Food Biophysics (2021) 16:306–316
https://doi.org/10.1007/s11483-020-09661-9

ORIGINAL ARTICLE

Storage Stability of Oleogels Made from Monoglycerides and High Oleic Sunflower Oil

Anabella S. Giacomozzi [1,2] · María E. Carrín [1,2] · Camila A. Palla [1,2]

Received: 19 August 2020 / Accepted: 23 November 2020 / Published online: 26 March 2021

Abstract

The aim of this study was to investigate the effects of storage time on physicochemical properties of oleogels from high oleic sunflower oil and monoglycerides (6.6 wt%). Oleogels were stored for eight weeks at 5 °C and tested weekly. The analyzed properties were: oil binding capacity, elastic modulus described by parameters of a power law equation, textural properties (hardness, adhesiveness, and cohesiveness), melting behavior, mean crystals length (L_c), polymorphism, peroxide value, and color. The main physicochemical parameters were not significantly altered over the first three weeks of storage, evidencing some changes thereafter. Hardness, cohesiveness, elasticity and L_c were the most affected properties. In addition, oleogelation allowed to improve the oil oxidative stability. No changes in melting behavior nor in polymorphism were found during storage time. The results of this work contribute to a better knowledge of the storage stability of monoglycerides oleogels, improving prospects for using them as *trans*-free substitutes for foods production.

Keywords Crystal network · Oil binding capacity · Semisolid fat · Storage time · Physicochemical properties · Textural properties

Abbreviations

AD	adhesiveness
CO	cohesiveness
ΔH_M	melting enthalpy
D	fractal dimension
DSC	differential scanning calorimetry
G'	elastic modulus
GLC	gas liquid chromatography
HA	hardness
HOSO	high oleic sunflower oil
L_c	mean crystals length
MG	monoglycerides
OBC	oil binding capacity
PLM	polarized light microscopy
PV	peroxide value
S	span
T_o	melting onset temperature
T_p	melting peak temperature
XRD	X-ray diffraction
$\log G'_0$, $m_{G'}$, and n,	constants of the elastic modulus model.

✉ Camila A. Palla
cpalla@plapiqui.edu.ar

[1] Departamento de Ingeniería Química, Universidad Nacional del Sur (UNS), Bahía Blanca, Argentina

[2] Planta Piloto de Ingeniería Química - PLAPIQUI (UNS-CONICET), Bahía Blanca, Argentina

Introduction

In recent years, the harmful effects of the excessive consumption of high levels of saturated and *trans* fats on human health have been extensively demonstrated [1, 2]. The food industry and research communities have taken a clear trend towards the use of alternative processes and materials aiming to increase the nutritional properties of foods. Thus, there has been great interest in incorporating oleogels in food formulations. Oleogels are a novel class of structured lipids which may have the functionality of fats and the nutritional profile very similar to liquid oils [3]. These materials are formed as the result of the oil entrapment in a three-dimensional network of structuring molecules. In particular, oleogels obtained from monoglycerides (MG) are promising materials since the MG form crystalline networks that allow the formation of a solid structure that gives elasticity and avoids oil migration [4–7].

Lipids are essential components of food products, as they affect their physicochemical properties, structure, stability, and sensory quality. One of the most important challenges in

the replacement of solid fats is to develop healthy alternatives minimizing the impact on product organoleptic properties and consumer acceptance over time. A bulk fat consists of a crystalline network composed of nano-structural elements that make up the microstructure, which is the result of interactions of different types, as hydrogen bonds and van der Waals-London forces [8]. The crystal network is a dynamic entity undergoing many changes during storage [9]. Moreover, this network affects the rheological properties, which are extremely important in some foods, such as shortenings, margarines, chocolates, and other spreads. This is because many of the sensory attributes, such as elasticity, mouthfeel and texture, depend directly on the rheological properties [8, 10].

Due to the characteristics of the oleogelation process, it may not cause any changes in the fatty acid composition or isomery of the oils [10]. However, the most used procedure for oleogels preparation consists of mixing oil and gelator molecules under continuous agitation at relatively high temperature (≥ 80 °C), which may play a negative role in the final quality of these materials [11]. In fact, an important deteriorative reaction induced by heating and mixing oil is the lipid oxidation, which can produce adverse effects in the quality and shelf life of food products by changing not only the chemical composition but also the organoleptic characteristics (flavor and/or color) and textural properties [12].

Based on these considerations, the physicochemical stability of oleogels is a key feature for their applicability [13]. Taking into account that changes in physicochemical properties of oleogels occurring during the time from production to utilization can greatly affect the properties of foods where they are included, a deeper understanding of the storage stability of these materials is necessary. Although many studies have been done to understand the structure and properties of oleogels from MG and different vegetable oils, studies of their storage stability are relatively limited. Ojijo et al. [14] investigated changes in microstructural, thermal and rheological properties of oleogels from MG and olive oil during eight weeks of storage at 25 °C. They found that upon this storage period, the olive oil/MG networks showed a decrease in the size of microstructural elements and a consequent increase in the material hardness. Chen and Terentjev [4] explained the aging process by analyzing the melting behavior, polymorphism, and microstructure of MG/hazelnut oil mixtures. They demonstrated that the MG arranged in sub-alfa crystalline phases (26 °C) lose their emulsified ability in hydrophobic environments as consequence of gradual rearrangement of hydrogen bonds over time (5–7 days), leading to the leakage of oil from the crystalline network. Da Pieve et al. [15] studied the influence of MG oleogel structure on the oxidative stability of cod liver oil at 4 and 20 °C for up to 40 days, concluding this oil-structuring method can be a promising strategy to extend the shelf life of the oil. Yılmaz and Öğütcü [16] investigated oleogels from hazelnut oil with beeswax and MG stored at 4 °C for 3 months, proving that there was no important change in their textural properties and they were very stable against oxidation during storage.

We are particularly interested in the production of MG oleogels using high oleic sunflower oil (HOSO). This vegetable oil is considered a high-quality lipid source to obtain oleogels for food application due to its high oxidative stability, availability, and cost [17]. In previous studies, we obtained optimized oleogels from MG and HOSO with a high oil binding capacity and with elastic modulus and hardness values very close to the ones of a commercial semisolid fat product [5, 18]. Then, these materials were used as saturated and *trans* fat replacers in a muffin formulation. The obtained products showed improved quality in comparison with those obtained using the commercial semisolid fat with the added benefit of a healthier nutritional profile [18]. Based on these positives results, the aim of the present work was to study the stability of these oleogels by evaluating their physicochemical properties as well as structure and oxidation over eight weeks of storage at 5 °C. To the best of our knowledge, there is no study in the literature focused on the stability of oleogels from MG and HOSO, so this work provides new and novel results for these solid fat replacers.

