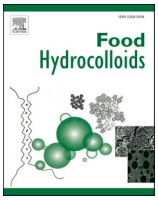
Food Hydrocolloids 143 (2023) 108893

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd



Food-grade bigels: Evaluation of hydrogel:oleogel ratio and gelator concentration on their physicochemical properties

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ARTICLE INFO

Keywords:
Food ingredient
Multi-component
Biphasic gel
Biopolymers
Organogel
Fat replacement

ABSTRACT

Soft-matter formulations like bigels (i.e., hybrid systems) usually exhibit superior properties than single-structured emulsion gels. In this study, a bigel system was developed using a carrageenan/locust bean gum-based hydrogel (1:1 ratio with concentrations of 0.5–2.5 wt%) and a glyceryl monostearate-based oleogel (5, 10 and 20 wt%), obtaining self-standing semi-solid structures. Solvent holding capabilities, micro-structural, rheological and textural properties, a X-ray diffraction and infrared spectroscopy (FTIR) were accessed. Furthermore, bigel formulations starting at 70 wt% of hydrogel fraction showed elevated structural matrix continuity, linked to self-standing ability. The most balanced properties, concerning microstructural stability were exhibited by the formulations containing 2 wt% of biopolymers. These formulations also demonstrate an increased capacity to arrest the oil phase even compared to formulations with high biopolymer concentrations. Rheology studies showed a certain level of destabilization among the sol-gel transition at higher temperatures for high polymer concentrations. The higher storage and loss modulus values were recorded at the end of the non-isothermal sweeps and positively correlated with the glyceryl monostearate (GM) concentration. Despite that, texture analysis did not evidence any increase of bigel hardness when concentrations of GM surpassed 5% (w/w), probably due to a lack of interfacial stabilization. Major differences in bigel hardness due to increased oleogel content were only seen for higher biopolymer concentrations. Also, the non-chemical arrangement was confirmed through FTIR. These results guide the development of bigel systems towards their use in novel food products.

1. Introduction

The widespread consumption of trans and saturated fats is associated with a higher prevalence of cardiovascular disease and other adverse consequences, such as detrimental effects on lipoprotein profile, increased body weight, augmented inflammation and oxidative stress, as well as type 2 diabetes and metabolic syndrome (López-Pedrouso et al., 2021; Roche, 2005). Partially hydrogenated oils were determined not Generally Recognised as Safe (GRAS) by the U.S Food and Drug Administration (FDA), and from 2015 on, efforts have been made to promote the substitution of animal fat for healthier alternatives (Bhandari et al., 2020). In line with that, the European Food Safety Authority (EFSA) produced a report on the consistent association between higher intakes of *trans*-fats and the increased risk of coronary heart disease (Authority, 2018). Fat-like mimics are sought now by academia and industry to answer the demands on health and sustainability issues, and oleogelation technologies seem to be in place to reach the desired purposes. At this point, the main challenge is to use food-grade gelators and materials in a straightforward manner in certain food products. To address that need, we aimed to develop a fat-mimetic material with tailoring capabilities concerning their mechanical and functional properties.

3D building blocks with a high oil binding capacity, able to form thermo-reversible oleogels, are the most suitable molecules to perform oil gelation. Edible gelators like natural waxes, shellac, mono and di-glycerides, as well as lecithin and sorbitol tristearates, long-chain fatty

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https://doi.org/10.1016/j.foodhyd.2023.108893
Received 28 December 2022; Received in revised form 11 May 2023; Accepted 17 May 2023
Available online 17 May 2023
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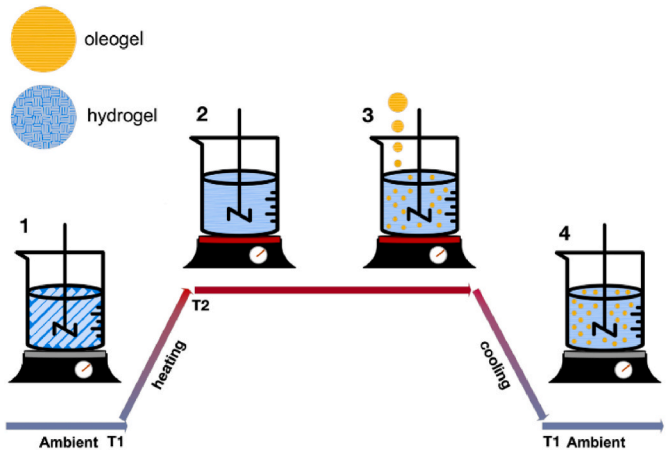

