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Development of chitosan-based oleogels via crosslinking with vanillin using an emulsion templated approach: Structural characterization and their application as fat-replacer

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ABSTRACT

In this study, we aimed to develop chitosan-based oleogels via crosslinking with vanillin using an emulsion-templated approach to allow the physical trapping of oil in the chitosan matrix. The role of Tween®60 to modulate the gelled network strength and the use of oleogels as a shortening substitute in cookies was also investigated. Four oleogels were structured using 0.75% chitosan by emulsion templates exclusively when vanillin was used (1.0% or 3.0%), resulting in a network able to entrap oil via the formation of Schiff bases with chitosan. All chitosan-oleogels presented adequate moisture (<3%), lipid content (>92%), high thermostability and moderate oil binding capacity (OBC). At 3.0% vanillin, the chitosan-oleogel displayed a more open microstructure and resulted in a weaker gel (↓G′) with lower OBC and mechanical resistance than the chitosan-oleogel with 1.0% vanillin. Tween®60 led to unstable oleogels during shearing, despite their higher hardness and adhesiveness due to a more compact microstructure. Chitosan-oleogel with 1.0% vanillin (without Tween®60) exhibited the best performance as a shortening substitute in cookies. Therefore, the emulsion-templated approach combined with the crosslinking modification of chitosan by vanillin enabled chitosan to form an edible oleogel to provide processed foods that are *trans*-fat-free with potential health-promoting properties.

1. Introduction

Solid fats are used in processed foods because they provide the desired functional properties to industrialized food products, such as plasticity, crispy texture, improved flavor retention, handling convenience, and oxidative stability, which are necessary for the manufacture of bakery and confectionery products, margarine, ice creams, and other products (Patel, Nicholson, & Marangoni, 2020). However, these fats contain moderate to high contents of saturated and/or industrial *trans*-fatty acids that increase the risk of non-communicable diseases, mainly cardiovascular disease, when consumed frequently. Consequently, there is a sustained demand to eliminate *trans*-fatty acids from the global food supply by 2023, while the content of saturated fatty acids should be limited (Astrup et al., 2020; Islam et al., 2019; WHO, 2018a).

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In this sense, health organizations recommend replacing these fatty acids in processed foods with mono- or polyunsaturated fatty acids from healthier vegetable oils (WHO – World Health Organization, 2018a, 2018b). Nevertheless, vegetable oils can compromise food texture and body structure properties, usually producing more greasy and less crispy or aerated products with reduced shelf life due to oil oxidation (Patel et al., 2020; Demirksen & Mert, 2020). Therefore, replacing solid fats with liquid oil has been challenging because of the difficulty in achieving the desired quality of food products, especially in bakery products.

A promising alternative to overcome this limitation is to entrap vegetable oil into a gelator self-assembling network to obtain oleogels, which are solid-like materials suitable for mimicking the technological properties of solid fats that incorporate unsaturated fatty acids and bioactive compounds from vegetable oils. Hence, oleogels can add health benefits besides technological value to processed food products. Several compounds with distinct chemical properties have been used as gelators, providing oleogels with varied mechanical and chemical properties for food applications (Scharfe & Flöter, 2020; Singh, Auzanneau, & Rogers, 2017).

The use of polysaccharides currently used as food additives is an emerging research field because they may provide several features missing in other oleogelators. They are derived from renewable sources, have low cost, and are positively perceived by consumers. In addition, polysaccharides can gel oil at low concentrations due to their high molecular weight, therefore meeting most of the criteria required of a good gelator despite being hydrophilic (Bascuas, Morell, Hernando, & Quiles, 2021; Davidovich-Pinhas, 2019). Nevertheless, information on the use of chitosan, a biologically active polysaccharide commonly used as an emulsifier and thickening agent in food products, as an oil gelator is limited to inedible oleogels and gel-like dispersions used for biolubricant applications (Sánchez, Alonso, Valencia, & Franco, 2015; Gallego, Arteaga, Valencia, & Franco, 2014; Gallego, Arteaga, Valencia, & Franco, 2013; Sánchez, Stringari, Franco, Valencia, & Gallegos, 2011).

Chitosan has attracted attention because it is a value-added marine by-product that is non-toxic, biocompatible, and biodegradable. Chitosan is a natural cationic polysaccharide derived from the deacetylation of chitin extracted from the exoskeleton of shellfish, insects, and fungal cell walls. Chitosan has well-documented bacteriostatic activity and can function as a dietary fiber. It can acts as a fat absorption inhibitor, immunomodulator, and anti-tumor agent (Wang, Xue, & Mao, 2020; May, Tangso, Hawley, Boyd, & Clulow, 2020). Therefore, chitosan is a promising candidate to structure healthier oleogels. However, its low oil solubility must be overcome to promote the formation of an adequate tridimensional network suitable for oil retention, thus forming a gel.

The emulsion-templated approach has been used to form a three-dimensional scaffold of hydrophilic hydrocolloids that effectively oleogelate. In this process, an emulsion stabilized by these polymers must be dried, leading to the formation of a tight network to which the oil will be physically retained (Bascuas, Hernando, Moraga, & Quiles, 2020; Meng, Qi, Guo, Wang, & Liu, 2018a; Meng, Qi, Guo, Wang, & Liu, 2018b; Patel et al., 2014), which can enable chitosan to be used as an oil structuring agent. Moreover, chitosan can be chemically modified by crosslinking with vanillin (3-methoxy-4-hydroxybenzaldehyde), which is a natural flavor, is non-toxic, and is also an antioxidant. Cross-linking with vanillin improves the balance of chitosan between affinity and insolubility in oil due to the hydrophobic methoxyphenyl group in the vanillin aromatic ring. Additionally, these cross-links stabilize the polymer network via Schiff bases formed from the reaction between the aldehyde groups in vanillin molecules and the nucleophilic amino groups from chitosan (Tomadoni, Ponce, Pereda, & Ansorena, 2019; Kamaraj et al., 2018; Zhang, Han, Zeng, Xiong, & Liu, 2015). Moreover, the addition of Tween®60 to crosslinked vanillin-chitosan-based films provided a more compact and stiffer structure via the formation of new interactions between its polar group and the hydrophilic groups of the chitosan scaffold (Stroescu et al., 2015). Therefore, these features might allow modulation of the mechanical properties of the polymeric network formed by chitosan chains, possibly improving its oil binding capacity and oil gelling efficiency.

Therefore, we hypothesized that by combining the emulsion-templated approach with chitosan crosslinking with vanillin and/or incorporating Tween®60 would allow the development of edible oleogels with the potential to replace solid fats in food. It is worth investigating this innovative food application for chitosan, which might provide processed foods that are trans-fat free and/or reduced in saturated fat with improved health-promoting properties and sensory attributes, creating alternatives to extend the use of chitosan by the food industry. The present study aimed to develop oleogels by combining the vanillin cross-linking of chitosan and the emulsion-templated approach and characterize the oleogels by ATR-FTIR, oil binding capacity, and their micro- and macro-structures. The use of Tween®60 as an emulsifier beyond chitosan was also tested in the formulations. Moreover, the developed oleogels were used in cookies as a proof-of-application to investigate the role of chitosan-based oleogels as solid fat replacer considering their impact on the physicochemical and textural properties of the cookies. To the best of our knowledge, this is the first attempt to structure edible oleogels using chitosan as a gelator by an emulsion-templated approach with the value-added innovation of using vanillin as an oleogelator adjuvant.

2. Materials and methods

2.1. Materials

Chitosan (75–85% deacetylation degree, 190–310 kDa; 200–800 Cp), 3-methoxy-4-hydroxybenzaldehyde (vanillin, 99% purity, GRAS 182.60) (FDA – Food and Drug Administration, 2021a, 2021b), and polyethylene glycol sorbitan monostearate (Tween®60, GRAS 172.836, FDA – Food and Drug Administration, 2021a, 2021b) were purchased from Sigma-Aldrich (São Paulo, Brazil). Ethanol and glacial acetic acid were analytical grades (Biograde Chemicals, Goiania, Brazil). Commercial refined canola oil (without added synthetic antioxidants) and commercial vegetal shortening were purchased at the local market (Rio de Janeiro, Brazil). Canola oil contained (CG-FID, g/100 g of fatty acids) 8.76 ± 0.04 of saturated, 63.7 ± 3.26 (61.9% oleic acid) of monounsaturated, and 27.5 ± 3.13 (20.1% linoleic acid) of polyunsaturated fatty acids. Commercial vegetal shortening contained equivalents contents of saturated and trans-fatty acids (27%), according to the data provided by the supplier.

2.2. Cross-linked-chitosan-based oleogels preparation by emulsion-templated approach

Chitosan-based oleogels were prepared by the emulsion-templated approach (Patel et al., 2014) using vanillin as a crosslinker with or without Tween®60 added as an emulsifier (Stroescu et al., 2015). Firstly, a stock solution of chitosan (1.5% w/v) was prepared in aqueous acetic acid solution (1.0% v/v) under magnetic stirring (50 °C/2 h). Ethanolic vanillin solutions (10% and 30%; w/v) were prepared. Oil in water (O/W) emulsions (40:60 v/v) were prepared by dispersing canola oil into a chitosan solution (50 mL) using a high-speed dispersing unit (Ultraturrax® T25 digital model, IKA®-Werke GmbH & Co. KG, Staufen, Germany) at 9000 rpm followed by the addition of vanillin solution (10 mL) under continuous shearing (4 min). Final emulsions contained 0.75% (w/v) chitosan and 1.0 or 3.0% (w/v) vanillin. Tween®60 was added, when necessary, at maximum usage concentration allowed for food emulsions (0.4 wt%) (FDA – Food and Drug Administration, 2021a, 2021b, CFR172836). The emulsions were freeze-dried (−60 °C, 0.101 mBar) (L108 Lyophilizer Liotop, São Paulo, Brazil), and the dried products were sheared (9000 rpm/2 min) to prepare the oleogels. Five oleogels were formulated as follows: 1) oleogel structured only by chitosan (0.75%); 2) cross-linked-chitosan-based oleogel with 1.0% vanillin; 3) cross-linked-chitosan-based oleogel with 3.0% vanillin; 4) cross-linked-chitosan-based oleogel with 1.0% vanillin + Tween®60, and 5) cross-linked-chitosan-based oleogel with 3.0% + Tween®60. All oleogels were made in triplicate and stored at 4 °C for 48 h before further analysis.