Materials

High oleic sunflower oil (HOSO) was purchased from a local grocery store and Myverol 18–04 K SG, the mixture of saturated MG used as gelator agent, was kindly donated by Kerry (Ireland). The fatty acid composition of HOSO and MG was determined as fatty acid methyl esters (FAME) by GLC analysis according to AOCS Official Methods Ce2–66 and Ce1–62 (AOCS, 2009), being: C16:0 (3.48 ± 0.07%), C18:0 (2.37 ± 0.01%), C18:1 (87.09 ± 0.08%), and C18:2 (6.85 ± 0.02%) for HOSO, and C16:0 (43.91 ± 0.07%) and C18:0 (53.65 ± 0.07%) for MG. The melting point of MG (68.9 ± 0.2 °C) was determined by DSC.

Methods

Preparation of Oleogels

Oleogels were prepared with a 6.60 wt% of MG according to the methodology described by Giacomozzi et al. [18]. In this previous work, we obtained a set of optimal preparation conditions (MG concentration, stirring speed and cooling ambient air temperature) that allows to produce oleogels with similar properties to a commercial margarine. Briefly, the mixture of HOSO and MG was kept at 80 °C during 30 min under magnetic agitation at 200 rpm. Afterwards, the molten sample was transferred to rectangular containers (85 mm length, 54 mm

width, 35 mm height) and cooled under static conditions at a controlled cooling ambient air temperature (17.5 °C) in an incubator until complete crystallization was achieved (~150 min). Three independent preparations were performed for each evaluated time.

The obtained oleogels were stored in the closed containers in darkness at the usual storage temperature for this type of product (5 °C) [15, 16], and kept there until analysis.

Measurement of Oleogel Physicochemical Properties

The time zero (t = 0) point was defined when the first 24 h of oleogels storage at 5 °C were accomplished. This point was selected as t = 0 because it is the most reported tempering time in studies about oleogels characterization to ensure structure stabilization prior to further testing [19, 20]. The oleogels physicochemical properties were weekly measured at the temperature at which fats are typically used for products elaboration, 20 °C, unless specifically stated otherwise. For this purpose, samples were kept at a controlled ambient air temperature (20 °C) for 1 h until analysis [18]. They were evaluated over a time period of up to eight weeks. This time was selected based on a previous contribution focused on MG oleogels stability [14] and practical considerations. All the techniques described in the following sections were performed taking the oleogel samples from the containers where they were formed. The oleogel properties are highly dependent on the heat transfer experimented during its formation, which in turn depends on the container where the molten material is gelified. Thus, the mentioned methodology is necessary to ensure that materials with comparable characteristics are analyzed by the different techniques used, avoiding influences that could be generated by gelifying molten samples in different containers according to each technique [5, 18].

Elastic Modulus

An Anton Paar Physica MCR 301 rheometer (Anton Paar GmbH, Austria) was used for elastic modulus (G') measurements using a parallel plate geometry (50 mm diameter) and a computerized data acquisition system (Rheoplus/32 V3.40). Oleogel samples were taken from the containers by cutting thin sheets that were used to obtain disk-shaped samples (50 mm diameter, 3 mm height) using a round metallic cutter. Oscillatory frequency (ω) sweep tests were performed at 20 °C at a strain of 0.5%, where all samples were in the linear viscoelasticity region, using a frequency range of 10 to 100 rad/s. The gap was set to produce a normal force of 10 N. The obtained G' curves were adjusted using a power law model ($\log G' = \log G'_0 + m_{G'} \log \omega^n$) following the methodology described by Palla et al. [5]. The parameter $log\ G'_0$ represents the $log\ G'$ value at $\omega = 1$ rad/s, while $m_{G'}$ indicates the frequency dependent behavior of samples, and n determines the shape of the log G' curve (concave: $n > 1$, convex: $n < 1$, and linear: $n = 1$). The advantage of using this model is the possibility to obtain the parameters that describe the G' behavior over all the frequency range instead of at a specified ω value [5].

Textural Properties

Oleogel textural properties, hardness (HA), adhesiveness (AD), and cohesiveness (CO), were measured by applying a texture profile analysis (TPA) test using a Texture Analyzer TA Plus (Lloyd instruments, England) equipped with a 50 N load cell. The TPA test consisted of a two cycles penetration of the sample with 10 s waiting time between the cycles. A round cylindrical probe (12.5 mm diameter, 56 mm length) was used to penetrate into samples at 1 mm/s to a depth of 10 mm. Samples were kept at 20 °C for 1 h in their containers prior to textural measurements. TPA curves, recorded as Force (N) vs. time (s), were used to determinate the aforementioned mechanical parameters. HA (N) is defined as the maximum force recorded at the first penetration cycle. CO is calculated as the ratio of the positive force area under the second (W2) and first compressions (W1), and is associated with the strength of internal bonds in the sample. AD (N.s) is related to the negative force area of the first bite [21]. Measurements were performed in triplicate for each independent experiment to the same analyzed storage time.

Oil Binding Capacity

The oil binding capacity (OBC) of the oleogels as a function of storage time was determined by weighing approximately 1 g of oleogel in an Eppendorf tube. Then, samples were centrifuged at 9000 rpm for 15 min using a microcentrifuge (Giumelli z-127-D, Argentina). After centrifugation, the Eppendorf tube was turned over onto a tissue paper to drain the released oil from the sample for 3 min. OBC was calculated as function of percent of oil released from the sample after centrifugation [18]. Five replicates of each independent experiment to the same analyzed storage time were measured.

Melting Behavior

The melting behavior of oleogels was measured in a Discovery DSC equipment (TA Instruments, USA). Samples (10–15 mg) were hermetically sealed in aluminum pans and placed in the DSC. They were kept at 5 °C for 1 min and then melted to 80 °C at a rate of 5 °C/min. The thermograms were analyzed using the TRIOS software (TA Instruments, USA) to record the melting onset temperature (T_o), melting peak temperature (T_p), and the change in enthalpy associated with the melting process (ΔH_M).