Fig. 1. Different stages and set-up procedures used for the development of bigels: (1) biopolymers dispersion; (2) stabilization at high temperature (90 ± 2 °C); (3) slow dispersion of heated oleogel phase at 2000 rpm, (4) homogenization of the mixture during 10 min.

acids, fatty alcohols and their mixtures and phytosterols can form stable oleogels, therefore are considered as the most promising food-grade oleogelators (Blach et al., 2016; Manzoor et al., 2022). However, the ability to produce fat-like materials with increasing versatility can also be fostered using bigel systems, where both aqueous and oily phases are gelled. This enables the droplet entrapment within a 3D network and as a consequence, no aggregation or flocculation occurs (Lupi et al., 2015; Martín-Illana et al., 2022). Hydrocolloids have become increasingly trendy for developing bigels, due to their interesting gelation, thickening and texture-tailoring properties (Wang et al., 2022). Incorporating a gelled oil phase into an hydrogelled matrix will allow the formation of a strong enough emulsion-based system, with both hydrophilic and lipophilic constituents (Pinto et al., 2021). The strong interactions provided by the hydrogen bonding that originates the hydrogel network formation are essential for the maintenance of the overall gel structure (Rehman et al., 2014). Such versatility allows these structures to serve as a backbone for developing diverse bigels/hydro-olleocolloid matrices (e.g., κ-carrageenan and monoglycerides; protein-based; lecithin and whey; gelatin and stearic acid and others) (Behera et al., 2015; Golodnizky & Davidovich-Pinhas, 2020; Wakhet et al., 2015; Zheng et al., 2020). If enough gelling material is added, these systems can display improved (and tailored) mechanical properties, broadening their applicability within food product development steps. At the same time, they can also be dynamically used to vehiculate bioactives with added protection (e.g., deterioration from oxidation) (Martín-Illana et al., 2022). The ability to tailor both the continuous phase and the filling content of the bigels, through combinations of gelator material and hydrogel:oleogel (HG:OG) ratio variations, is a significant feature concerning the future applicability of these structures in different food matrices. Interestingly, the development of bigel inks expands their application range as high-oil materials with the capability to be broadly used in 3D food printing (Chen et al., 2023; Qiu et al., 2022; Zhai et al., 2022) alongside oleogels (Oliveira et al., 2022). Despite the several works performed in the field, the evaluation of the hydrogel:oleogel (HG:OG) ratio combined with the effect of gelator concentrations needs further studies foreseeing its use in foods. Therefore, our objective was to develop a self-sustained bigel system and evaluate its physicochemical properties when different concentrations of gelators and HG:OG ratios were tested. The developed bigels were evaluated through polarized microscopy, rheology, texture, solvent holding capacity, X-Ray diffraction and ATR-FTIR spectroscopy.

2. Materials and methods

κ-carrageenan (CEAMGEL 30–316 - with maximum water gel strength at 90 °C of 400 g) and clarified locust bean gum (CEAMGUM 3080 - with max viscosity of 1600.00) were purchased from Ceamsa® (Pontevedra, Spain). Glyceryl monostearate (90% purity) was acquired from Mosselman Oleochemicals (Mons, Belgium). The sunflower oil Fula Equilíbrio® (Sovena, Algés, Portugal), with up to 12% of saturated fatty acids was purchased from a local supermarket.