2.3. *Physical-chemical characterization of cross-linked-chitosan-based oleogels*

2.3.1. *Visual appearance, moisture, total lipid content, and oil binding capacity (OBC)*

Visual appearance was assessed in emulsions, dried products, and oleogels using a digital camera (Canon EOS Rebel T5, DS126491, EFS 18-55, Manaus, Brazil). The lipid system was classified as "oleogel" when the material did not flow with the inversion (180°) of the storage becher (Rocha et al., 2013). Moisture and total lipid contents were determined according to the AOAC: Association of Official Analytical Chemists (2012) methods. The OBC of oleogels was determined by the centrifuge method (Meng et al., 2018a) and the oil loss (Eq. 1) and OBC (Eq. 2) values were calculated, where *m* was the mass of the empty vial, *m1* was the mass of the vial with the initial sample, and *m2* was the mass of the vial with the sample after centrifugation.

$$\mathrm{Oil\ Loss}(\%) = [(\mathrm{m1} - \mathrm{m}) - (\mathrm{m2} - \mathrm{m})]/(\mathrm{m1} - \mathrm{m}) \times 100\% \tag{1}$$

$$\mathrm{OBC}(\%) = 100 - \mathrm{oil}\ loss(\%) \tag{2}$$

2.3.2. *Chemical characterization by Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) spectra analysis*

The infrared spectra of the canola oil, chitosan, vanillin, Tween®60, and oleogels were recorded using an ATR-FTIR spectrometer (Nicolet-IS50, ThermoScientific, Waltham, Massachusetts, USA) equipped with a diamond ATR module and a detector of deuterated triglycine sulfate. Before each measurement, a small amount of each sample was deposited directly on the flat surface of the diamond crystal cell at controlled ambient temperature (25 °C). All spectra were acquired at ambient temperature in a wavenumber range from 4000 cm^{-1} to 600 cm^{-1}. Each with 32 scans at a resolution of 4 cm^{-1} (Xu et al., 2018).

2.3.3. *Microstructure studies*

The microstructure of oleogels was assessed using a polarized light microscope (Olympus BX51, Tokyo, Japan), coupled with a QImaging digital color camera (Media Cybernetics, USA). The real-time images were transmitted to the computer using Image-Pro Plus 7.0 Software (Media Cybernetics, USA). A single drop of oleogel was placed on a glass microscope slide, covered with a coverslip to form a thin film. The samples were equilibrated at 25 °C on a hot stage connected to a Linkam PE-120 System Controller (Linkan Scientific Instrument Ltd, Surrey, UK) and visualized under bright-field and polarized light at the magnification of ×40 (Silva et al., 2017).

2.3.4. *Macrostructure studies (mechanical properties): rheological and instrumental textural measurements*

The small amplitude oscillatory rheological tests and steady-flow measurements of the oleogels were performed using a controlled stress rheometer (AR-G2, TA Instruments, New Castle, USA) equipped with a *Peltier* system for temperature control. A parallel plate cross-hatched geometry of 60 mm diameter was used, and a gap of 1000 μm was set for samples. Oscillatory tests of frequency (0.1–10 Hz) and temperature (5–80 °C; 80–5 °C) were measured within the linear viscoelastic region (LVR) determined by the strain sweep test using a strain range of 0.01–100%, at a frequency of 1 Hz. The storage (G') and loss (G'') modulus were determined. The flow measurements were performed at shear rates in the range of 1–100 s^{-1} to analyze the shear-thinning behavior and at oscillatory strains of 0.1% (interval 1), 10% (interval 2), and 0.1% (interval 3) to analyze the thixotropy and structure-recovery properties of the samples. The structure recovery at 0.1% was calculated by dividing the viscosity value in interval 3 by the end of interval 1 (Meng et al., 2018a).

Textural profile analysis (TPA) was carried out using a TA-XT *plus* Texture Analyzer (Stable Micro Systems, Surrey, UK) equipped with a 5 kg load cell with a cylindrical probe (36 mm of diameter). After 48 h of refrigeration at 4 °C, oleogel samples (40 g) in 100 mL beaker (height × internal diameter= 75 × 50 mm) were equilibrated at room temperature before analysis. Each sample was subjected to a double compression test at speed of 13 mm/s in pre- and post-test, and 10 mm/s, during-test. Trigger force was 0.1 N, and the interval between compressions was 5.0 s (Meng et al., 2018a). The texture parameters measured were: hardness (N; the peak force of the first penetration), cohesiveness (dimensionless; the ratio between the area of work during the second and the first compressions), adhesiveness (g.s; the total negative area in the first compression), elasticity (dimensionless; the distance to recovery original height between the end of the first compression and the start of the second compression) and resilience (dimensionless; the ratio between the area during probe withdrawal from the first compression by the area of the first compression). The texture properties of oleogels (hardness and adhesiveness) were also monitored during a storage assay at 35 °C, in the dark for 30 days to evaluate the mechanical stability of oleogels. The hardness and adhesiveness of oleogels were measured at 0, 15, and 30 days of storage. The stability assay was done in duplicate and all experimental analysis at least in triplicate from three independent oleogel processing.

2.4. *Cross-linked-chitosan-based oleogel application as fat-replacer in cookies*

2.4.1. *Cookie making procedure*

Six formulations (control with commercial vegetable shortening and formulations replacing 100% of shortening by liquid oil (canola oil)) or each chitosan-based oleogel on a weight basis were prepared using the following ingredients: wheat flour (325 g), sugar (280 g), vegetal shortening, canola oil or chitosan-based oleogel (160 g), whole eggs (110 mL), salt (4.5 g), sodium bicarbonate (3.9 g), and vanillin essence (7 mL). All the solid ingredients were manually mixed and gathered with the liquid ingredients until complete homogenization. The dough was kneaded, molded and cut in a cylindrical shape (4.5 cm diameter × 0.7 cm thickness), and weighed on average 13 g. Height and diameter of cookies doughs were measured using a manual pachymeter, and doughs out of standard were not used in this study. The cookie doughs were baked in a conventional oven (180 °C/10 min). After, cookies were allowed to cool down at room temperature and sealed in plastic bags until analysis. The baking procedure for each cookie formulation was made in duplicate (20 cookies in each one) on the same day.

2.4.2. *Measurements of cookie geometry and proximate composition*

The dimensional properties of cookies ($n = 12$) were evaluated in terms of weight, diameter, thickness, and spread ratio (diameter/thickness). The spread factor (%) of cookies replacing with oleogels was calculated compared to the control cookies (Eq. 3).

$$\text{Spread factor }(\%) = \left(\text{Spread ratio}_{\text{oleogel cookie}} / \text{Spread ratio}_{\text{control cookie}}\right) \times 100 \tag{3}$$

The proximate composition of cookies was evaluated for moisture (method 925.10), crude ash (method 923.03), crude protein (method 920.87), and crude fat (method 922.06) (AOAC: Association of Official Analytical Chemists, 2012). The carbohydrate content was obtained by subtracting the sum of moisture, ash, protein, and fat contents from 100 g. The results were expressed as g/ 100 of wet base (w.b) and energy value (Kcal/100 g) was estimated by the sum of % available carbohydrate (× 4 kcal/g), protein (× 4 kcal/g), and fat (× 9 kcal/g). Proximate composition (except for moisture content) of cookies replaced with canola oil was based on data available for each ingredient in the Food Brazilian Food Composition Table available at http://www.fcf.usp.br/tbca.