Microstructure

The microstructure of the samples was evaluated using a 25X magnification objective in a Karl Zeiss optical microscope with polarized light (Phomi III Pol, Germany). A small amount of oleogel sample was placed between a slide and a cover slide. Considering that particle size distribution analysis is the most adequate approach to determine differences in MG crystals size when samples show not homogeneous size distribution and irregular shape [22], this technique was used to characterize oleogels microstructure. It was performed using the Image Pro-Plus software 7.0 (National Institutes of Health, Bethesda, MD, USA) and the mean crystals length (L_c) and the Span (S) were reported. L_c is defined as the particle size where half of the population resides below this value. It was calculated based on the individual MG crystals, even in the case of clusters formed by the aggregation of these crystals. S describes the width of the size distribution and is calculated as (D90 – D10)/D50, being D10, D50, and D90 the particle size where 10%, 50%, and 90% of the population, respectively, reside below these points [23]. At each specified storage time, five images were recorded for each independent experiment, and from each picture, at least five L_c measurements were taken; so, a total of 75 measurements per storage time were used to obtain a frequency histogram. This methodology was followed in order to obtain L_c values as representative for the individual needle-like crystals, even in the case of spherulitic clusters formed by the aggregation of these elongated crystals [6].

In addition, the fractal dimension (D) parameter was calculated for each storage time by analyzing the images with the FracLac plugin from Image Pro-Plus software 7.0 following the methodology described by Palla et al. [6].

Polymorphism

In order to diminish oil interference in diffraction analysis, oleogel samples were filtered prior to specific test. For this purpose, a small portion of oleogel was directly taken from the container with a spoon and immediately placed into a Büchner funnel and filtered using a glass microfiber filter (Whatman CAT N°1823–047, pore diameter 0.7 μm). Filtration was performed under vacuum for 30 min at room temperature (~20 °C). The crystals retained in the filter were collected in a beaker and used to determine polymorphism. Diffraction peaks in the scattering angle (2θ) region of 3–30° were obtained using a X'pert Pro-Diffractometer (Philips, The Netherlands) which had a single goniometer PW 1710 (θ/2–θ, voltage 45 kV, current 40 mA) using a Cu Anode X-ray tube. The short spacings are widely used to characterize the various polymorphic forms in the crystalline structure and refer to cross sectional packing of the hydrocarbon chain.

Peroxide Value

The oxidative stability of the oleogels and HOSO was determined by measuring the peroxide value (PV, meq O_2/kg) according to AOCS Official Methods Cd 8–53 [24].

Color

A HunterLab UltraScan XE spectrophotometric colorimeter (Hunter Associates Laboratory Inc., Reston, USA) was used to measure the total surface color of the oleogels, without removing them from their containers. The reflected color was measured at 10° observer angle with D65 illuminant and specular component excluded. Results were expressed in terms of the CIELab scale parameters: lightness (L*, 0: black / 100: white), redness (a*, − green / + red), and yellowness (b*, − blue / + yellow). Measurements were performed at three different points of the oleogel surface. The total color difference (ΔE*) between the color parameters of oleogels at t = 0 and those of the oleogels measured at subsequent storage times was calculated as follows: $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$. A value of $\Delta E^* < 3$ means that the differences are not visible to the human eye [25].

Statistical Analysis

Results are reported as mean ± standard deviation of the number of replicate measurements in one representative experiment of three independent experiments. Statistical analysis on the data was performed using the Infostat software [26]. Data were analyzed by one-way ANOVA and significant differences ($p < 0.05$) over storage time (t) were determined using the Fisher post-hoc test.

Results and Discussion

Elastic Modulus

Table 1 shows the evolution of the parameters used to describe the G' behavior, $\log G'_0$, $m_{G'}$, and n, of oleogels evaluated over eight weeks of storage at 5 °C. The R^2 values obtained from fitting of the G'curves over the frequency sweep test were higher than 0.992 for all the samples tested, indicating that the frequency dependence of G'can be properly adjusted by the power law model. The parameter $\log G'_0$, associated to the G' magnitude [5], did not show significant differences over the first four weeks of storage, evidencing a decrease from this point. However, this decrease did not continue, since no significant differences were observed in this parameter during the second month of storage. These results could be associated

Table 1 Evolution over time of the elastic modulus (G') model parameters ($\log G'_0$, $m_{G'}$, n), the oil binding capacity (OBC), and the fractal dimension (D) of the monoglycerides oleogels stored at 5 °C

Time (weeks)	$\log G'_0$ (Pa)	$m_{G'}$ (Pa.s)	n	OBC (%)	D
0	4.56 ± 0.03^{a}	0.044 ± 0.001^{c}	0.90 ± 0.05^{cd}	90.14 ± 0.45^{a}	2.73 ± 0.01^{a}
1	4.56 ± 0.04^{a}	0.046 ± 0.001^{bc}	0.82 ± 0.01^{d}	90.63 ± 0.36^{a}	2.73 ± 0.01^{a}
2	4.57 ± 0.02^{a}	0.046 ± 0.001^{bc}	0.88 ± 0.05^{cd}	90.72 ± 0.53^{a}	2.74 ± 0.01^{a}
3	4.53 ± 0.00^{ab}	0.055 ± 0.002^{a}	0.88 ± 0.05^{b}	90.51 ± 0.31^{a}	2.63 ± 0.01^{c}
4	4.55 ± 0.02^{a}	0.053 ± 0.004^{a}	0.96 ± 0.07^{ab}	88.56 ± 0.39^{b}	2.65 ± 0.01^{bc}
5	4.38 ± 0.06^{c}	0.042 ± 0.001^{c}	0.91 ± 0.01^{ab}	88.31 ± 0.56^{b}	2.66 ± 0.01^{b}
6	4.43 ± 0.02^{bc}	0.052 ± 0.001^{ab}	0.95 ± 0.03^{a}	88.12 ± 0.62^{b}	2.65 ± 0.01^{bc}
7	4.34 ± 0.02^{c}	0.053 ± 0.004^{a}	0.96 ± 0.01^{a}	87.99 ± 0.58^{b}	2.60 ± 0.01^{d}
8	4.38 ± 0.03^{c}	0.057 ± 0.005^{a}	0.88 ± 0.02^{bc}	87.93 ± 0.68^{b}	2.55 ± 0.01^{e}

Mean values ± standard deviation within the same column with different superscripts are significantly different ($p < 0.05$)

with a decrease in the strength of the bonds that stabilize the microstructure of the crystalline network [27]. Regarding to the parameter that defines the stability of the structure, the values found for $m_{G'}$ were low over the whole storage time studied, so it could be stated that the oleogels obtained over this storage time showed an elastic modulus with slight dependence upon frequency, which is typical of strong gels [5]. Although it was found that $m_{G'}$ values significantly increased, these changes were not enough to observe important modifications in the shape of G' curves (Fig. 1S). The parameter n showed some significant changes over storage time, but its final value did not show significant differences with the initial one.