2.1. Bigel development

Bigels consisting of an aqueous (hydrogel) phase and an oleogel phase were prepared independently under different environmental conditions and then mixed (Fig. 1). In the first stage, the LBG was dispersed (at room temperature ~21 °C) in distilled water using a mechanical mixer, followed by the addition of κ-carrageenan (κ-car) (1:1 ratio) at different concentrations (0.5–2.5 wt%). The 1:1 ratio of the LBG and κ-car combination has been reported as the one responsible for reaching the maximum hydrogel-breaking strength (He et al., 2017). After mixing, the solutions (50–90 wt%) were heated until reaching approx. 90 ± 2 °C to enforce the synergism between both gelators (holding for 2 min). Then independently prepared oleogels, with sunflower oil and GM, were added in selected concentrations to the first solution while still hot. In the first stage, 5 wt% of GM was used, but for viscoelastic measurements and polarized microscopy also 10 and 20 wt % of GM were used for the production of the oleogel. GM concentrations equal or above 5 wt% were used to guarantee the minimum gelling concentration (Cerqueira et al., 2017). The mixing procedure was performed using a Heidolph HEi-Tec mechanical mixer (HeiDolph Instruments, Germany) for 10 min at 2000 rpm. After that period, the bigel samples were left to cool at room temperature and then stored at ~4 °C before testing.

2.2. Optical microscopy

Hot bigel samples (immediately after the emulsification stage) were poured in glass slides and were gently compressed by a glass coverslip to avoid complete stretching, thus allowing the sample to gel on the glass surface. Then, samples were analysed at room temperature using an optical microscope (Olympus System Microscope model BX51TF, Olympus America Inc., Centre Valley, PA, USA) equipped with a polarizer and a digital camera (Olympus EX300, Olympus America Inc., Centre Valley, PA, USA). The micrographs were acquired at a magnification of 50x, 200x and 500x. For particle size frequency analysis, a set of four pictures (with a magnification of 200x) depicting different parts of the sample were selected for particle size measurements. A total of 200 particles were analysed for each of the formulations tested.

2.3. Solvent holding capacity

The solvent holding capacity (SHC) of the bigels was evaluated under the methodology proposed by Yilmaz42 et al. (2014), with some modifications. This method permitted the quantification of the solvent retention levels within the bigel structure and comprises the placement of approximately 1 mL of freshly prepared bigels in Eppendorf tubes at 4 °C for 24 h. Then all tubes were centrifuged at 10 *g* for 15 min at room temperature. After that, the tubes were turned over and the released solvent was captured in a filter paper. The solvent holding capacity (SHC) was calculated using Equation (1).

$$\text{SHC } \% = [1 - (wi - wf) \,/\, wi)] * 100 \quad (1)$$

Where *wi* and *wf* are the initial and the final recorded weight, respectively.

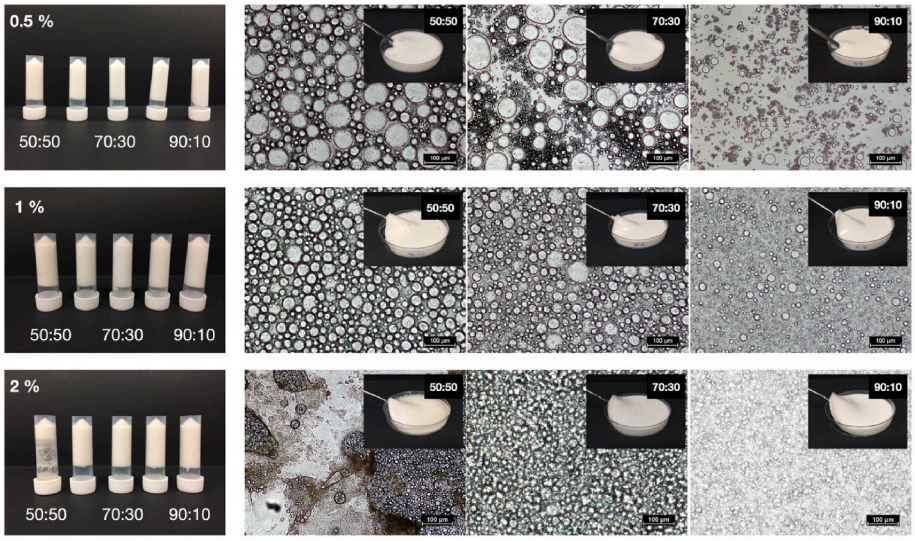

Fig. 2. Images (photographs and micrographs) of the prepared bigels with GM 5 wt% and stored at 4 °C. On the left are displayed the images of the inverted tubes, representing all the tested ratios. On the right are the brightfield micrographs, obtained at a magnification of 200x with the illustration of the samples that were stored in Petri dishes.

