effectiveness as a phosphorus vehicle within an internal model in vitro.

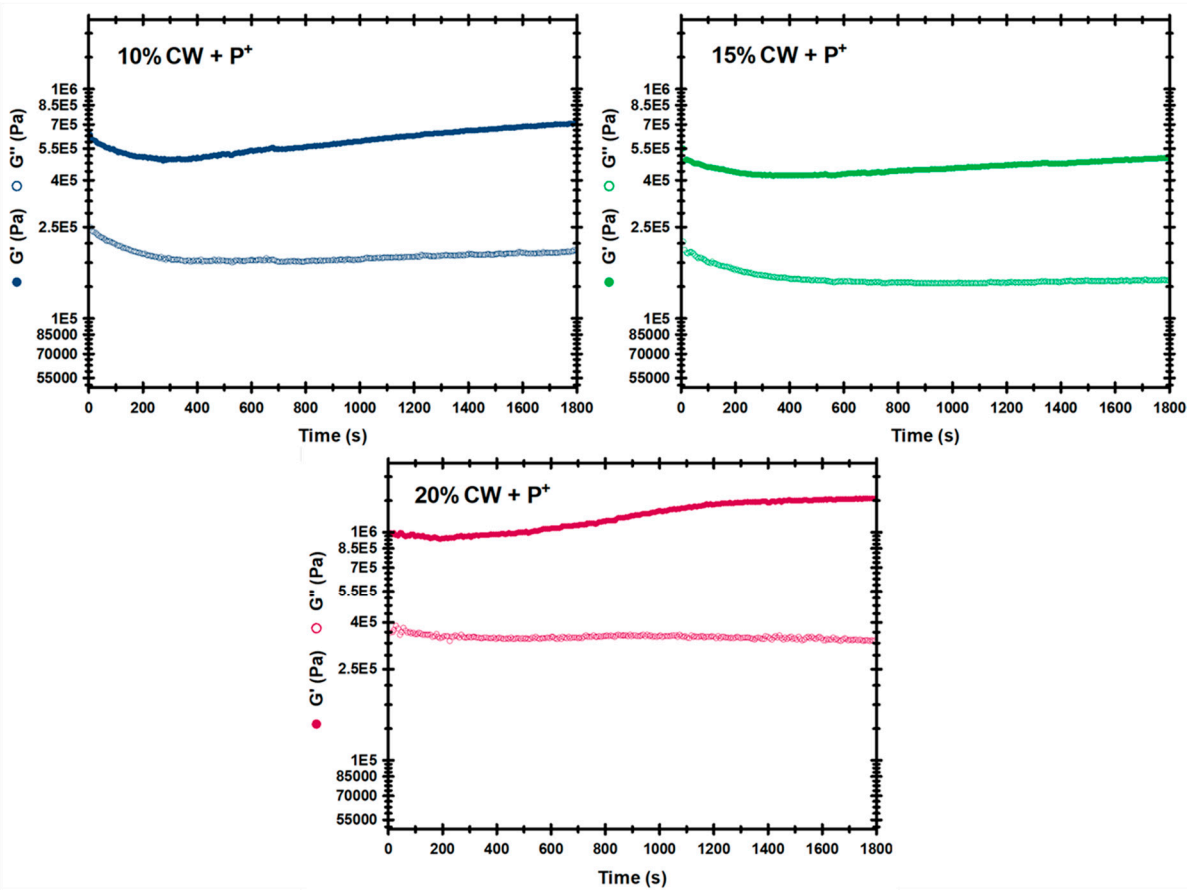

Figure 3. Time sweeps of the oleogels developed with different concentrations of candelilla wax (CW) with the addition of phosphorus (P^+) at 40 °C.