Thus, based on the results of the G' model parameters, it is possible to ensure that the elasticity of the oleogels remained stable during the first four weeks of storage, and decreased (~35%) thereafter.

Textural Properties

Textural parameters measured in this work (HA, AD, CO) have been recognized as important indicators of oleogels potential as fat replacers [16, 18]. In addition, it was demonstrated that these textural properties instrumentally measured correlate well with sensory perceptions [28].

HA refers to the strength of the oleogel structure [21]. As it can be noted from Fig. 1, HA of MG oleogels significantly decreased over time from 1.58 ± 0.03 N at $t = 0$ to 0.83 ± 0.02 N at $t = 8$ weeks, even though no significant changes were found during the first three weeks of storage. It is important to point out that this finding provide support for the time period selected in this work. Since HA was significantly reduced over $t = 8$ weeks of storage, it would not worth measuring it thereafter. The stability of the oleogels structure is given by non-covalent bonds, such as hydrogen bonds and van der Waals interactions [29]. From the results shown in Fig. 1, it could be possible to think that the links between crystalline structures of different levels could be significantly affected after $t = 3$ weeks, causing a decrease in the total force that keeps the network structure of the oleogels and therefore generating a weaker crystalline network. Yılmaz and Ögütcü [16] reported similar results in their study of the hardness of oleogels from MG and olive oil stored for 90 days at 4 and 20 °C.

While a decrease of near 35% ($\Delta G'$) was found in the elastic modulus over the whole analyzed storage time (Table 1), HA was reduced to about 50% (ΔHA, Fig. 1(a)). These findings are consistent taking into account that hardness and rheology results provide different interpretations of the oleogel structure behavior. Narine and Marangoni [8] explained that HA is related to all levels of structure conforming the network, whereas G' is associated to the microstructural level of the structure. So, in this study near the 70% (calculated as the ratio $\Delta G'/\Delta HA$) of changes in all levels of structure conforming the network could be explained by the changes at the microstructural level.

AD represents the work required to overcome the sticky forces between the sample and the probe [21]. No significant differences were found in AD of the oleogels over storage time (Fig. 1(b)), with the exception of the samples at $t = 3$ and $t = 4$ weeks, where significantly higher AD values were observed. This increment in AD values could be associated with the decrement in the oil binding capacity determined at this point. However, after $t = 4$ weeks, the AD decreased even though OBC did not change. This was an unexpected behavior and may be related to other unidentified factors at higher storage times.

The effect of storage time on the CO of MG oleogels is shown in Fig. 1(b). This textural parameter significantly increased over time. CO is related to the strength of internal bonds making up the structure of oleogels and, therefore, it allows to characterize the global integrity of the product. These results were related to those obtained for HA. W1 is a

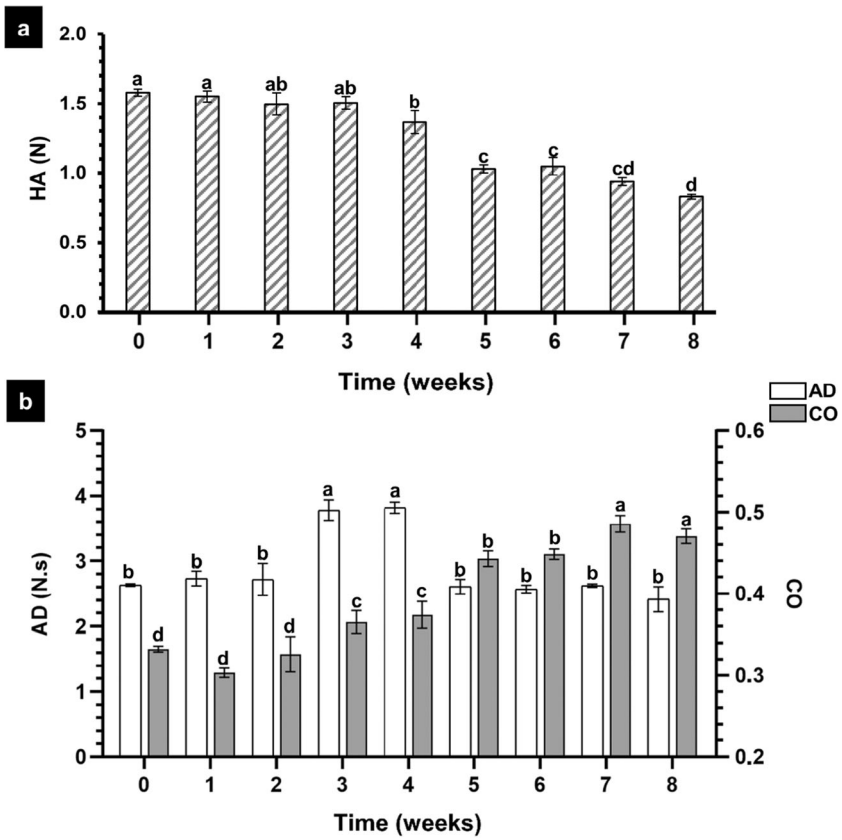

Fig. 1 Evolution over time of the (a) Hardness (HA) and (b) Adhesiveness (AD) and Cohesiveness (CO) of the monoglycerides oleogels stored at 5 °C. Same color bars with the same lowercase letters are not significantly different ($p > 0.05$). Error bars represent mean values ± standard deviations

measure of the network resistance showed in the first deformation work performed in the textural measurement and it is proportional to HA [22], whereas W2 gives information about the presence of more persistent bonds after the analysis [5]. From the analysis of W1 and W2 values, it was found that W1 significantly decreased over time from t = 0 to t = 8 week (from 8.20 ± 0.26 to 5.38 ± 0.35 N.s), in accordance with the significant change observed in HA over this period, whereas W2 did not change (from 2.85 ± 0.22 to 2.89 ± 0.13 N.s). As a consequence, the CO increased as W1 decreased. However, an important finding to emerge from W2 results is that the more persistent bonds conforming the MG oleogels remained stable during the analyzed period.