2.4. Rheological properties

The rheological study was performed under a Discovery Hybrid Rheometer (DHR1) from TA Instruments (New Castle, USA) equipped with a 40 mm stainless steel parallel plate with a truncation gap of 1 mm. The linear viscoelastic regime (LVR) was determined under oscillatory tests. The LVR was accessed, at first, on bigels produced with 5 wt % of GM on the oleogel phase. This two-step oscillation-temperature ramp followed a conditioning stage at 22 °C. The non-isothermal stage consisted of two (up and down) ramps with a 5 °C.min^{-1} rate until reaching 90 °C with a resting time of 10 s, ending at 22 °C. Strain and frequency were at 0.01% and 1 Hz, respectively.

2.5. Mechanical properties

Texture profile analysis (TPA) and penetration textural experiments were performed using a Shimadzu AGX-10kN Texture Analyzer (Shimadzu, Japan) equipped with a 500 N load cell and a 50 mm (diameter) probe. The evaluation of the results was done using at least five replicates (32 mm in diameter and 14 mm in height). The tested conditions involved a compression speed of 0.5 mm s^{-1} (with equivalent probe pulling speed) with a maximum strain of 50%. This strain value was unable to endure fractural damage within the bigels' structure, as desired, allowing the acquisition of an overall response during the two-bite process. The bigels' preparation for TPA consisted in placing the gel (hot) after mixing in a mold (acrylic plate) to form cylindrical-shaped samples, with 32 mm diameter and 18 mm height. For the penetration tests, plastic containers with a diameter of 1.5 mm were used as sample holders. For these tests, a 5 mm stainless steel compression probe was selected and the data was recorded during at least a 15 mm travel distance. The speed parameters for single compression were similar to the ones described above.

2.6. Fourier transform infrared spectroscopy (FTIR)

FTIR spectra of the oil, GM and bigels' samples were determined using ATR (Attenuated Total Reflection) mode using a VERTEX 80v vacuum FTIR spectrometer (Bruker, USA). A piece of each bigel sample was sliced and placed on the spectrometer lens. Measurements were made under a vacuum atmosphere, in the wavenumber range of 400 and 4000 cm^{-1} using 64 scans with a resolution of 4 cm^{-1}. An open beam was used as a blank measurement.

2.7. X-ray diffraction (XRD)

XRD measurements were done with an X-ray Diffractometer X Pert PRO MRD from Malvern Panalytical Ltd. (Royston, UK). X-ray scans were recorded at room temperature (~ 22 °C) in the range of 10–60° ($2\boldsymbol{\theta}$ degrees), with a Cu source, the X-ray tube ($\gamma = 1.54056$ Å) at 45 kV and 40 mA with $\boldsymbol{\theta}$ set to −0.0372° for fine calibration offset. The diffraction parameters were acquired from the minimum of the 2nd derivative with parameters set for peak search in HighScore Plus software. Under Bragg's law the lattice parameter d was determined; λ is the wavelength of the X-ray used, $\boldsymbol{\theta}$ is the half of the diffraction Bragg angle ($2\boldsymbol{\theta}$) and d is the space between planes.

$$n\,\lambda = 2d\,\sin\,\theta \tag{2}$$

2.8. Software and statistical analysis

Prism 10 (GraphPad Software, Inc., USA) was used to perform an analysis of variance, through Tukey's mean comparison test ($p < 0.05$). Rheology device control was performed by TRIOS Software Version: 4.1.1.33073 (TA Instruments, New Castle, USA), which also assisted in the calculation of rheological parameters. X'Pert HighScore Plus software (PANanalytical, Netherlands) was used to gather XRD data and perform peak diffraction analysis. The parameters were calculated using the TrapeziumX software (Shimadzu, Japan). FTIR spectrometer control and data gathering were both done using OPUS software.