3.2. Liquid Phase Release Capacity of Oleogels

The determination of the liquid phase release capacity of the CW oleogels, with and without phosphorus, was carried out at room temperature (i.e., ~25 °C), quantifying the percentage of liquid loss in the selected samples. Table 2 shows that, in all systems, the percentage of liquid phase release was the same regardless of the presence of phosphorus ($p < 0.05$). It can also be seen that systems with 10% CW and 15% CW (with and without phosphorus) followed the same trend over time, even releasing similar percentages of the liquid phase ($p < 0.05$), while in the systems with 20% CW (with and without phosphorus), the liquid phase release was greater than in the previous systems. After 84 h, the 10% CW oleogel released ~27.6% of liquid phase, the 15% CW released ~28.8% and the 20% CW released ~47.1%. This phenomenon could be attributed to the type of molecular aggregation of CW—which has shape and distribution transitions—in the liquid phase caused by the chemical nature of the molecules due to the storage conditions [21,45]. It

has been reported that in CW oleogels, a phase transition, known as the rotatory phase, may occur at 25 °C [40]. This transition is commonly observed in n-alkanes (the main components of CW) and is characterized by the formation of a crystal lattice of molecular centers while the molecules still rotate around their axes. The rotatory phase causes a change of structural order (i.e., the molecules pass to a higher order state as a function of time) through a solid-solid transition. In oleogels, this type of transition gives rise to the expulsion of the liquid phase [40], as happened with the systems developed in the present work. In addition, it was observed that by containing a higher concentration of CW (10% < 15% < 20%), the oleogels expelled more liquid phase in less time (Table 2); that is, the solid-solid transition (or rotatory phase) was a function of the gelator concentration in this type of system. Another factor that could influence the release of the liquid phase is the size of the pores formed between the network of CW molecules. In this regard, Blake et al. [46] reported that the diameter of these pores is also directly related to the type of molecular aggregation that is formed and the concentration of the gelator. The results of the evaluation of the liquid phase release were congruent with those of the rheological studies (i.e., time sweeps), where it was observed that the values of G´ at concentrations of 10% and 15% of CW were similar over time. In contrast to the concentration of 20% CW, there was a greater hardness that was related to a greater liquid phase release.

Our results on the percentage of liquid phase release in the developed oleogels allowed us to approximate the phosphorus release that could occur within the rumen. It is worth mentioning that within the rumen, fermentation conditions (i.e., the bacterial load, temperature and pH) and peristaltic movements could affect the release of the active component. For this reason, a future study to explain the effect of the fermentation conditions of the rumen on the retention of the liquid phase of oleogels for use as boluses has been considered. However, as a first approximation in this work, the results of studies carried out using a model system at the laboratory level are presented. Two mediums were used: an aqueous medium was used for the first one, and the second was a model with goat ruminal fluid.

Table 2. Liquid phase loss (%) in oleogels developed with candelilla wax (CW) with and without the addition of phosphorus (P^+).