2.4.3. *Instrumental texture and color properties of cookies*

Texture properties of hardness and crispness of cookies (n = 12) were measured by penetration texture analysis using a TA-XT *plus* Texture Analyzer (Stable Micro Systems, Surrey, UK) under speed conditions of pre-test, test, and post-test at 2.0, 2.0, and 5.0 mm/s,

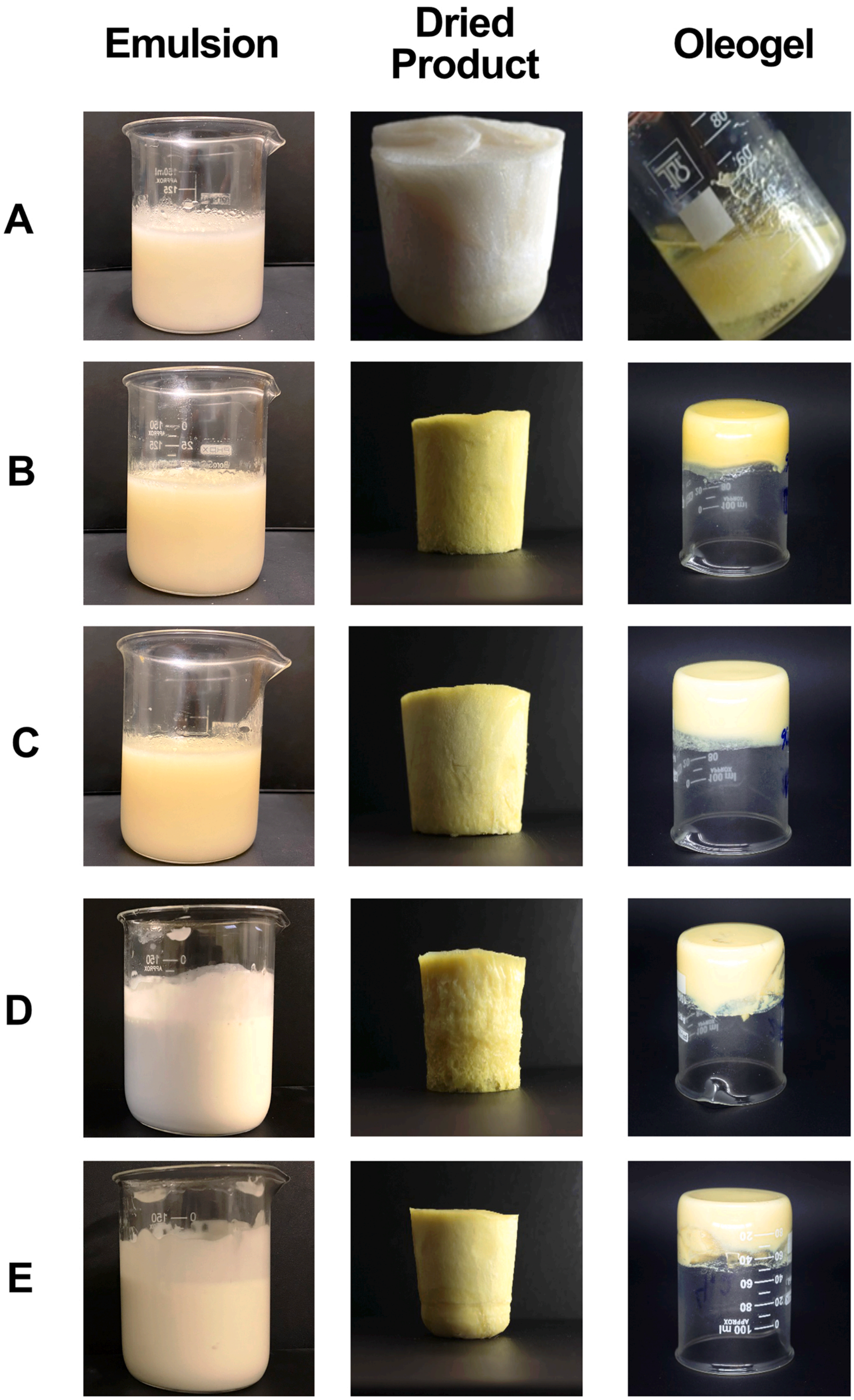

Fig. 1. Photographs of emulsion, dried product and oleogel samples. A) 0.75% Chitosan; B) 1.0% Vanillin; C) 3.0% Vanillin; D) 1.0% Vanillin + Tween®60; E) 3.0% Vanillin + Tween®60.

respectively. The trigger force was 0.01 kg, and the trigger type was "auto" (Silva et al., 2018).

The instrumental color of the surface of the cookies was measured at different points (n = 12) using the Minolta CR-400 colorimeter (Konica Minolta, Osaka, Japan) with illuminant D65 and 2° viewing angle. The CIElab L^* a^* b^* color components were measured and used to calculate the total color difference (ΔE^*) before and after the cooking procedure (Eq. 4).

$$\Delta E^* = \sqrt{(\Delta L*)^2 \quad + \quad (\Delta a*)^2 \quad + \quad (\Delta b*)^2} \tag{4}$$

Texture and color analyses were performed at room temperature with cookies from two batches of banking.

2.5. Statistical analysis

All data were presented as mean ± standard deviation (coefficient of variation $< 10\%$) after testing for normality distribution using the Shapiro-Wilk test ($\alpha = 0.05$). Data were obtained from at least three independent samples from each oleogel and two independent samples from each cookie formulation. One-way analysis of variance (ANOVA) followed by the Duncan multiple range test was used to determine the significant differences in physical-chemical and mechanical properties among chitosan-based oleogels and cookies. Values with $p < 0.05$ were considered statistically different. GraphPad Prism version 8.0.0 for Windows (GraphPad Software, San Diego, California, USA) was used to perform all statistical analyses.

3. Results and discussion

3.1. Visual appearance, moisture, total lipids, and oil biding capacity of chitosan-based oleogels

In the present study, we used the emulsion-templated approach combined with chitosan modification by crosslinking with vanillin (with or without Tween®60 added as an additional emulsifier) to obtain chitosan-based oleogels (Fig. 1). The use of chitosan as the sole structuring agent was able to stabilize canola oil droplets in oil/water emulsions due to its emulsifying nature. However, a two-phase system (oil and polymer) was formed after the freeze-drying process, rather than an oleogel (Fig. 1A). Therefore, this sample was not used in subsequent experiments. However, we observed that using vanillin as a crosslinking agent was essential to promote physical oil entrapment by chitosan chains, resulting in crosslinked-chitosan-based oleogels with a solid-like appearance after the shearing step (Fig. 1B–E). Additionally, incorporation of Tween®60 in systems containing 1% or 3% vanillin altered the appearance of the emulsions, but the oleogels did not appear to be different (Fig. 1D,E).

The amino group spacing between the chitosan molecular chains was probably reduced after water removal by the freeze-drying process, resulting in strong repulsion between the cationic molecules. Therefore, the highly concentrated oil droplets aggregate, resulting in phase separation (Wang et al., 2020; Pareda, Amica, & Marcovich, 2012). Other surface-active polysaccharides such as methylcellulose (MC) and hydroxypropyl-methylcellulose (HPMC) were also unable to generate stable oleogels as unique oil gelators when used at low concentrations (< 1 wt%) by the emulsion-template method. The combination with non-surface-active polysaccharides (e.g., carrageenan and xanthan gum) is necessary to increase the viscosity of a continuous aqueous phase, preventing the coalescence of oil droplets while forming a polymeric network capable of entrapping oil through both polysaccharides (Bascuas et al., 2020; Meng et al., 2018a, 2018b; Patel et al., 2014).

Chitosan crosslinks with aromatic aldehydes to form Schiff bases, protecting its amino groups and avoiding chain repulsion, and providing an ideal balance between the affinity and solubility of chitosan in the oil (Lei et al., 2015; Zhang et al., 2015). Consequently, vanillin causes rearrangement of the chitosan chains, ensuring an adequate distance between the polymer molecules and increasing the emulsion stability during freeze-drying, similar to a cryoprotectant (Morais et al., 2016). Therefore, it was possible to structure chitosan-based oleogels crosslinked with vanillin, independent of the presence of Tween®60. Furthermore, the chitosan-based oleogel with 1.0% vanillin showed the most translucial and brightest appearance (Fig. 1B). Additionally, the yellow color intensity from emulsions containing vanillin is related to the formation of Schiff bases between the chitosan and vanillin molecules (Stroescus et al., 2015), although the presence of Tween®60 masked this coloration.

All crosslinked-chitosan-based oleogels had the requisite moisture ($< 3\%$) and total lipid content ($> 92\%$) to be classified as "oleogels" (Patel et al., 2015) (Table 1). The moisture content increased significantly with an increase in the amount of vanillin. In both oleogels formulated with 3.0% vanillin, free vanillin might form crystals during freeze-drying, and the crystals accumulated on the emulsion surface prevented water sublimation (Zhang et al., 2015). The incorporation of Tween®60 significantly reduced the moisture content of the chitosan-based oleogel with 1% vanillin, while increasing that of crosslinked-oleogel with 3% vanillin. When an emulsifier is used in a polymer solution, interactions between the polymer and emulsifier tend to be formed (Stroescu et al., 2015). Therefore, at 1% vanillin, Tween®60 may establish chemical interactions with chitosan, limiting the access of water molecules to hydrophilic chitosan sites. Consequently, the mass of water that evaporated during drying was higher, reducing the moisture content of the oleogel. However, at 3% vanillin, this water loss was hindered by the accumulation of vanillin crystals on the emulsion surface.

The OBC of the oleogels varied from 63.6% to 67.9%. In the absence of Tween®60, a crosslinked-oleogel with 1% vanillin exhibited a higher OBC than the oleogel with 3% vanillin, suggesting the formation of a more stable network when a lower content of vanillin was used. According to our microstructure images discussed in *Section 3.3*, the excess of vanillin impaired network continuity, resulting in a weaker structure with a lower capacity to entrap oil. However, with the addition of Tween®60, no significant differences were observed among oleogels with 1% and 3% vanillin. Tween®60 slightly reduced the OBC of the oleogel with 1% vanillin, impairing its network stability (Table 1).

The chitosan-based oleogels showed intermediary OBC, and this parameter should be improved because it is related to the physical stability of the system. Other polysaccharide-based oleogels made by emulsion-templates exhibited an OBC varying from 57% to 95%. Alizadeh, Abdolmaleki, Nayebzadeh, and Hosseini (2020) reported an OBC of 57% for oleogels structured with 1% HPMC and sunflower oil, which increased in the presence of sodium caseinate. Moreover, Espert, Salvador, and Sanz (2020) and Meng et al. (2018a) reported that the OBC varied from 78% to 95% when HPMC concentrations varied from 0.1% to 1.0%. Increased polysaccharide concentration might improve the

Table 1
Initial characterization of crosslinked-chitosan-based oleogels.