3. Results and discussion

3.1. Visual observation and brightfield microscopy

The formulations with structural matrix continuity and self-standing ability were considered to be the successfully developed gels. The tube inversion method was used to evaluate the bigel self-standing aptitude and allowed to detect phase separation phenomena. Despite being classified as self-standing, under the tube inversion method, the consistency of some formulations (particularly those made with the lowest biopolymer concentration - 0.5 wt%) was fairly weak after being

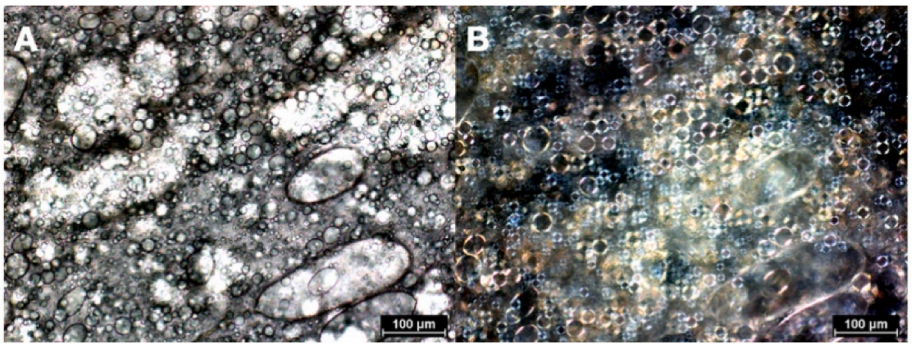

Fig. 3. Micrographs of: A) brightfield and B) polarized microscopy of bigels with 2.5% of biopolymer concentration with an 80:20 ratio (GM fixed at 5 wt%). Images taken with 200X magnification.

stabilized in Petri dishes. The ones with 2.5 wt% were only tested for solvent holding capacity and their microstructure was evaluated under the polarized microscope.

Since no surface active agents were added to assist in the emulsification process, it is not expected any interference on the crystallization arrangement/mechanics of the oleogel entrapment (inside hydrogel matrix). A progressive increase in polymer concentration impacted the influence of the HG:OG ratio in the development of the bigel, as it became increasingly hard to disperse the oleogel fraction even at high temperatures. The use of higher oleogel proportions induced a more disordered-like particle distribution, and in particular circumstances, gel structural instability was detected. Bigels' fluidity and malleability decreased alongside the upsurge of hydrogel strength. As visible in Fig. 2, the obtained brightfield micrographs displayed an increase in the hydrogel content producing smaller-sized oleogel particles. The increase in polymer concentration led to the formation of smaller, more evenly dispersed oleogel droplets, which contributed to the structural macrostability of the resulting bigels. Polymer concentrations ranging from 0.5 to 2 wt% within the hydrogel phase result in a homogeneous structure of the bigel, however, signs of structural destabilization were perceived. For higher oleogel contents in the bigel's formulations (starting at 70:30 HG:OG) some oleogel particles/droplets were visible outside of the hydrogel continuous phase. Phase separation (at the highest degree) was observed for 50:50 samples as a result of poor oil binding performance. These behaviour can be attributed to structural disruption, possibly caused by the absence of a surface active agent or crystallization modifier, which impaired the stabilization of the interfacial space resulting in the lack of incorporation of the oleogel droplets into the polymeric network.

Due to the development of highly viscous gels, it became increasingly difficult to disperse/homogenize the oleogel fraction in the hydrogel matrix, even using high processing temperatures. Bigels produced with a polymer concentration of 2.5 wt% evidenced phase separation at 70:30 ratio or lower, and for the ratios that still produced homogeneous bigels (80:20 and 90:10), it was demonstrated that the oleogel particles evidenced different size distribution among the entire sample, partially due to coalescence events and lack of dispersibility/oil particle breakdown. Apart from observed circular/spherical shaped particles, a core of ellipse-like elements was formed during the oleogel physical entrapment (Fig. 3). This behaviour can be explained by the high viscosity of the continuous (aqueous) medium.