	Liquid Phase Loss (%)							
	0 h	**12 h**	**24 h**	**36 h**	**48 h**	**60 h**	**72 h**	**84 h**
10% CW	$0 \pm 0.00^{A,a}$	$2.82 \pm 0.10^{B,a}$	$2.92 \pm 0.04^{B,a}$	$3.08 \pm 0.05^{C,a}$	$3.27 \pm 0.17^{C,a}$	$4.56 \pm 0.09^{D,b}$	$5.72 \pm 0.08^{E,b}$	$6.76 \pm 0.08^{F,a}$
15% CW	$0 \pm 0.00^{A,a}$	$2.76 \pm 0.15^{B,a}$	$3.09 \pm 0.12^{B,a}$	$3.33 \pm 0.06^{C,c}$	$3.40 \pm 0.06^{C,a}$	$4.69 \pm 0.04^{D,b}$	$5.80 \pm 0.20^{E,b}$	$6.93 \pm 0.11^{F,a}$
20% CW	$0 \pm 0.00^{A,a}$	$3.28 \pm 0.15^{B,b}$	$5.37 \pm 0.31^{C,b}$	$5.94 \pm 0.06^{D,d}$	$6.75 \pm 0.11^{E,c}$	$6.77 \pm 0.14^{E,c}$	$8.79 \pm 0.04^{F,c}$	$10.87 \pm 0.3^{G,b}$
10% CW + P^+	$0 \pm 0.00^{A,a}$	$2.80 \pm 0.26^{B,a}$	$2.95 \pm 0.09^{B,a}$	$3.19 \pm 0.04^{C,b}$	$3.20 \pm 0.04^{C,a}$	$4.28 \pm 0.15^{D,a}$	$4.48 \pm 0.4^{D,a}$	$6.66 \pm 0.34^{E,a}$
15% CW + P^+	$0 \pm 0.00^{A,a}$	$2.94 \pm 0.10^{B,a}$	$3.08 \pm 0.15^{B,a}$	$3.36 \pm 0.03^{C,c}$	$3.42 \pm 0.19^{C,a}$	$4.48 \pm 0.04^{D,ab}$	$4.74 \pm 0.13^{E,b}$	$6.87 \pm 0.09^{F,a}$
20% CW + P^+	$0 \pm 0.00^{A,a}$	$3.12 \pm 0.02^{B,b}$	$5.34 \pm 0.01^{C,b}$	$6.05 \pm 0.11^{D,d}$	$6.46 \pm 0.06^{E,b}$	$6.74 \pm 0.29^{E,c}$	$8.47 \pm 0.30^{F,c}$	$10.95 \pm 0.3^{G,b}$

CW: Candelilla wax; P^+: phosphorus. Values are shown as the mean ± standard deviation of four replicates. Means are represented by different lowercase letters in all columns at each time, and uppercase letters in lines at different oleogel formulations indicate that the samples are statistically different ($p < 0.05$).

3.3. Evaluation of the Release of Phosphorus in an Aqueous Medium

The release of phosphorus from oleogels was evaluated in a model composed of an aqueous medium that simulated rumen conditions (i.e., temperature, pH and stirring). Starting with an initial phosphorus concentration of 226.7 mg/L, the phosphorus concentration released within the model systems was measured every 12 h. The results are shown in Figure 4, where it can be seen that the accumulated amount of phosphorus released by all the oleogels studied increased as a function of time. The oleogel with 10% CW had a total percentage of phosphorus release of 39.5% (~89.5 mg), that of 15% CW had a phosphorus release of 76.1% (~172.5 mg), and that of 20% CW showed a phosphorus release of 83.3% (~188.8 mg). Thus, in the present work, it could be deduced that both the type of molecular self-assembly formed by the CW as well as the diffusion coefficient of the molecules present in the liquid phase and also of the medium in which they were deposited (i.e., the aqueous solution of the model system) had an effect on the effective

release of phosphorus. In addition, as mentioned in Section 3.2, another factor that could have influenced the release of the liquid phase in these systems is related to solid-solid transitions caused by a molecular rearrangement as a function of time and also with the pore size of the network of CW molecules. With these results, it is demonstrated that the developed oleogels released phosphorus slowly in the medium. Therefore, if these systems were used as feed boluses, they could guarantee a supply of phosphorus to the animals for up to 4 days, thereby meeting the requirements of this mineral in grazing goats [10]. As the release is slow, the dosage of the oleogel to the animals could be spaced out, avoiding stress due to frequent encounters and also lowering labor costs. In the present work, the release of phosphorus in ruminal fluid extracted from the digestive system of a recently slaughtered goat was also evaluated, which replaced the aqueous medium in the model system used previously.