Chitosan-based Oleogel[1]	Physico-chemical properties		
	Moisture (g/100 g)	Total lipids (g/100 g)	Oil Binding Capacity (%)
1.0% Vanillin	1.35 ± 0.07^{a}	96.1 ± 0.65^{a}	67.9 ± 1.56^{a}
3.0% Vanillin	2.31 ± 0.16^{b}	94.8 ± 0.34^{a}	63.6 ± 3.11^{b}
1.0% Vanillin + Tween®60[2]	1.02 ± 0.09^{c}	94.2 ± 0.21^{a}	66.1 ± 3.24^{ab}
3.0% Vanillin + Tween®60[2]	2.97 ± 0.24^{d}	92.8 ± 2.80^{a}	64.3 ± 1.38^{ab}

Results are expressed as mean ± standard deviation of independent triplicate for each oleogel. [1]0.75% (w/v) chitosan. [2]0.4% (wt%). Different superscript letters in the same column indicate significant differences among different formulations of chitosan-based oleogels. ANOVA one-way followed by Duncan post-test ($p < 0.05$).

OBC by forming a stronger physical network with a high capacity to entrap oil. High OBC (>95%) is a desirable property in oleogels because of its relationship to the physical stability of the network (Blake & Marangoni, 2015). Hence, the OBC of chitosan-based oleogels could be improved with a higher chitosan concentration (> 0.75%), and this hypothesis deserves further investigation.

3.2. *Chemical characterization by ATR-FTIR spectroscopy*

The ATR-FTIR spectra of chitosan, vanillin, Tween®60, canola oil, and oleogels are presented in Fig. 2. The spectrum of chitosan showed characteristic peaks at 3356, 1647, and 1570 cm^{-1} corresponding to the overlapping stretching vibrations of -OH and -NH$_2$, the C=O of amide groups, and the symmetrical stretching vibration absorption of -NH$_2$, respectively (Sánchez et al., 2015; Stroescu et al., 2015). The spectrum of vanillin displayed the -OH stretching and C-H stretching absorptions of the methyl group at 3166 and 2861 cm^{-1}, respectively. The C=O stretching vibration of the aldehyde group corresponds to the peak at 1661 cm^{-1}, and the bending vibration of the phenolic hydroxyl group corresponds to the peak at 1263 cm^{-1} (Xu et al., 2018; Peng et al., 2010). The spectrum of Tween®60 exhibited a peak at 3477 cm^{-1}, corresponding to the -OH stretching vibration. The symmetric and asymmetric stretching vibrations of aliphatic -CH$_2$ correspond to the peaks at 2916 and 2850 cm^{-1}, respectively, and the stretching vibration of C=O corresponds to the peak at 1735 cm^{-1}. The peaks at 1643 and 1466 cm^{-1} can be attributed to the vibration of the tetrahdropyrane ring. The strong and sharp peak at 1094 cm^{-1} corresponds to the C-O-C vibration in (CH$_2$CH$_2$O) (Branzoi & Branzoi, 2017). The spectrum of canola oil exhibited the most prevalent triglyceride functional groups composed of long-chain carbon, ester linkages, and double bonds. The peak at 3007 cm^{-1} corresponds to the stretching vibration of the adjacent double bonds (C=C). The peaks at 2922 and 2853 cm^{-1} correspond to the asymmetrical and symmetrical stretching vibrations of multiple methylene peaks. The strong and sharp peak at 1743 cm^{-1} corresponds to the stretching vibration of C=O of the ester linkage. In addition, multiple overlapping stretching vibration absorption peaks of C-O, C-C, and C-H were observed between 1300 and 1000 cm^{-1} (Pan, Sun, Zhou, & Chen, 2018).

The chitosan-based oleogel spectra confirmed the interaction between chitosan and vanillin by Schiff bases at 1643 cm^{-1}, corresponding to the C=N stretching vibration, resulting in an entangled network capable of trapping oil (Xu et al., 2018). As expected, an increasing vanillin concentration caused an increase in the intensity of the characteristic Schiff base peaks. However, the peak at 811 cm^{-1}, which corresponds to the benzene ring, can also be assigned to excess free vanillin (Peng et al., 2010). Consequently, 3.0% vanillin seemed to be in excess of the amount required to provide crosslinking of 0.75% chitosan when structuring chitosan-based oleogels by the emulsion-templated approach. Otherwise, this excess of vanillin changed the characteristic -OH peak to lower frequencies at 3171 and 3158 cm^{-1}, suggesting the increased formation of hydrogen bonds between the 4-hydroxyl group of vanillin and the hydroxyl or amino groups of chitosan (Zhang et al.,

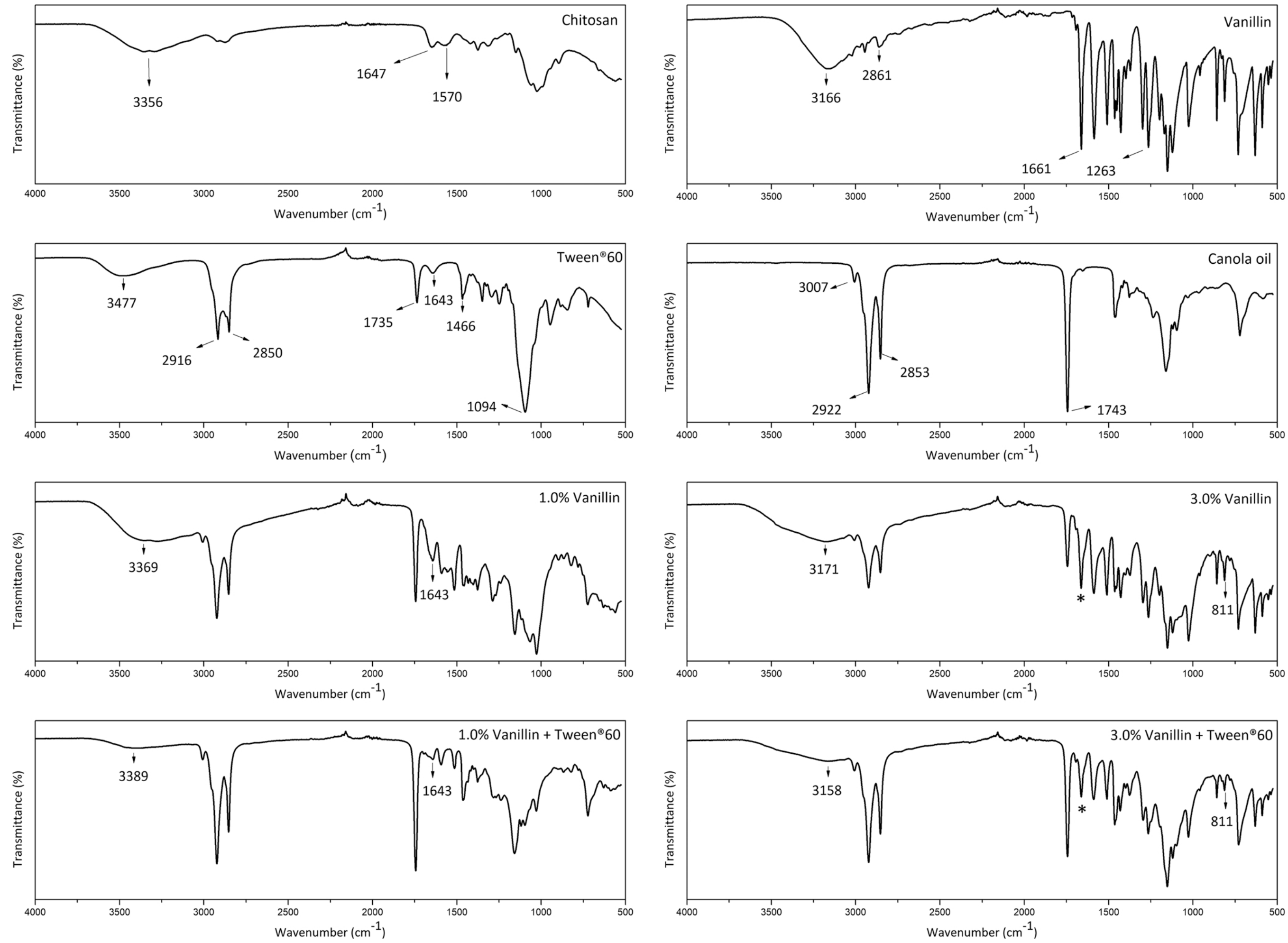

Fig. 2. FTIR spectra of chitosan, vanillin, Tween®60, canola oil, chitosan-based oleogels with vanillin (1.0% or 3.0%) without Tween®60 and chitosan-based oleogel with vanillin (1.0% or 3.0%) with Tween®60. (*) Schiff-base peak.

2015; Xu et al., 2018). Therefore, we confirmed the essential role of vanillin in the formation of a tridimensional structure, enabling the production of chitosan-based oleogels.

Additionally, the effects of using Tween®60 in the structure of chitosan-based oleogels have not been disclosed by FTIR. Tween®60 and canola oil have similar chemical groups; hence, they probably overlapped, resulting in no noticeable presence of Tween®60 in oleogels (Branzoi and Branzoi, 2017). In addition, interactions between Tween®60 and chitosan were not observed. Stroescu et al. (2015) also reported no interaction between chitosan and Tween®60 in chitosan-based films, corroborating our results.