Oil Binding Capacity

OBC is one of the most important properties used to determine the physical stability of oleogels. It represents the ability of the crystalline matrix to entrap liquid oil efficiently [29]. The effect of storage time on the OBC of oleogels is presented in Table 1. It can be observed that the OBC of the oleogels did not show significant differences during the first three weeks of storage (average value: 90.5%), indicating that these materials had a stable network which avoids the oil migration over that period of time. It was observed a slight decrease (~ 3%) in the OBC of oleogels at t = 4 weeks, but no subsequent changes occurred thereafter, keeping an average value of 88.2%. This reduction could be associated with the increase in the AD values shown by oleogels at t = 3 and t = 4 weeks of storage. Furthermore, this finding partially agrees with those found for the hardness: after the first month of storage (t = 4 weeks), a possible decrease in the strength of the bonds that keep the crystalline network could reduce the stability of the structure leading to a lower capacity to retain oil during the centrifugation process. Taking into account that OBC is one of the key factors determining the performance of a new fat formulation [30], these results would allow to ensure that MG oleogels stored for less than three weeks from their production have potential to be used as fat replacers in the food industry.

Melting Behavior

The melting behavior of oleogels stored at 5 °C for eight weeks is shown in Fig. 2S. The thermograms of the initial and stored oleogels showed a single endothermic peak, which corresponds to the melting of the MG.

No significant differences ($p > 0.05$) in the parameters associated with the melting behavior of oleogels (ΔH_M, T_o and T_p) were identified among the different storage times (Table 2), indicating that there was no change in the amount of crystallized material during storage. Moreover, no differences in ΔH_M over storage times indicates that the relation

Table 2 Evolution over time of the melting behavior parameters: enthalpy (ΔH_M), onset temperature (T_o) and peak temperature (T_p), and the color parameters (L*, a*, b*) of the monoglycerides oleogels stored at 5 °C

Time (weeks)	ΔH_M (J/g)	T_o (°C)	T_p (°C)	L*	a*	b*
0	9.69 ± 0.27^a	54.68 ± 0.95^a	60.42 ± 1.23^a	45.6 ± 0.3^a	−4.0 ± 0.0^b	3.0 ± 0.1^d
1	10.42 ± 0.84^a	55.60 ± 0.21^a	60.90 ± 0.88^a	45.8 ± 0.3^a	−3.9 ± 0.0^b	2.9 ± 0.0^d
2	10.27 ± 0.46^a	54.25 ± 1.20^a	61.53 ± 0.42^a	45.8 ± 0.5^a	−4.0 ± 0.0^b	3.0 ± 0.1^d
3	9.59 ± 0.27^a	52.94 ± 0.82^a	61.29 ± 0.15^a	46.0 ± 0.1^a	−4.0 ± 0.1^b	3.6 ± 0.1^b
4	10.65 ± 0.97^a	52.99 ± 0.21^a	61.59 ± 0.76^a	44.4 ± 0.0^b	−4.1 ± 0.1^b	3.4 ± 0.1^c
5	10.30 ± 1.32^a	54.38 ± 0.48^a	62.38 ± 0.10^a	43.8 ± 0.2^{bc}	−4.0 ± 0.0^b	3.9 ± 0.1^a
6	10.32 ± 1.49^a	55.09 ± 2.05^a	60.87 ± 2.18^a	43.6 ± 0.2^c	−4.4 ± 0.0^a	3.0 ± 0.0^d
7	10.45 ± 1.19^a	54.18 ± 0.59^a	61.96 ± 0.70^a	43.7 ± 0.6^{bc}	−4.3 ± 0.1^a	2.6 ± 0.2^e
8	9.97 ± 0.08^a	54.35 ± 0.99^a	61.56 ± 0.42^a	43.5 ± 0.4^c	−4.3 ± 0.0^a	2.9 ± 0.1^d

Mean values ± standard deviation within the same column with different superscripts are significantly different ($p < 0.05$)

between ΔH_M of aged oleogel ($t > 0$) and ΔH_M of initial ($t = 0$) oleogel, known as coagel index, would be maintained around 1, reflecting the thermodynamic stability of the analyzed material [4]. Similar results were observed by Ojijo et al. [14] when they studied the effects of storage for nine weeks at 25 °C on thermal characteristics of oleogels from olive oil and MG.

Microstructure

Figures 2(a) and (b) show the PLM images and the crystals size distributions, respectively, of oleogels kept at 5 °C over eight weeks of storage. L_c and S were the parameters used to describe the crystal size distribution. As it can be observed from these figures, MG crystals appeared as irregular elongated, needle-like arrangements composing the oleogel matrix dispersed in the black liquid oil as the background. It is possible to identify a certain degree of heterogeneity in the crystals size, evidenced by the polydispersity found in the crystals size distribution for each storage time.

During the analyzed storage period, L_c decreased from 31.6 μm ($t = 0$) to 18.4 μm ($t = 8$ weeks), which resulted in a decrease in crystals size of approximately 40%; however, some variations to this behavior were observed in the overall storage period. Regarding to S values, which is an indicator of the width of a particle size distribution, the results showed that, in general, all oleogels samples exhibited narrow distributions of crystal size [23]. The oleogel samples stored for $t = 5$ weeks showed the highest S value, which implies a lower degree of uniformity of the crystals that compose this network [31]. However, no trend was identified in the analyzed period. Palla et al. [6] reported the influence of L_c on oil migration through the network of oleogels from MG. These authors concluded that OBC increases as L_c decreases as a result of the increase in the surface area available to trap a larger amount of oil, evidenced by the higher fractal dimension (D) values. This relationship between L_c and OBC was not verified in the present work. Likewise, the decrease in L_c was not reflected in an increase in the hardness of the oleogels as it has been observed by other authors [32, 33]. However, it should be noted that these authors evaluated the change in the crystal size during the gelation process, whereas in the present work we analyzed the effect of storage on the network structure already formed. This finding would support the previously mentioned idea that the decrease in hardness and OBC could be associated to changes in the interactions at another structural level of the crystalline network.

On the other hand, D is known as a structural parameter of the network which provides a numerical indication of the homogeneity of the crystalline mass distribution within the oleogel network [8, 34]. It was observed a slight reduction in D over time (Table 1), reaching a reduction of 6.6% at the eighth week. According to Narine and Marangoni [8], this parameter is an important indicator of the elasticity of the network, and therefore an indicator of the hardness of the crystal network, which was confirmed in this work by comparing the decrease observed in D values with those in G' and HA. Considering that lower fractal dimensions indicate that the network structure presents a more disordered molecular organization with less evenly distributed (or clustered) mass [8], the oleogels structure showed a less homogeneous packing order of the microstructural elements after $t = 3$ weeks of storage. It allows to think that D would be a better estimative parameter than L_c to find a relationship between microscopic and macroscopic behavior.