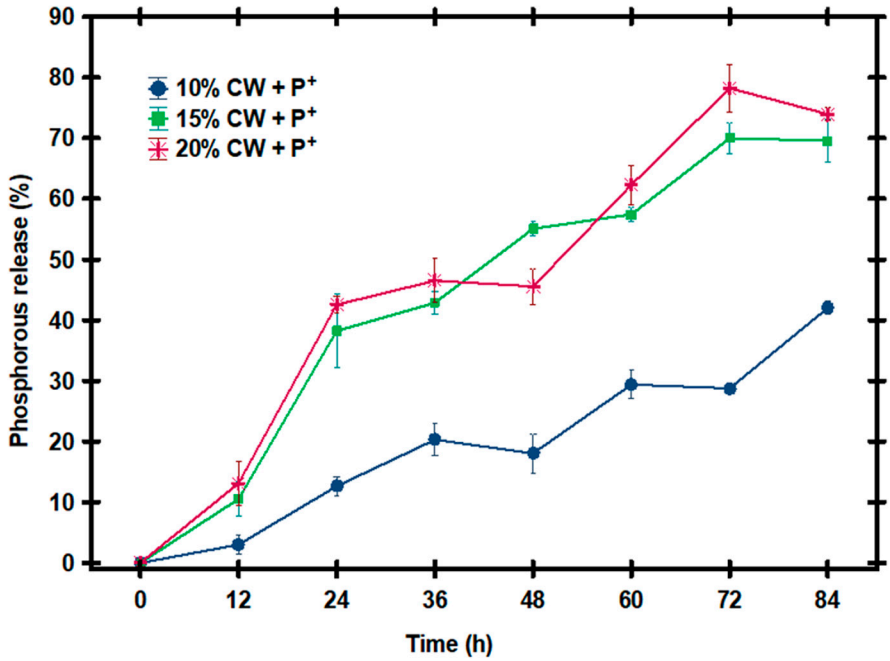

Figure 4. Cumulative percentages of phosphorus release (P^+) of oleogels with 10%, 15% and 20% candelilla wax (CW) in an aqueous medium for 84 h.

3.4. Evaluation of Phosphorus Release in Ruminal Fluid

The amounts of phosphorus released in the ruminal fluid from oleogels with 10%, 15% and 20% CW are presented in Table 3 (and in Figure S1 for cumulative percentage comparisons). The system with 10% CW released 55.6% (126.11 ± 1.03 mg/L) of the initially added phosphorus after 84 h, while the oleogel with 15% CW released 92.07% (208.73 ± 3.56 mg/L) and the oleogel with 20% CW released 98% (221.85 ± 1.13 mg/L). The phosphorus release trends as a function of the CW concentration were similar to those of the systems evaluated in the model with an aqueous medium. However, the results obtained in the model with ruminal fluid reflected that there was a greater release of phosphorus in this medium, which may be due to the effect of the conditions that generated the presence of bacteria, enzymes and other natural components of the ruminal fluid. Currently, there are no methodologies or reports on the evaluation of the nutrient release efficiency of commercial supplements in goats, so this work's methodological proposal is the first in this area. Likewise, the bolus formulation based on a CW oleogel, as well as its use and functional efficiency as a phosphorus vehicle, are also presented for the first time in this research. One of the main problems with commercial mineral supplements is that, due to their low concentration, it is difficult for goats to consume enough of the supplement to cover their requirements for this mineral [46]. Because of the formulation of oleogels

formulated with vegetable oil and CW, it is possible to provide a better vehicle to supply phosphorus to goats and ensure the required phosphorus contribution. Evaluating the effectivity of phosphorus release in goats in vivo is the next step to investigate.

Table 3. Phosphorus concentration (P^+) released into the ruminal fluid by the oleogels of CW for 84 h.