3.3. Microstructure of chitosan-based oleogels

As shown in Fig. 3, the chitosan-based oleogel network formed by the crosslinking reaction between the amino groups of chitosan and the 1-aldehyde group in the vanillin molecule and by hydrogen bonds between the 4-hydroxyl group of vanillin and the hydroxyl or amino groups of chitosan can be seen under bright field. In addition, the influence of Tween®60 on the microstructure was visualized. In contrast, free vanillin crystals that have not undergone crosslinking can be observed under polarized light. Vanillin crystals were present as rods/ plates forms, resulting in a crystalline network capable of influencing

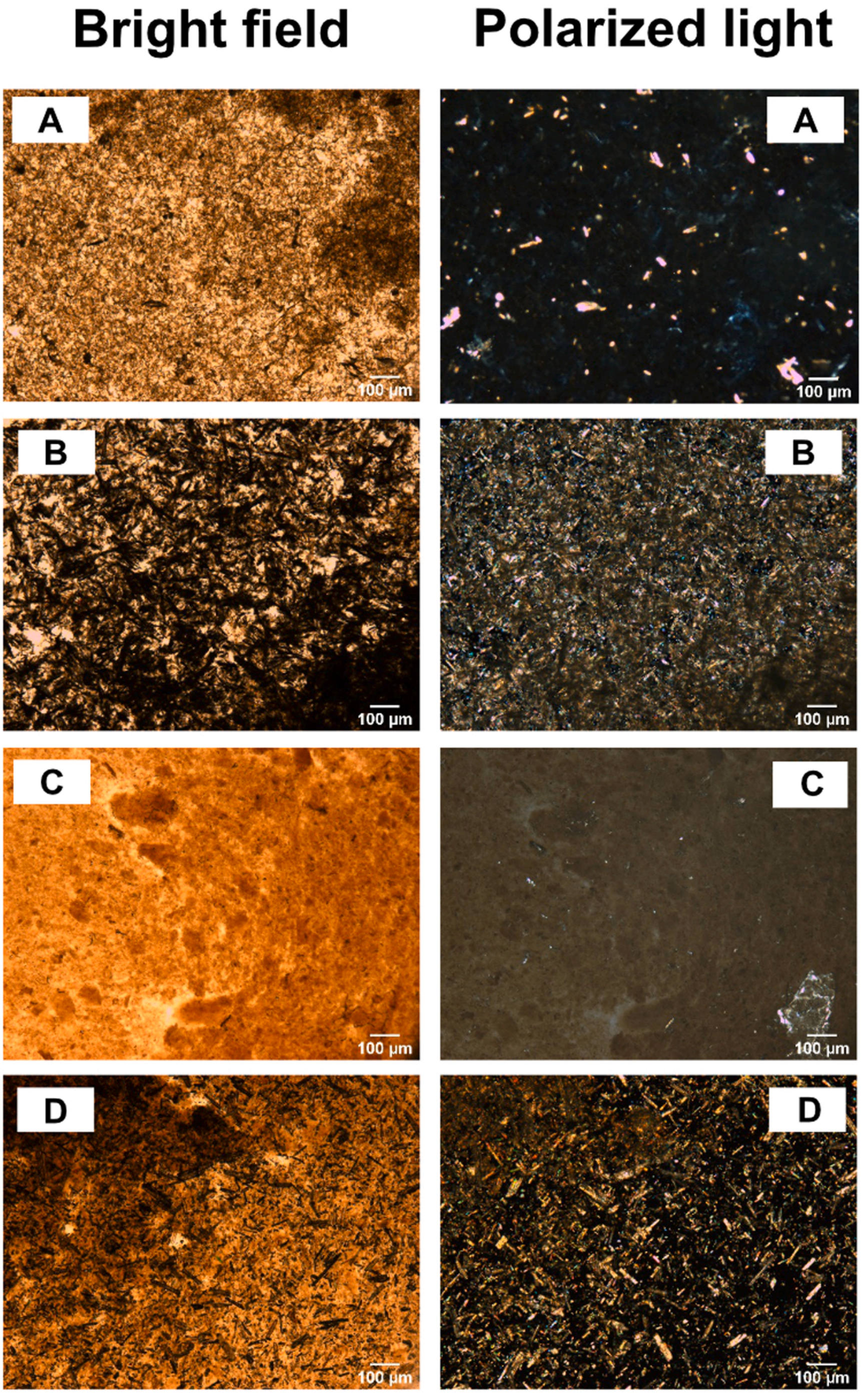

Fig. 3. Microstructure of the crosslinked-chitosan-based oleogels under bright field and polarized light at 25 °C. A) Chitosan-based oleogel with 1.0% vanillin (Tween®60-free); B) Chitosan-based oleogel with 3.0% vanillin (Tween®60-free); C) Chitosan-based oleogel with 1.0% vanillin and Tween®60; D) Chitosan-based oleogel with 3.0% vanillin and Tween®60.

the final structure of chitosan-based oleogels.

The chitosan-based oleogel containing 1.0% vanillin (Fig. 3A) showed a packed network with a few large vanillin crystals entrapped in the structure. However, this amount of crystals did not seem to hinder network continuity. In contrast, the oleogel containing 3.0% vanillin (Fig. 3B) showed a higher amount of vanillin crystals on the surface, resulting in a loose and, consequently, weaker structure explaining the lower OBC. The increased number of crystals agreed with the hypothesis of free vanillin when the oleogel was formulated with 3.0% of this flavor compound. Both oleogels formulated with Tween®60 (Fig. 3C and D) showed a more compact and homogeneous structure in which the chitosan network and vanillin crystals were not evident. Tween®60 probably contributed to increasing the viscosity of the emulsions, improving their physical stability during drying. Hence, after drying, the oil droplets tended to be tightly packed together in a more homogeneous distribution of the polymer matrix. Therefore, it seems that the role of Tween®60 as a surface-active molecule contributes to the modification of the structure of the vanillin-chitosan network formed (Bascuas et al., 2020).

3.4. *Macrostructure of chitosan-based oleogels*

3.4.1. *Rheological characterization*

The LVR region extension in the chitosan-based oleogels was up to 10 Pa (yield stress). All chitosan-based oleogels presented elastic modulus (G′) between 10^3 and 10^4 Pa, which were consistently higher than the viscous modulus (G″), confirming the solid-like dominant behavior (G′>G″) of oleogels (Meng et al., 2018a, 2018b) (Fig. 4A). The elastic modulus measures the elasticity of a material and quantifies its resistance to deformation. Therefore, materials with higher G′ are more resistant to deformation, which is related to a more structured material (Chen, Wen, Janmey, Crocker, & Yodh, 2010). In this sense, chitosan-based oleogels with 3.0% vanillin showed weaker structures (↓G′) than those containing 1% vanillin, especially when Tween®60 was incorporated. Additionally, the oleogel with 3.0% vanillin without Tween®60 showed the lowest extension of LVR, reflecting a weak gel structure since G′ became dependent on the stress applied earlier. This behavior was possibly affected by the open microstructure of the 3.0% vanillin oleogel without Tween®60 (Fig. 3A). HMPC-based oleogels (< 1%) combined with thickening agents showed similar yield stress (10 Pa) but lower G′ values (between 10^2 and 10^3 Pa) to those observed in our chitosan-based oleogels (Meng et al., 2018a, 2018b). However,