Polymorphism

The XRD analysis was performed with the aim to study the effect of storage time on the polymorphic forms present in the oleogel structures, which is associated with the molecular organization of MG crystals.

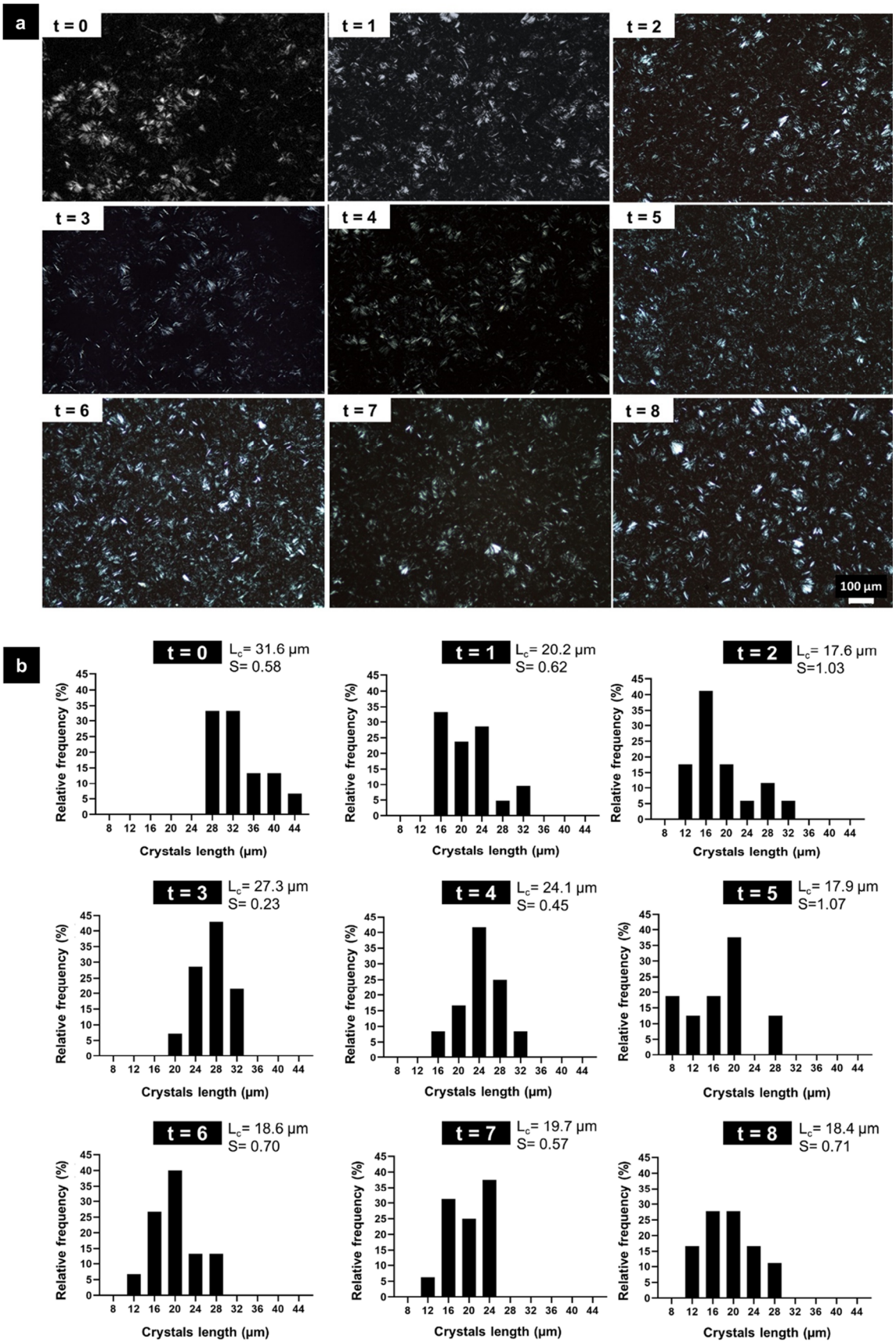

Fig. 2 (a) Polarized light microscopy (PLM) images and (b) Crystals size distributions of the monoglycerides oleogels stored at 5 °C over different storage times (t = weeks). PLM images were taken at 25X magnification. L_c: mean crystals length, S: span

The XRD patterns of the stored oleogels are presented in the Fig. 3. The polymorphic form of the crystals can be identified as: i) α, characterized by a short spacing at 4.15 Å, ii) β′, characterized by short spacings at 3.8 and 4.2 Å, and iii) β, if the peaks do not satisfy the conditions for α and β′ and has a strong short spacing at 4.6 Å [35]. Thus, four peaks were identified at 2θ = 25.0, 23.8, 22.8, and 20.4, which correspond to the characteristics short spacings of the β′ form (3.6, 3.8, 3.9 and 4.3 Å). The polymorphic forms α (2θ = 21.4°, d = 4.15 Å) and β (2θ = 19.3°, d = 4.60 Å) were not identified. Moreover, there was no change in the patterns between t = 0 and t = 8 weeks, indicating that the storage at 5 °C during eight weeks did not affect neither inter-plane spacing nor the subcell packing of the MG crystals [15]. This allow to affirm that the oleogel's nanoscale structure was kept stable. This finding was in agreement with those previously reported by DSC analysis, in which no changes were observed in the melting behavior of the stored oleogels. In contrast to these findings, Chen and Terentjev [4] reported that the MG molecules rearranged into the structure of the β polymorphic form over five days of storage at 26 °C, resulting in a denser structural packing that weakened the ability of MG crystals to retain oil and, as consequence, the system lost its rheological nature of a gel.

The presence of the β′ form in monoglycerides oleogels has been also identified by Ferro et al. [36] in oleogels formulated with sunflower oil and 5 wt% of glyceryl monostearate. This type of polymorphic form provides to fats the desired functionality to be incorporated in some foods, especially for aerated products where a smooth structure is required. Therefore, the β′ polymorphic form observed in these oleogels would allow the obtention of a material with similar structure to margarines and spreads, and thus MG oleogels could be used as effective fat replacers in the food industry.