	Concentration of Released Phosphorus (mg)							
	0 h	12 h	24 h	36 h	48 h	60 h	72 h	84 h
10% CW + P^+	$0 \pm 0.00^{A,a}$	$9.03 \pm 1.50^{B,a}$	$37.96 \pm 1.52^{C,a}$	$60.92 \pm 2.61^{D,a}$	$54.15 \pm 3.19^{E,a}$	$88.22 \pm 2.3^{F,a}$	$86.32 \pm 0.66^{F,a}$	$126.11 \pm 1.03^{G,a}$
15% CW + P^+	$0 \pm 0.00^{A,a}$	$31.59 \pm 2.81^{B,b}$	$144.77 \pm 6.1^{D,c}$	$128.62 \pm 1.8^{C,b}$	$165.23 \pm 1.1^{E,b}$	$172.32 \pm 1.2^{F,b}$	$209.89 \pm 2.6^{F,b}$	$208.73 \pm 3.56^{F,b}$
20% CW + P^+	$0 \pm 0.00^{A,a}$	$39.35 \pm 3.65^{B,c}$	$127.61 \pm 1.4^{D,b}$	$139.58 \pm 3.6^{E,c}$	$54.15 \pm 3.68^{C,a}$	$186.96 \pm 3.2^{F,c}$	$234.67 \pm 3.84^{H,c}$	$221.85 \pm 1.13^{G,c}$

CW: Candelilla wax; P^+: phosphorus. Values are shown as the mean ± standard deviation of four replicates. Means are represented by different lowercase letters in all columns at each time, and uppercase letters in lines at different oleogel formulations indicate that the samples are statistically different ($p < 0.05$).

4. Conclusions

The oleogels developed with 10%, 15% and 20% CW in vegetable oil presented thermomechanical properties (i.e., melting temperatures greater than 50 °C and viscoelasticity of the order of 10^6 Pa) that permitted preservation of their integrity during the digestive processes in the in vitro aqueous and ruminal models. The oleogels' physical and chemical characteristics also conferred to them the ability of releasing the liquid phase slowly over a long time. All oleogels studied could be used as a medium of the controlled release of phosphorus. However, the oleogels' functionality showed itself to be more efficient using the systems with 20% CW (i.e., oleogel with the highest viscoelasticity) that released 189–222 mg of the phosphorus in a period of 84 h in the aqueous model and in the one that contained goat rumen fluid, respectively. The results of this investigation show that oleogels are worth further exploration. The bolus formulation based on a CW oleogel, as well as its use and functional efficiency as a phosphorus vehicle, are presented for the first time in this research. In future works, it would be important to complement oleogel characterization with the study of their microstructure and also to investigate their effectiveness as a phosphorus delivery system in in vivo models.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/app11093815/s1, Figure S1: Percentages of phosphorus release (P^+) of oleogels with 10%, 15% and 20% candelilla wax (CW) in a ruminal medium, for 84 h, Table S1: Experimental design for the elaboration of the controlled release bolus (oleogel) with different concentrations of candelilla wax.

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References

1. Heffelfinger, J. *Deer of the Southwest: A Complete Guide to the Natural History, Biology, and Management of Southwestern Mule Deer and White-Tailed Deer*, 1st ed.; Texas A&M University Press: Austin, TX, USA, 2006; ISBN 1585445150.
2. Gobierno de México. Producción Agrícola. Servicio de Información Agroalimentaria y Pesquera. Available online: https://www.gob.mx/siap/acciones-y-programas/produccion-agricola-33119 (accessed on 21 February 2021).
3. McDowell, L.R.; Arthington, J.D. *Minerals for Grazing Ruminants in Tropical Regions*; McDowell, L.R., Arthington, J.D., Eds.; Institute of Food and Agricultural Sciences, University of Florida: Gainesville, FL, USA, 2005.
4. Moradi, H.; Musapuor, A.; Afsharmanesh, M.; Moradi Shahrbabak, H. Use of Microbial Phytase for Decrease of Pollutant Due to Environmental Poultry Excreta Phosphorus. *Int. J. Agric. Biol.* **2014**, *8*, 35–37.
5. NRC (National Research Council). *Nutrient Requirements of Dairy Cattle*, 7th ed.; National Academies Press: Washington, DC, USA, 2001; p. 381.