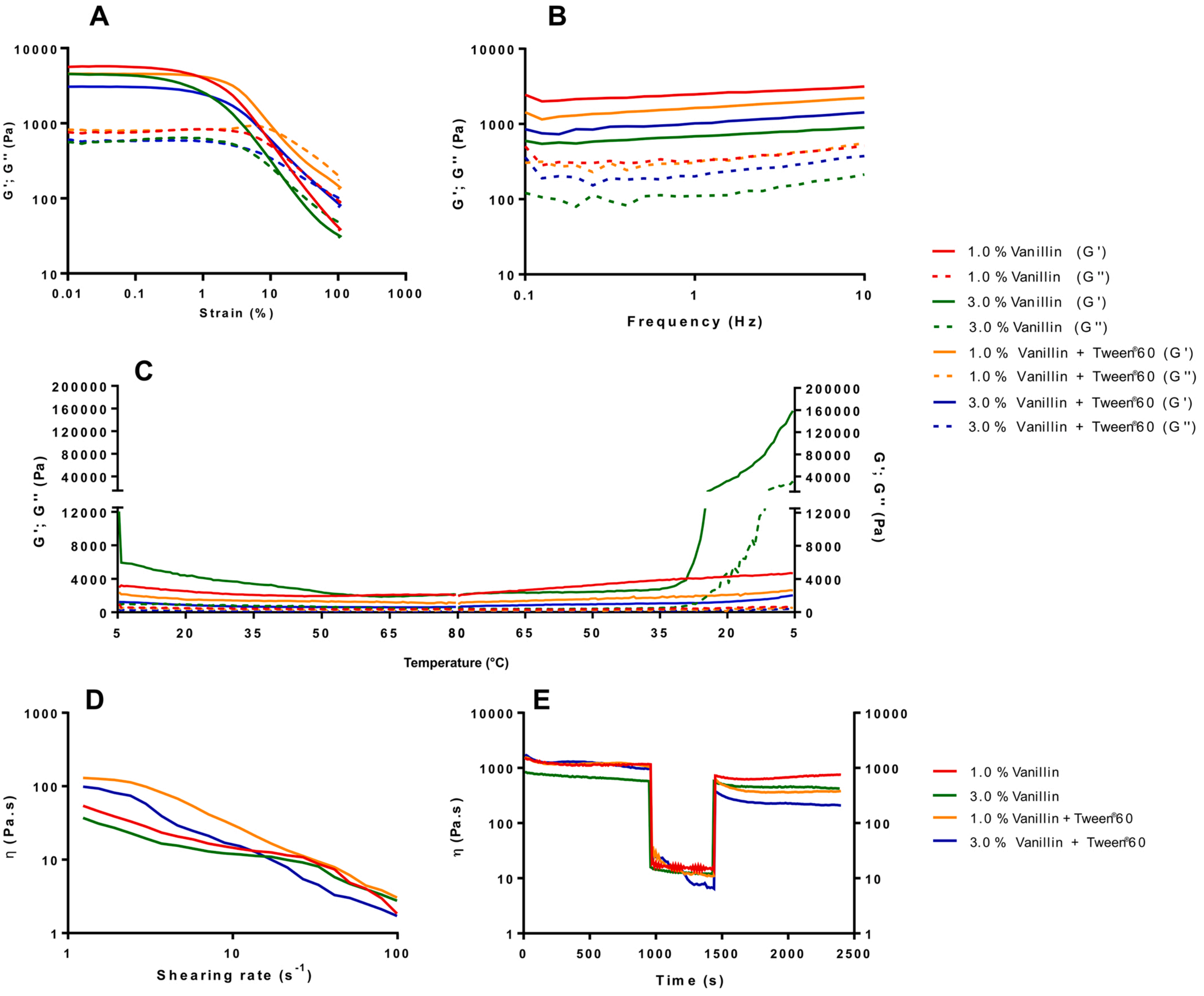

Fig. 4. Small oscillatory rheological and flow measurement tests for chitosan-based oleogels. A) Stress sweep test; B) Frequency sweep test; C) – Oscillatory temperature test; D) Shear-thinning behavior test; E) Oscillatory shear behavior (recovery test).

oleogels structured with HMPC or methylcellulose (MC) (0.5–2.0%) without additional thickeners showed 100-fold higher G′ values with a yield stress near 100 Pa (Espert et al., 2020). Therefore, our chitosan-based oleogels showed reasonable mechanical strength, with those containing 1% vanillin stiffer than those containing 3% vanillin, modified by the presence of Tween®60 as an additional emulsifier.

Additionally, the low dependence of G′ on the frequency indicated good tolerance of the gels to external forces (Fig. 4B). In addition, G′ remained higher than G″ over the entire frequency range. Therefore, the mechanical spectrum of the chitosan-based oleogels exhibited a "plateau-region" where G′ slightly increases with frequency and G″ displays a minimum, which is expected for physically stable polymeric gels with entangled networks. Our results were similar to those reported for inedible isocyanate-chitosan-based oleogels made by direct dispersion in castor oil (Gallego et al., 2013, 2014) and HPMC- and MC-based oleogels made by emulsion-templates (Espert et al., 2020; Bascuas et al., 2020; Meng et al., 2018a, 2018b) that also exhibited a "plateau-region".

The loss tangent ($\tan\delta = G''/G'$) values of chitosan-based oleogels were determined as the following: 0.12 (1.0% vanillin oleogel Tween®60-free)), 0.20 (3.0% vanillin oleogel Tween®60-free), 0.27 (1.0% vanillin oleogel with Tween®60) and 0.42 (3.0% vanillin oleogel with Tween®60). Tan δ indicates whether the viscous or elastic component is predominant in a material. Thus, low values of tan δ indicate that the elastic portion is prominent, whereas high (> 1) values indicate that the viscous portion is dominant. Therefore, all chitosan-oleogels exhibited a higher elastic portion than the viscous portion. Moreover, chitosan-oleogels with 1% vanillin were more elastic (lower tan δ) than their counterparts with 3% vanillin. However, the tan δ values of the oleogels increased in the presence of Tween®60, indicating a greater contribution to the viscous nature of the gels.

Notably, G″ in 3.0% vanillin oleogels showed brittle behavior over the frequency range, probably due to the rupture of a few individual physical bonds, although the entire surrounding material retained the whole gel structure. In general, all chitosan-oleogels were stable with no crossover point (G′=G''). Similarly, the gel-like structure of the oleogels was not affected by temperature variations, indicating high thermostability. Except for the chitosan-oleogel with 3.0% vanillin (Tween®60-free), which showed an increase in G′ and G″ during the cooling step, resulting in a stronger gel than the initial gel (Fig. 4C). Vanillin crystals in the oil phase can melt between 51 and 63 °C and re-crystallize in more than one polymorphic form with different physical properties, such as flow behavior and hardness. This phenomenon can vary according to the temperature and cooling rates, in addition to the solid/solvent ratio. It is likely that the rearrangement of the excess vanillin crystals during the cooling step resulted in the formation of unwanted polymorphism, which increased the stiffness of the oleogel with 3.0% vanillin (Tween®60-free) (Kavuru, Grebinoski, Patel, Wojtas, & Chadwick, 2016; Svärd, Gracin, & Rasmuson, 2007).

Tween®60 also affected the shear-thinning behavior of crosslinked-oleogels. Oleogels containing Tween®60 showed higher apparent viscosity, which decreased with increasing shear rate (10–100 s^{-1}). Otherwise, oleogels without Tween®60 showed lower apparent viscosity that gradually decreased under shear, being more stable gels (Fig. 4D). With increasing shear rate, high molecular weight molecules (chitosan) tend to align in the flow direction. Consequently, the viscosity and flow resistance are reduced, limiting the structuring capacity of the polymeric network of chitosan-oleogels to entrap oil. However, after structure destabilization by shear, Tween®60 can induce the formation of micellar structures in oil, disrupting networks, and leading to a faster decrease in viscosity (Petrovic, Sovijl, Katona, & Milanovic, 2010).

Accordingly, chitosan-oleogels without Tween®60 showed better structural recovery than oleogels with Tween®60 (Fig. 4E). The structure-recovery of crosslinked-chitosan-based oleogels (Fig. 4E) was as follows: oleogel containing 3.0% vanillin, Tween®60-free (99%) > 1.0% vanillin, Tween®60-free (65%) = 1.0% vanillin with Tween®60 (60%) > 3.0% vanillin with Tween®60 (39%). Therefore, Tween®60 combined with an excess of vanillin crystals (3%) sensibly hampered the re-stabilization of internal network structures when shearing rates slowed, causing more permanent changes in the gel structure, that would enable its recovery. Thus, Tween®60-free oleogels that showed slower deformation under shear and acceptable structure recovery (> 60%) expectedly would be more stable in food processing that applies high shear intensity, such as mixing, shaking, stirring, and pumping. Similarly, they could be considered for spreads and other food products that need to break down their structure to be distributed into a surface and then rebuild to guarantee adequate mouthfeel, avoiding phase separation or dripping.

3.4.2. TPA characterization

The textural properties of oleogels are related to their rheological and microstructural properties, which affect industrial food processing and consumer acceptance (Marangoni, van Duynhoven, Acevedo, Nicholson, & Patel, 2020). Herein, the TPA parameters of hardness, adhesiveness, cohesiveness, elasticity (springiness), and resilience were affected by the concentrations of vanillin and Tween®60 (Table 2).

Chitosan-oleogels containing Tween®60, independent of vanillin content, showed two-fold higher values of hardness and adhesiveness (Table 2) than oleogels Tween®60-free, reflecting their more compact microstructure (Fig. 3). Hardness is one of the most important parameters for characterizing the gel strength. Tween®60, as an emulsifier, might produce more stable emulsions with small oil droplets that, after the drying process, are related to the development of harder oleogels with high strength (Espert et al., 2020). Similarly, chitosan-oleogels without Tween®60 showed hardness values between two and three times higher than oleogels structured by MC or HPMC (2.0 wt%) obtained from emulsions with 46% oil (6.61–6.99 N) or 60% oil (3.40–3.45 N), respectively (Espert et al., 2020). However, these authors measured the oleogel texture using a protocol test with different experimental setup than in our study, for instance the probe and sample surface contact area, which could promoted these differences.

In contrast, chitosan-oleogels containing Tween®60 showed slightly lower cohesiveness and elasticity. Cohesiveness and elasticity are related to how the gel structure is damaged after a deformation force and its recovery capability, respectively. Consequently, oleogels with Tween®60 were harder but brittle, and their internal structure was more easily destroyed than those oleogels without Tween®60. Food hardness is inversely correlated with cohesiveness and elasticity (Liu et al., 2018).

Spreadability, one of the most desirable characteristics in solid fats, comprises the relationship between hardness, elastic constant, and post-yield stress flow characteristics (Patel et al., 2020). In this respect, hardness is inversely related to spreadability. Therefore, the harder the oleogel, the worse its spreadability. Consequently, chitosan-oleogels containing Tween®60 tended to spread less than those without Tween®60. Harder and more adhesive oleogels can impair the machinability of bakery dough products to achieve the desired shape (Pehlivanoglu, Demirci, & Toker, 2017). However, in some applications, the use of harder oleogels can be advantageous for strengthening the hardness of the finished product, such as frankfurts (Ye, Li, Lo, Fu, & Cao, 2019). Therefore, future effort should be made to set working limits to minimize or maximize the desired values for these texture attributes according to the use of chitosan-based oleogels as fat-replacers.

Additionally, the hardness and adhesiveness properties of all crosslinked-oleogels (except for the one containing 3.0% vanillin with Tween®60) were relatively stable during storage at 35 °C for 30 days (Fig. 5). At 3.0% vanillin with Tween®60, the hardness and adhesiveness of the oleogel significantly increased between 0 and 15 days of storage, but reduced between 15 and 30 days, suggesting that this oleogel may cause undesirable changes in the texture of the food product over time. Notably, chitosan-based oleogels exhibited higher thermal stability than solid fats, which melt near human body temperature (37 °C), avoiding the unpleasant residual mouthfeel (Hartel, von Elbe, & Hofberger, 2018). However, even fat-replacers with properties different

Table 2

Texture profile parameters of crosslinked-chitosan-based oleogels.