3.2. Solvent holding capacity

The solvent holding capacity was accessed while exploring polymer concentrations ranging from 0.5 to 2.5 wt% and was obtained through centrifugation stability tests for bigels with ratios from 50:50 to 90:10 (HG:OG). The degree of solvent retention within the bigel structure evidenced a great level of stability, as a consequence of the hydrogel polymeric network, even for formulations with lower GM concentrations. Table 1 presents the solvent holding capacity of the gels. Most of the formulations showed increased levels of stability, recording solvent holding values close to 100%. The entire range of 50:50 formulations registered values under 100%. Samples produced with equal HG:OG ratio and with 0.5 and 1 wt% of biopolymer concentration, showed an average capacity of 87.40 ± 0.23 and 86.90 ± 1.74. These values are within the ones reported for another type of bigels, produced using gelatin and waxes, where the solvent loss for samples produced with a

Table 1
Solvent holding capacity (%) of bigels with different HG:OG ratios.

Polymer concentration (wt %)	50:50	60:40	70:30	80:20	90:10
2.5	*	88.361	96.602	99.261	100
2	81.861	99.671	99.983	97.301	99.995
1.5	88.204	99.571	99.983	100	99.993
1	86.899	99.940	99.985	99.810	99.995
0.5	87.398	98.850	99.766	99.983	99.997

Notes: Greyscale table background is set up according to the exhibited phase separation profile.
Light grey – phase separation; Dark grey – partial phase separation; Black – No phase separation.
(*) not determined due to large phase separation.

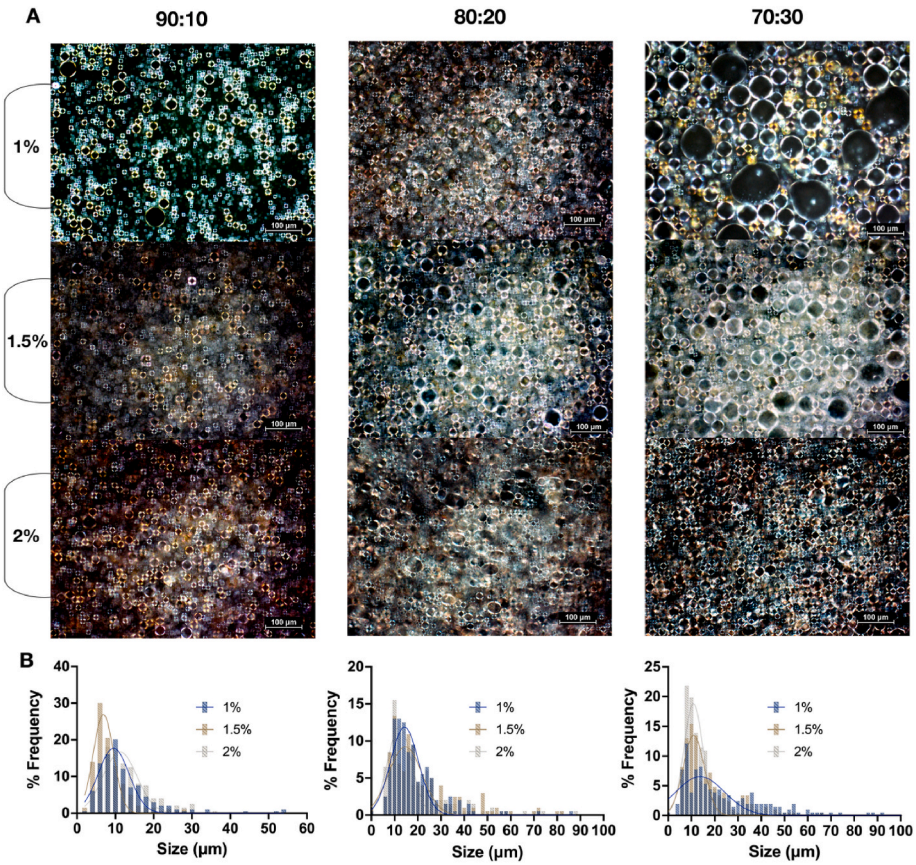

Fig. 4. Images of: A) Polarized micrographs and B) frequency distribution of bigels produced with 5 wt% GM concentration with 90:10, 80:20, 70:30 HG:OG rations and increasing biopolymer concentrations (1; 1.5; 2 wt%).