6. Coneglian, S.M.; Ferriani Branco, A.; Guimarães, K.C.; Mano, D.S.; Barreto, J.C.; Fávaro, V.R. Replacement of dicalcium phosphate by rock phosphate in cattle diets: Nutrients digestibility, plasma parameters, ruminal fermentation and microbial synthesis efficiency. *Rev. Bras. Zootec.* **2010**, *39*, 815–823. [CrossRef]
7. Olvera, C.G.; Kurt, A.; Suárez, S.; Rosiles Martínez, R.; Ducoing Watty, A.; Ortiz Hernández, A. Blood and fecal selenium in sheep with the use of inorganic intraruminal boluses. *Vet. México* **2005**, *36*, 313–324.
8. Revilla-Vázquez, A.; Ramírez-Bribiesca, E.; López-Arellano, R.; Marina Hernández-Calva, L.; Tórtora-Pérez, J.; García-García, E.; Cruz Monterrosa, R.G. Supplement of selenium with intraruminal bolus of sodium selenite in sheep. *Agrociencia* **2008**, *42*, 629–635.
9. Muñoz-Gonzalez, J.C.; Huerta-Bravo, M.; Ramírez-Valverde, R.; Gonzalez-Alcorta, M.J. Estado mineral y suplemento con alambre de óxido de cobre en cabras de San José Teacalco, Tlaxcala. *Ecosistemas Recur. Agropecu.* **2015**, *2*, 203–210.
10. Haenlein, G.F.W.; Anke, M. Mineral and trace element research in goats: A review. *Small Rumin. Res.* **2011**, *95*, 2–19. [CrossRef]
11. Wilkie, I.W. Mineral Levels in Animal Health—Diagnostic Data. *Can. Vet. J.* **1989**, *30*, 190.
12. Hart, S. Meat Goat Nutrition. In *Proceedings of the 23rd Anniversary. Goat Field Day*; Langston University: Langton, UK, 2008; pp. 58–83.
13. Soares, J.H. Phosphorus bioavailability. In *Bioavailability of Nutrients for Animals*; Clarence, B., Ammerman, D.H., Baker, A.J.L., Eds.; Elsevier: San Diego, CA, USA, 1995; pp. 257–294.
14. Avendaño-Vásquez, G.; De la Peña-Gil, A.; Charó-Alvarado, M.E.; Charó-Alonso, M.A.; Toro-Vazquez, J.F. Self-Assembly of Symmetrical and Asymmetrical Alkyl Esters in the Neat State and in Oleogels. *Front. Sustain. Food Syst.* **2020**, *4*, 1–21. [CrossRef]
15. López-Martínez, A.; Morales-Rueda, J.A.; Dibildox-Alvarado, E.; Charó-Alonso, M.A.; Marangoni, A.G.; Toro-Vazquez, J.F. Comparing the crystallization and rheological behavior of organogels developed by pure and commercial monoglycerides in vegetable oil. *Food Res. Int.* **2014**, *64*, 946–957. [CrossRef]
16. Lan, Y.; Rogers, M.A. 12-Hydroxystearic acid SAFiNs in aliphatic diols—A molecular oddity. *CrystEngComm* **2015**, *17*, 8031–8038. [CrossRef]
17. De La Peña-Gil, A.; Álvarez-Mitre, F.M.; González-Chávez, M.M.; Charó-Alonso, M.A.; Toro-Vazquez, J.F. Combined effect of shearing and cooling rate on the rheology of organogels developed by selected gelators. *Food Res. Int.* **2017**. [CrossRef] [PubMed]
18. Guo, S.; Lv, M.; Chen, Y.; Hou, T.; Zhang, Y.; Huang, Z.; Cao, Y.; Rogers, M.; Lan, Y. Engineering water-induced ceramide/lecithin oleogels: Understanding the influence of water added upon pre- and post-nucleation. *Food Funct.* **2020**, *11*, 2048–2057. [CrossRef]
19. Martínez-Ávila, M.; De la Peña-Gil, A.; Álvarez-Mitre, F.M.; Charó-Alonso, M.A.; Toro-Vazquez, J.F. Self-Assembly of Saturated and Unsaturated Phosphatidylcholine in Mineral and Vegetable Oils. *J. Am. Oil Chem Soc.* **2019**, *96*, 273–289. [CrossRef]