Chitosan-based oleogel[1]	Texture profile parameters				
	Hardness (N)	Adhesiveness (N.s)	Cohesiveness[3]	Elasticity[3]	Resilience[3]
1.0% Vanillin	10.2 ± 1.10^{a}	4.62 ± 0.31^{a}	0.65 ± 0.07^{ab}	0.98 ± 0.01^{a}	0.02 ± 0.00^{a}
3.0% Vanillin	9.75 ± 0.48^{a}	5.09 ± 0.41^{a}	0.71 ± 0.05^{a}	0.98 ± 0.01^{a}	0.02 ± 0.00^{a}
1.0% Vanillin + Tween®60[2]	20.9 ± 2.04^{b}	12.2 ± 0.47^{b}	0.63 ± 0.05^{b}	0.96 ± 0.01^{bc}	0.03 ± 0.00^{b}
3.0% Vanillin + Tween®60[2]	19.7 ± 2.03^{b}	11.4 ± 0.51^{b}	0.60 ± 0.05^{b}	0.97 ± 0.01^{ac}	0.02 ± 0.00^{a}

Results are expressed as mean ± standard deviation of independent triplicates for each oleogel. [1] 0.75% (w/v) chitosan. [2]0.4% (wt%). Different superscript letters in the same column indicate significant differences among different chitosan-based oleogels. ANOVA one-way followed by Duncan post-test ($p < 0.05$). [3]Dimensionless.

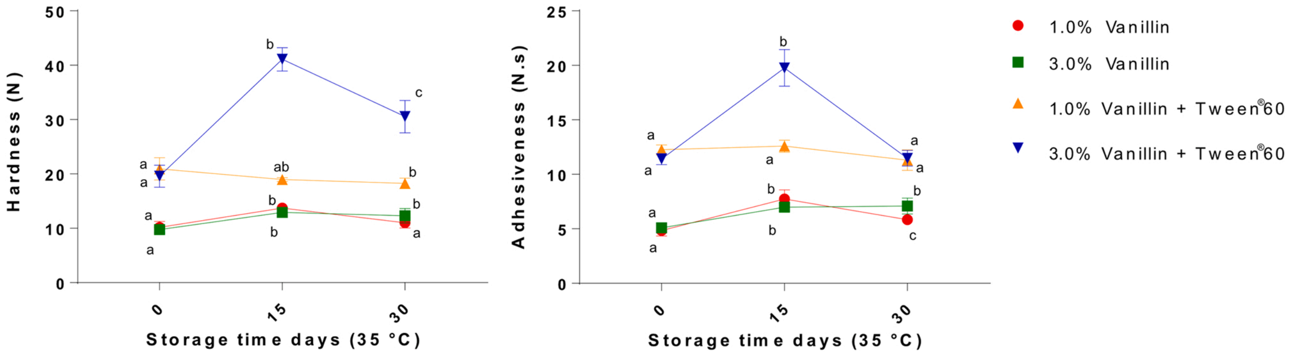

Fig. 5. Monitoring of hardness and adhesiveness of chitosan-based oleogels at 35 °C for 30 days. ANOVA one-way followed by Duncan post-test ($p<0.05$). ●($p < 0.05$). • chitosan-based oleogel 1.0% vanillin; ■ chitosan-based oleogel 3.0% vanillin; ▲ chitosan-based oleogel 1.0% vanillin + Tween®60; ▾chitosan-based oleogel 3.0% vanillin + Tween®60.

from those of solid fats can still create food products with technological properties similar to the conventional ones containing solid fats (Patel et al., 2020).

Herein, we report exploratory and a future stability study using more than one storage temperature for a prolonged time will be welcome to broaden the understanding of the stability of chitosan-based oleogels. Moreover, the small sample size ($n = 4$) in this study limits extrapolation of the effects of chitosan, vanillin, and Tween®60 and their interactions with the physicochemical and mechanical properties of crosslinked-chitosan-based oleogels in general, and further studies addressing this issue should be conducted to improve knowledge in this sense. However, these results might stimulate new insights into the oleogelation of the biopolymer chitosan.

3.5. Physical and chemical properties of cookies: cookies geometry, proximate composition, instrumental color and texture

Six formulations of cookies were prepared using either commercial

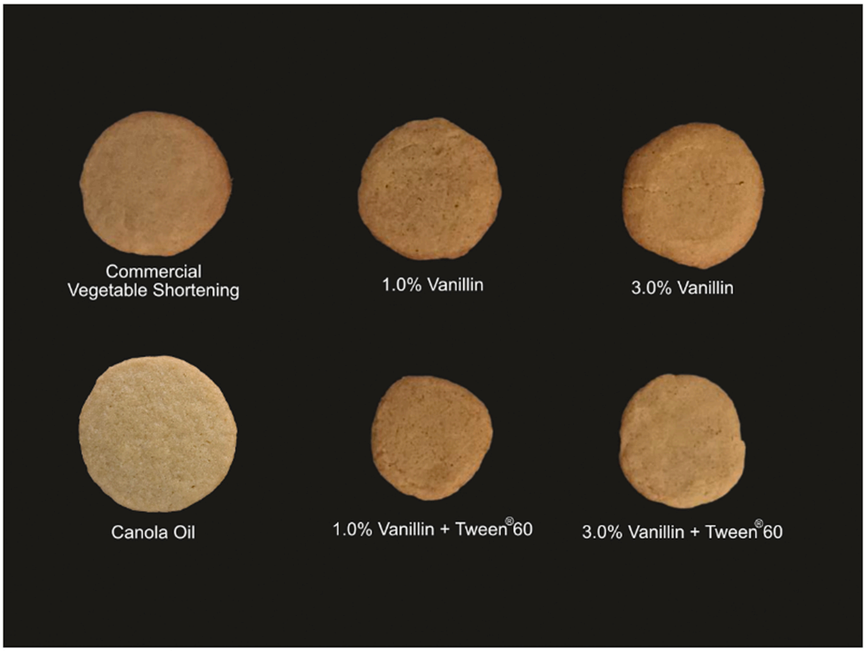

Fig. 6. Photograph of cookies produced with commercial vegetable shortening or chitosan-based oleogels crosslinked with 1% or 3% vanillin (with or without Tween®60).

vegetable shortening or replacing it with canola oil or crosslinked-chitosan-based oleogels (Fig. 6), and their physicochemical properties are shown in Table 3.

The weight of all the oleogel-cookies was lower than that of the shortening-cookies, which did not differ statistically from the oil-cookies. In addition, all replaced cookies showed less diameter expansion than the shortening-cookies, with lower expansion for the oleogel-cookies containing 1.0% vanillin (with Tween®60). Additionally, oil-cookies showed the lowest thickness while those containing oleogels with 3% vanillin (independent of the presence of the Tween®60) showed the highest ones. There was no significant difference between shortening-cookies and those containing oleogels with 1% vanillin. Consequently, oil-cookies showed the highest spread ratio (diameter/thickness), while cookies replaced by oleogel with 3% vanillin showed the lowest. Cookies replaced by chitosan-oleogels with 1.0% vanillin stood out as the most similar to shortening-cookies with similar spread ratios and a spread factor of 96.8% (Tween®60-free) and 82.6% (with Tween®60). Otherwise, oil-cookies exhibited the worst baking performance compared to shortening-cookies, with a spread factor of 175%.

During cookie manufacture, solid fat (especially shortening) can block the interaction between glutenin and gliadin proteins thus inhibiting the formation of an extensive gluten network. In addition, fat covers gluten and starch granules preventing their adherence, leading to the development of a machinable viscoelastic dough structure that provides the required spreading properties of cookies (diameter and thickness) (Demirksen & Mert, 2020; Yılmaz & Öğütcü, 2015). When liquid oil was used to replace shortening, dough formation was less viscous and unable to control gluten development, forming an extensive and cohesive network related to a higher spread ratio of cookies during baking (Yılmaz & Öğütcü, 2015). Otherwise, when oil was trapped in a chitosan network crosslinked with 3% vanillin, shrinkage was favored. It is possible that the elastic nature of the doughs was impaired. Hence, the chitosan-oleogel with 1% vanillin (Tween®60-free) provides a significant improvement over liquid canola oil and chitosan-oleogel with 3% vanillin.

Additionally, oleogel-cookies, especially vanillin 3.0%, and oil-cookies, showed significantly higher moisture content than shortening-cookies. It is likely that the vanillin-chitosan-crosslinked chains, especially when combined with an excess of free vanillin crystals, created a physical barrier on the cookie surface, which restricted water evaporation during baking. Usually, cookies should have a moisture content below 5%. Therefore, cookies made with canola oil or chitosan-oleogels with 1% vanillin provide final products with moisture content near this target. Otherwise, there was no significant difference in the total lipid content among cookies. However, the unsaturated fatty acid content, especially oleic acid, might have been increased in the oil-cookies and oleogel-cookies due to the use of canola oil, improving the fatty acid profile and the nutritional value of these cookies. Cookies replaced with chitosan-based oleogels showed slightly higher ash and protein content than shortening-cookies, with total carbohydrate content ranging from 64.4 to 71.6 g/100 g. However, oil-cookies exhibited the lowest protein, ash, and total carbohydrate content (Table 3).