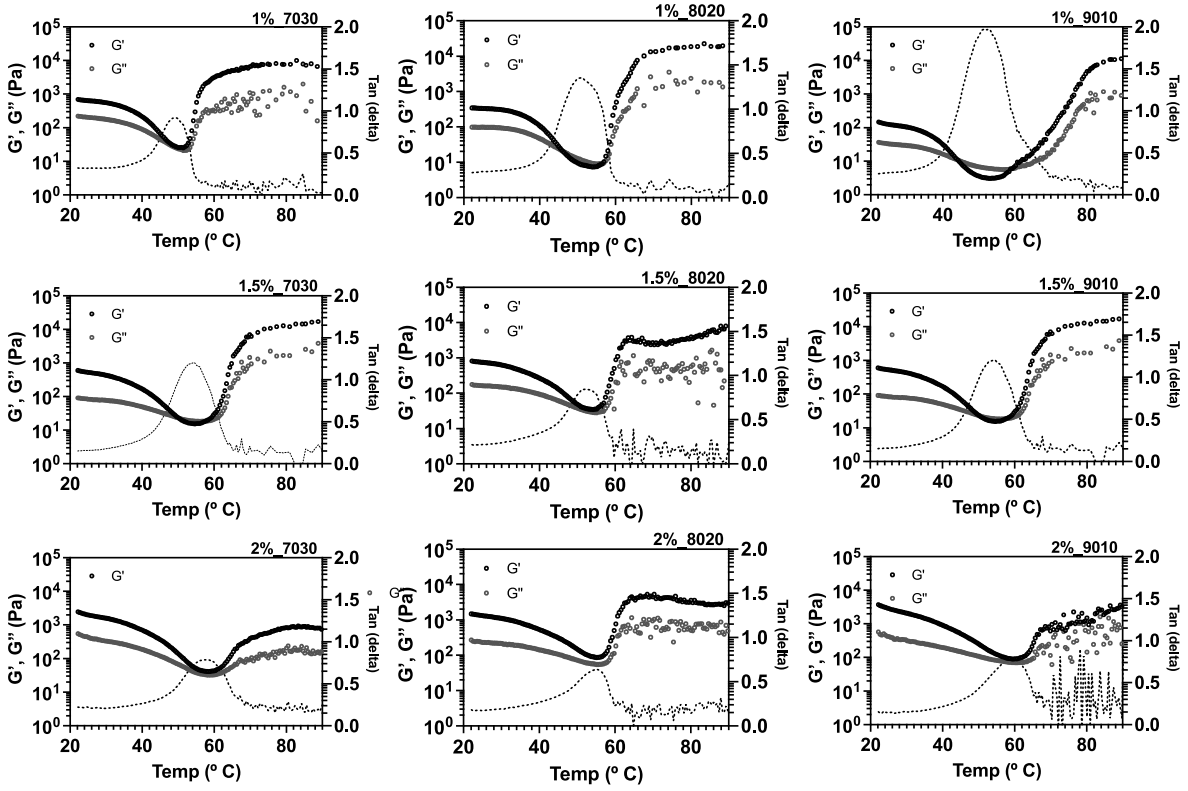

Fig. 5. Non-isothermal rheological (heating) sweeps for bigels with fixed GM concentration at 5 wt%, increasing biopolymer concentration (i.e., 1, 1.5, 2 wt%) and different HG:OG ratios (i.e., 70:30, 80:20, 90:10). *G'* (●) and *G''* (○) are displayed in black and grey, respectively, and tan (delta) is represented by the dashed dots.