20. Aguilar-Zárate, M.; De La Peña-Gil, A.; de Álvarez-Mitre, F.M.; Charó-Alonso, M.A.; Toro-Vazquez, J.F. Vegetable and Mineral Oil Organogels Based on Monoglyceride and Lecithin Mixtures. *Food Biophys.* **2019**, *14*, 326–345. [CrossRef]
21. Toro-Vazquez, J.F.; Morales-Rueda, J.A.; Dibildox-Alvarado, E.; Charó-Alonso, M.; Alonzo-Macias, M.; González-Chávez, M.M. Thermal and Textural Properties of Organogels Developed by Candelilla Wax in Safflower Oil. *J. Am. Oil Chem. Soc.* **2007**, *84*, 989–1000. [CrossRef]
22. Alvarez-Mitre, F.M.; Toro-Vazquez, J.F.; Moscosa-Santillán, M. Shear rate and cooling modeling for the study of candelilla wax organogelsâ€™ rheological properties. *J. Food Eng.* **2013**, *119*, 611–618. [CrossRef]
23. Onacik-Gür, S.; Żbikowska, A. Effect of high-oleic rapeseed oil oleogels on the quality of short-dough biscuits and fat migration. *J. Food Sci. Technol.* **2020**, *57*, 1609–1618. [CrossRef]
24. Samuditha, L.; Dassanayake, K.; Kodali, D.R.; Ueno, S.; Sato, K. Physical Properties of Rice Bran Wax in Bulk and Organogels. *J. Am. Oil Chem Soc.* **2014**, *86*, 1163–1173. [CrossRef]
25. Tang, S.; Liu, X.Y.; Strom, C.S. Producing supramolecular functional materials based on fiber network reconstruction. *Adv. Funct. Mater.* **2009**, *19*, 2252–2259. [CrossRef]
26. Li, X.; Wang, X.R.; Wang, S.-Q.; Wang, X. Nonlinearity in large amplitude oscillatory shear (LAOS) of different viscoelastic materials. *J. Rheol. J. Rheol.* **2009**, *531*. [CrossRef]
27. Aguilar-Zárate, M.; Macias-Rodriguez, B.A.; Toro-Vazquez, J.F.; Marangoni, A.G. Engineering rheological properties of edible oleogels with ethylcellulose and lecithin. *Carbohydr. Polym.* **2019**, *205*, 98–105. [CrossRef]
28. Alvarez-Mitre, F.M.; Morales-Rueda, J.A.; Dibildox-Alvarado, E.; Charó-Alonso, M.A.; Toro-Vazquez, J.F. Shearing as a variable to engineer the rheology of candelilla wax organogels. *Food Res. Int.* **2012**, *49*, 580–587. [CrossRef]
29. Wang, F.C.; Gravelle, A.J.; Blake, A.I.; Marangoni, A.G. Novel trans fat replacement strategies. *Curr. Opin. Food Sci.* **2016**, *7*, 27–34. [CrossRef]
30. Yılmaz, E.; Öğütcü, M. The texture, sensory properties and stability of cookies prepared with wax oleogels. *Food Funct.* **2015**, *6*, 1194–1204. [CrossRef]
31. Yılmaz, E.; Öğütcü, M. Oleogels as spreadable fat and butter alternatives: Sensory description and consumer perception. *RSC Adv.* **2015**, *5*, 50259–50267. [CrossRef]
32. Manzocco, L.; Calligaris, S.; Da Pieve, S.; Marzona, S.; Nicoli, M.C. Effect of monoglyceride-oil-water gels on white bread properties. *Food Res. Int.* **2012**, *49*, 778–782. [CrossRef]
33. Plamen, K.; Cong, L.E.; Khanh, A.N.H.; Alice, D.; Halima, R.; Silvia, R.U.M.; Carla, V.; Marek, H.; Fabrice, P. Organogels for cosmetic and dermo-cosmetic applications. Classification, preparation and characterization of organogel formulations. Part 1. *Househ. Pers. Care Today* **2015**, *10*, 15–19.