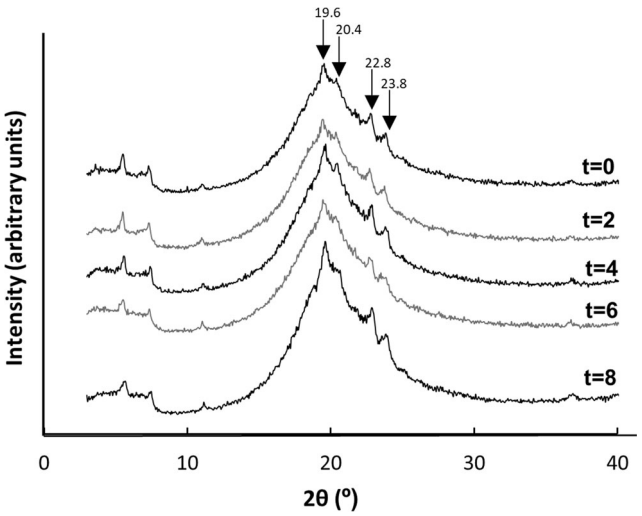

Fig. 3 Effect of storage time (t = weeks) on the X-ray diffraction pattern of monoglycerides oleogels stored at 5 °C

Peroxide Value

The oxidative stability of stored oleogels was evaluated by the PV measurement. The oxidative stability of stored HOSO was also determined every two weeks for a total of t = 8 weeks in order to analyze the effect of the oleogelation process on the oil oxidation.

All oleogeles samples showed very low PV. A significant increase ($p < 0.05$) in the PV of oleogels was observed from t = 0 to t = 8 weeks, remaining unchanged during the first month of storage (Fig. 4). Moreover, it was found that oleogels presented significantly lower ($p < 0.05$) PV than HOSO from at least the fourth week of storage, representing a reduction from 16% at t = 4 weeks until 9% at t = 8 weeks, respectively. This means that the oxidative stability of HOSO was improved by structuration through MG self-assembly, indicating that the structural characteristics of MG oleogels may delay the occurrence of the initial stages of oxidation reactions. Taking into account that the rate of peroxide formation depends on the availability of oxygen in the system, the presence of the MG crystalline network would represent a barrier that obstruct the entry of oxygen at the reaction sites. Similar results have been previously reported by Yılmaz and Ögütcü [16] in their study about oleogels based on hazelnut oil, MG and beeswax stored for three weeks at 5 °C.

Color

The color is a critical quality parameter of processed foods related to consumer's acceptance. The color of the oleogels is usually dependent on the liquid oil used [16]. Table 2 shows the initial values and the evolution over time of the surface

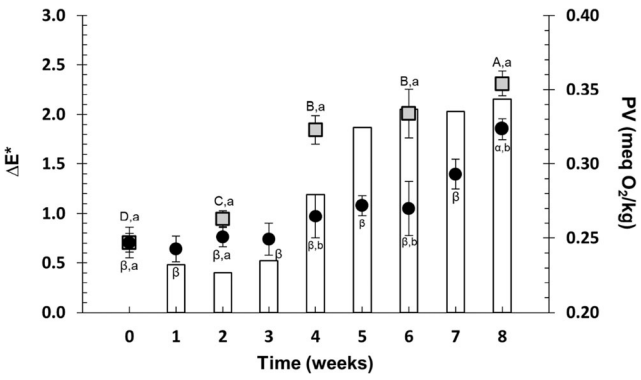

Fig. 4 Evolution over time (t) of the total color difference (ΔE*, white columns) between the color parameters of oleogels at t = 0 and those of the oleogels measured at t > 0, and of the peroxide value (PV) of the monoglycerides oleogels (black circle symbols) and high oleic sunflower oil (HOSO) (grey square symbols) stored at 5 °C. Error bars in PV values indicate standard deviation. Same capital and greek letters indicate non-significant differences ($p > 0.05$) between values at different storage times for HOSO and oleogels, respectively. Same lowercase letters indicate non-significant differences ($p > 0.05$) between oleogeles and HOSO within the same storage time

color of the oleogels stored at 5 °C, expressed according to the CIELab scale. The measured values were similar to those previously reported by Gur et al. [37] for oleogels from HOSO, waxes, and MG. Regarding the effect of time, it was found a slight but significant decrease in L* from t = 0 to t = 8 weeks, indicating that oleogel lightness was reduced. No significant differences were found in the a* values over the first weeks of storage. However, the redness increased, in terms of its absolute values, at t = 6 weeks. The parameter b* increased from t = 0 and reached a maximum value at t = 5 weeks, decreasing slightly from this week until reaching a value similar to the initial one. We hypothesized that the changes in the color parameters could be related at least to two identified factors. Firstly, it could be associated with the peroxide content. As it can be seen from Fig. 4, ΔE* increased as PV raised. As previously mentioned, lipid oxidation produces the deterioration of food products by altering their organoleptic characteristic, such as color. The primary oxidation products, lipid hydroperoxides, exist only transiently and decompose to alkoxy radicals and then form aldehydes, ketones, acids, esters, alcohols, and short-chain hydrocarbons. All these compounds are responsible for the color changes in the oxidized edible oils [38]. In addition, changes in the oleogel microstructure (size and distribution of MG crystals) could alter the way or amount of reflected light and so, the color parameters. However, it is important to mention that even though some significant changes in the surface color of oleogels were found over storage time, the value of ΔE* was lower than 3 in all samples (Fig. 4), so these changes could not be visible to the human eye [25].

Conclusions

Results demonstrated that the studied monoglycerides oleogels showed great stability during at least the first three weeks of storage at 5 °C, evidencing no significant changes in their main physicochemical properties, such as oil binding capacity, hardness, elasticity, and color. From a structural perspective, it is likely that interaction forces between the structural elements that compose the oleogels network structure have remained stable over the first three weeks of storage. However, it seems they were weakened and/or changed thereafter, evidenced by the results from rheology, textural, OBC, and microstructure analysis. Nevertheless, no changes neither the polymorphism nor melting behavior were observed over storage, indicating that crystals packing remained thermodynamically stable.

Overall, the hardness was the most affected among all analyzed properties, since it was reduced by half from its initial to final value after eight weeks of storage at 5 °C. Even though significant changes were observed in the crystals size and the elastic modulus, oil binding capacity slightly decreased. The β′ form was the predominant arrangement, which is the desired polymorphism in fat substitutes where margarine-like functionality is sought. As an important result, it was found that the oxidation process was reduced by structuration of the high oleic sunflower oil with monoglycerides.