All oleogel-cookies showed similar hardness and crispness to shortening-cookies, except for cookies replaced by chitosan-oleogel with 3.0% vanillin (Tween®60-free), which were two- and three-fold harder and crispier, respectively (Table 3). Chitosan-oleogels with 3.0% vanillin displayed a hardening phenomenon during the cooling step (35–5 °C) in the structure recovery test (Fig. 4), which clearly influenced the technological properties of the cookies. Oil-cookies were the hardest, being 2.5-fold harder than shortening-cookies, but less crispy, which is expected for cookies with higher spread ratios.

Finally, we evaluated the color parameters of cookies (Table 3), an important quality attribute of food products, influencing consumer choices and preferences. Moreover, the formulation of baking products may affect the rates and pathways of chemical reactions that take place during baking, such as Maillard reaction and Streacker degradation, and both can lead to formation of pigments known as non-enzymatic browing. Therefore, monitoring the instrumental color of cookies can be a proxy of these browning reactions that lead to desirable changes in color of baked products. Furthermore, color changes in oleogel-cookies can provide information about the behavior of chitosan-oleogels during baking and the perception of these changes in the human eye (Pathare, Opara, & Al-Said, 2013). In addition, the chitosan-oleogels showed a yellow color that might cause color differences in the final product.

Table 3

Physicochemical and mechanical properties of cookies with replacement of vegetal shortening by crosslinked-chitosan-based oleogels or canola oil.

Measures	Cookies Formulations[1]					
	Shortening	Canola oil	1.0% Vanillin	3.0% Vanillin	1.0% Vanillin + Tween®60	3.0% Vanillin + Tween®60
Physical characteristics						
Weight (g)	11.2 ± 0.61^{a}	12.0 ± 0.26^{a}	10.2 ± 0.11^{b}	8.12 ± 0.94^{c}	8.04 ± 0.26^{c}	10.3 ± 0.67^{b}
Diameter (cm)	6.34 ± 0.15^{a}	6.04 ± 0.18^{b}	5.84 ± 0.13^{b}	5.06 ± 0.19^{d}	4.94 ± 0.04^{d}	5.28 ± 0.04^{c}
Thickness (cm)	0.97 ± 0.01^{b}	0.58 ± 0.09^{c}	0.92 ± 0.04^{b}	1.03 ± 0.04^{a}	0.91 ± 0.03^{b}	1.11 ± 0.04^{a}
Spread ratio	6.56 ± 0.14^{b}	11.5 ± 1.06^{a}	6.35 ± 0.36^{b}	4.90 ± 0.14^{c}	5.44 ± 0.14^{bc}	4.75 ± 0.11^{c}
Spread factor (%)	–	175.8	96.8	74.4	82.8	72.3
Proximate composition *(g/100 g)*						
Moisture	4.74 ± 0.08^{f}	5.78 ± 0.05^{c}	5.36 ± 0.13^{d}	6.02 ± 0.01^{b}	5.10 ± 0.03^{e}	7.29 ± 0.07^{a}
Total lipid[2]	21.5 ± 0.07^{a}	19.8	19.9 ± 0.05^{a}	20.2 ± 0.25^{a}	20.7 ± 0.43^{a}	21.7 ± 1.42^{a}
Protein	6.09 ± 0.29^{c}	5.34	6.97 ± 0.22^{ab}	7.11 ± 0.03^{a}	6.67 ± 0.23^{bc}	6.50 ± 0.23^{bc}
Ash	1.38 ± 0.07^{c}	0.39	1.51 ± 0.11^{c}	1.79 ± 0.12^{ab}	1.60 ± 0.12^{bc}	2.00 ± 0.09^{a}
Carbohydrate	71.0	59.6	71.6	70.9	70.9	64.6
Energy value (kcal/100 g)	478.1	434.9	467.1	464.3	471.9	464.4
Instrumental color						
L^*	59.2 ± 0.67^{c}	68.4 ± 0.87^{a}	57.4 ± 0.78^{d}	58.9 ± 0.63^{c}	56.6 ± 0.38^{e}	61.2 ± 1.20^{b}
a^*	4.45 ± 0.01^{b}	-1.62 ± 0.15^{e}	5.87 ± 0.34^{a}	5.99 ± 0.55^{a}	5.93 ± 0.31^{a}	3.77 ± 0.39^{d}
b^*	28.9 ± 1.14^{d}	26.7 ± 0.70^{e}	31.1 ± 0.29^{c}	33.7 ± 0.99^{a}	30.9 ± 0.42^{c}	32.9 ± 0.94^{b}
ΔE	–	11.2	3.17	5.09	3.63	4.57
h°	81.2	86.5	79.3	79.9	79.1	83.4
Instrumental texture						
Hardness (N)	24.7 ± 1.59^{c}	61.4 ± 6.35^{a}	26.9 ± 1.60^{c}	54.1 ± 4.22^{b}	25.5 ± 2.21^{c}	28.1 ± 1.73^{c}
Crispness[3]	10.5 ± 0.61^{b}	6.42 ± 0.69^{d}	9.10 ± 0.49^{c}	27.3 ± 0.95^{a}	9.01 ± 0.45^{c}	9.50 ± 0.09^{bc}

Results are expressed as mean ± standard deviation of triplicates (n = 12 cookie from two baking processes for each formulation). [1] 0.75% (w/v) chitosan, and 0.4% (w/w) Tween®60, when added. [2]Promixate composition of control-cookies with canola oil were calculated based on the Food Brazilian Food Composition Table available at http://www.fcf.usp.br/tbca. Different superscript letters in the same line indicate significant differences among control-cookies with shortening or canola oil-cookies and cookies with oleogels. ANOVA one-way followed by Duncan post-test ($p < 0.05$). [3]Dimensionless.

There was a significant difference in the lightness (L* value) between the cookies. Oil-cookies and oleogel-cookies with 3.0% vanillin (with Tween®60) showed a lighter surface color than shortening-cookies. Otherwise, oleogel-cookies with 1% vanillin were darker. Moreover, oleogel-cookies had a significantly higher redness surface color than shortening-cookies and oil-cookies (mainly), except when chitosan-based oleogel with 3% vanillin (with Tween®60) was used as a solid fat replacer. All oleogel-cookies showed a more yellowish surface color than shortening- and oil-cookies.

During baking, browning coloration is an important color change due to non-enzymatic browning, mainly attributed to the Maillard reaction that takes place between reducing sugars and amino acids, producing brown-yellow pigments. In this sense, the baking temperature (near 180 °C) might have accelerated chemical reactions between the free vanillin in the oleogels and other compounds formed during baking, creating new Schiff bases that subsequently lead to the formation of new browning pigments (Laceta, Guerrero, & de la Caba, 2013). This phenomenon was more intense in cookies that were replaced with a chitosan-based oleogel with 3% vanillin than in those with 1% vanillin.

Oil-cookies showed the highest total color difference ($\Delta E = 11.2$) compared to shortening-cookies, followed by cookies replaced with oleogels containing 3% vanillin (with and without Tween®60) and 1% vanillin (with and without Tween®60). $\Delta E > 3$ indicates very distinct colors, which are more perceivable at increasing values. Therefore, the oil-cookies exhibited a notable color difference. However, although oleogel-cookies with 3% vanillin showed ΔE near 5.0, it was barely noticeable with the naked eye (Fig. 6). Even so, a ΔE value < 3 is preferable as a sign of color stability because it is only perceptive through close observation (Pathare et al., 2013). Therefore, crosslinked-oleogels with 1% vanillin (especially Tween®60-free) provided the cookies closest to this attribute, suggesting a total color appearance most similar to cookies made with shortening.

In the present work, crosslinked-chitosan-based oleogels containing 1.0% vanillin showed the best technological performance as a shortening replacement in a proof-of-application using cookies. However, further optimization of the chitosan-based oleogel formulation to match all the quality properties of a desirable fat replacer is needed. In addition, future sensory analyses should be carried out to validate the consumer perspective.

4. Conclusion

The emulsion-templated approach combined with the crosslinking of chitosan with vanillin proved to be an effective approach to physically entrap oil in the chitosan matrix, creating chitosan-based oleogels (independent of Tween®60 presence as an emulsifier). The mechanical properties of the chitosan-oleogels were modified by vanillin and Tween®60 content. At a low concentration (1.0%), vanillin structured stronger and more mechanically stable chitosan-based oleogels, whereas at a high concentration (3.0%), the oleogels showed a less stable tridimensional network due to the excess of vanillin crystals. The use of Tween®60 modified the strength of the network, resulting in harder and more adhesive oleogels, but with a low recovery structure after deformation. Herein, we conducted an exploratory study to provide convincing evidence for the development of edible chitosan-based oleogels. Future investigations aimed at elucidating the chemical and physical interactions between chitosan, vanillin, and Tween®60 to tailor the mechanical properties of chitosan-oleogels are welcome and should further help to design a versatile fat-replacer. From the present work, it is clear that crosslinked-chitosan-based oleogel containing 1.0% vanillin is promising candidate as saturated or *trans*-fat replacers in processed foods and have been successfully used in a proof-of-application set of experiments with cookies.

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CRediT authorship contribution statement

Gabriela B. Brito: Methodology, Formal analysis, Investigation, Writing – original draft. **Vanessa O. Di-Sarli:** Investigation. **Matheus T. Martins:** Investigation. **Denes K.A. Rosário:** Investigation. **Juliana N. Ract:** Investigation, Writing – review & editing. **Carlos A. Conte-Júnior:** Writing – review & editing. **Alexandre G. Torres:** Writing – review & editing. **Vanessa N. Castelo-Branco:** Conceptualization, Writing – review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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