34. Beri, A.; Pichot, R.; Norton, I.T. Physical and material properties of an emulsion-based lipstick produced via a continuous process. *Int. J. Cosmet. Sci.* **2014**, *36*, 148–158. [CrossRef]
35. Vintiloiu, A.; Leroux, J.-C. Organogels and their use in drug delivery—A review. *J. Control. Release* **2008**, *125*, 179–192. [CrossRef]
36. Lo Nostro, P.; Ambrosi, M.; Ninham, B.W.; Baglioni, P. Effect of Headgroup Chirality in Nanoassemblies. 2. Thermal Behavior of Vitamin C-Based Surfactants. *J. Phys. Chem. B* **2009**, *113*, 8324–8331. [CrossRef]
37. Davidovich-Pinhas, M.; Gravelle, A.J.; Barbut, S.; Marangoni, A.G. Temperature effects on the gelation of ethylcellulose oleogels. *Food Hydrocoll.* **2015**, *46*, 76–83. [CrossRef]
38. U.S. Food and Drug Administration CFR—Code of Federal Regulations Title 21. Available online: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm (accessed on 31 January 2021).
39. Kuznesof, P.M.; Whitehouse, B. Candelilla wax. In *Japan's Specifications and Standards for Food Additives*; Ministry of Health and Welfare: Tokyo, Japan, 2003; Volume 7.
40. Morales-Rueda, J.A.; Dibildox-Alvarado, E.; Charó-Alonso, M.A.; Weiss, R.G.; Toro-Vazquez, J.F. Thermo-mechanical properties of candelilla wax and dotriacontane organogels in safflower oil. *Eur. J. Lipid Sci. Technol.* **2009**, *111*, 207–215. [CrossRef]
41. Castro-Costa, A.; Salama, A.A.K.; Moll, X.; Aguiló, J.; Caja, G. Using wireless rumen sensors for evaluating the effects of diet and ambient temperature in nonlactating dairy goats. *J. Dairy Sci.* **2015**, *98*, 4646–4658. [CrossRef] [PubMed]
42. Da Silva, T.L.T.; Barrera Arellano, D.; Martini, S. Interactions between candelilla wax and saturated triacylglycerols in oleogels. *Food Res. Int.* **2019**, *121*, 900–909. [CrossRef]
43. León-Cruz, M.; Ramírez-Bribiesca, E.; López-Arellano, R.; Miranda-Jiménez, L.; Rodríguez-Patiño, G.; Díaz-Sánchez, V.M.; Revilla-Vázquez, A.L. Trace mineral controlled-release intraruminal boluses. *Revision. Rev. Mex. Ciencias Pecu.* **2020**, *11*, 498–516. [CrossRef]
44. Mezger, T.G. *The Rheology Handbook*, 4th ed.; Vincentz Network: Hanover, Germany, 2014; ISBN 978-3-86630-842-8.
45. Blake, A.I.; Co, E.D.; Marangoni, A.G. Structure and Physical Properties of Plant Wax Crystal Networks and Their Relationship to Oil Binding Capacity. *J. Am. Oil Chem Soc.* **2014**, *91*, 885–903. [CrossRef]
46. Guo, Y.Q.; Tong, B.X.; Wu, Z.G.; Ma, W.Q.; Ma, L. Dietary manipulation to reduce nitrogen and phosphorus excretion by dairy cows. *Livest. Sci.* **2019**, *228*, 61–66. [CrossRef]