These findings allow to estimate that studied oleogels stored at 5 °C by a maximum period of three weeks could be incorporated in food formulations generating final products of similar quality. However, additional work will be necessary to corroborate this idea as well as to completely understand the interactions of stored oleogels, especially those stored by more than three weeks, with other ingredients composing a food matrix.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11483-020-09661-9.

Acknowledgments The authors acknowledge the financial support by the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, PIP 2015-2017 N°0156), the Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT, PICT 2016-0520, PICT 2017-1522), and the Universidad Nacional del Sur (UNS, PGI 24/M152) in Argentina.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

References

1. R. Chowdhury, S. Warnakula, S. Kunutsor, F. Crowe, H.A. Ward, L. Johnson, O.H. Franco, A.S. Butterworth, N.G. Forouhi, S.G. Thompson, K.T. Khaw, D. Mozaffarian, J. Danesh, E. di Angelantonio, Ann. Intern. Med. **160**(6), 398–406 (2014)
2. F.C. Wang, A.J. Gravelle, A. Blake, A.G. Marangoni, Curr. Opin. Food Sci. **7**, 27–34 (2016)
3. A. R. Patel, Alternative Routes to Oil Structuring. (Springer, Switzerland, 2015)
4. C.H. Chen, E.M. Terentjev, Langmuir **25**(12), 6717–6724 (2009)
5. C. Palla, A. Giacomozzi, D. Genovese, M.E. Carrín, Food Struct. **12**, 1–14 (2017)
6. C. Palla, J. de Vicente, M.E. Carrín, M.J.G. Ruiz, Food Res. Int. **125**, 108613 (2019)
7. J. Rosen-Kligvasser, M. Davidovich-Pinhas, Food Chem. 127585 (2020)
8. S.S. Narine, A. Marangoni, Food Res. Int. **32**(4), 227–248 (1999)
9. L.S.K. Dassanayake, D.R. Kodali, S. Ueno, Curr. Opin. Colloid Interface Sci. **16**(5), 432–439 (2011)
10. A. G. Marangoni, N. Garti, Edible Oleogels: Structure and health implications, 1st edn. (AOCS Press Urbana, 2011), pp. 1–18
11. J. Lim, H.S. Hwang, S. Lee, Appl. Biol. Chem. **60**(1), 17–22 (2017)
12. F. D. Gunstone, J. L. Harwood, A. J. Dijkstra, The lipid handbook with CD-ROM. (CRC press, 2007)
13. I.F. Almeida, M.F. Bahia, Int. J. Pharm. **327**(1–2), 73–77 (2006)
14. N.K. Ojijo, E. Kesselman, V. Shuster, et al., Food Res. Int. **37**(4), 385–393 (2004)
15. S. Da Pieve, S. Calligaris, A. Panozzo, G. Arrighetti, M.C. Nicoli, Food Res. Int. **44**(9), 2978–2983 (2011)

16. E. Yılmaz, M. Öğütcü, J. Am, Oil Chem. Soc. **91**(6), 1007–1017 (2014)
17. K.F. Chaves, D. Barrera-Arellano, A.P.B. Ribeiro, Food Res. Int. **105**, 863–872 (2018)
18. A. Giacomozzi, M.E. Carrín, C. Palla, J. Food Sci. **83**(6), 1505–1515 (2018)
19. S. Da Pieve, S. Calligaris, M.C. Nicoli, A.G. Marangoni, Food Biophys. **5**(3), 211–217 (2010)
20. O. G. Rocha-Amador, J. A. Gallegos-Infante, Q. Huang, N. E. Rocha-Guzman, M. R. Moreno-Jimenez, R. F. Gonzalez-Laredo, Int. J. Food Sci. **2014** (2014)
21. M. Bourne, Food texture and viscosity: concept and measurement. (Elsevier, 2002)
22. A. Giacomozzi, C. Palla, M.E. Carrín, S. Martini, J. Food Sci. **84**(9), 2549–2561 (2019)
23. A.L.R. Rattes, W.P. Oliveira, Powder Technol. **171**(1), 7–14 (2007)
24. AOCS, Official Methods and Recommended Practices of the American Oil Chemists' Society, 6th edn. (Champaign, USA, 2009)
25. F. J. Francis, F. M. Clydesdale, Food colorimetry: theory and applications. (AVI Publishing Co. Inc., 1975)
26. A. J. Di Rienzo, F. Casanoves, M. G. Balzarini, L. Gonzalez, M. Tablada, C. W. Robledo, InfoStat version 2011. (Universidad Nacional de Cordoba, 2011), http://www.infostat.com.ar. Accessed 05 March 2020
27. C.D. Doan, D. Van de Walle, K. Dewettinck, A.R. Patel, J. Am, Oil Chem. Soc. **92**(6), 801–811 (2015)
28. E. Yılmaz, M. Öğütcü, Food Funct. **6**(4), 1194–1204 (2015)
29. E.D. Co, A.G. Marangoni, J. Am, Oil Chem. Soc. **89**(5), 749–780 (2012)
30. C.D. Doan, A.R. Patel, I. Tavernier, N. de Clercq, K. van Raemdonck, D. van de Walle, C. Delbaere, K. Dewettinck, Eur. J. Lipid Sci. Technol. **118**(12), 1903–1914 (2016)
31. S. Dyankova, M. Doneva, Y. Todorov, M. Terziyska, IOSR J. Pharm. **6**, 1–8 (2016)
32. J. Toro-Vazquez, J.A. Morales-Rueda, E. Dibildox-Alvarado, M. Charó-Alonso, M. Alonzo-Macias, M.M. González-Chávez, J. Am, Oil Chem. Soc. **84**(11), 989–1000 (2007)
33. A.I. Blake, A.G. Marangoni, J. Am, Oil Chem. Soc. **91**(6), 885–903 (2014)
34. A.I. Blake, A.G. Marangoni, Food Biophys. **10**(4), 403–415 (2015)
35. Y. Pomeranz, Functional properties of food components, 2nd ed. (Academic Press Inc., 2012)
36. A.C. Ferro, P.K. Okuro, A. Badan, R.L. Cunha, Food Res. Int. **120**, 610–619 (2019)
37. S.O. Gur, A. Zbikowska, M. Przybysz, M. Kowalska, Mater. Plast. **54**(4), 800–805 (2017)
38. E. Choe, D.B. Min, Compr. Rev. Food Sci. Food Saf. **5**(4), 169–186 (2006)