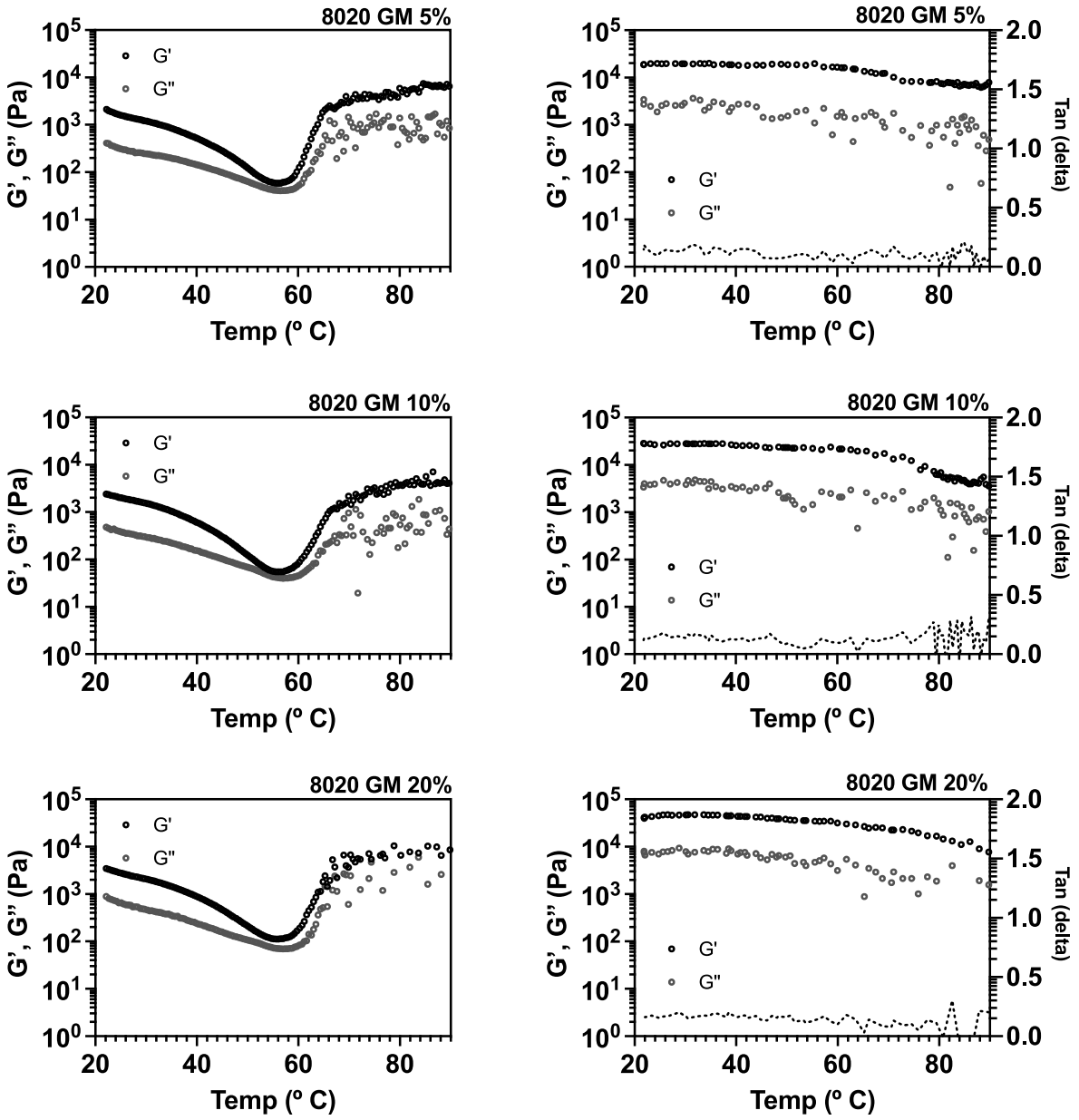

Fig. 6. Heating-cooling ramps for 80:20 bigels (2 wt% of biopolymers) with increasing GM content. On the right, it is visible the cooling profile sweep. *G'* (●) and *G''* (○) are displayed in black and grey, respectively, and tan (delta) is represented by the dashed dots.

50:50 (HG:OG) ratio was less than 10% (Saffold & Acevedo, 2022).

3.3. Polarized microscopy

Considering the bigels' microstructure, the increase of biopolymers for the hydrogel phase development was accountable for the intensification of the oleogel droplet packing (Fig. 4a). Particle size distribution was performed on bigel formulations that exhibited solid-like behaviour, a high sphericity level of the oleogel droplets and no phase separation (Fig. 4b). It was verified that the oleogel content influenced the distribution of the droplets' population. The size distribution for the 90:10 formulation (less oleogel content) barely exceeded the 30 μm size range. On the other hand, bigel formulations of 80:20 and 70:30 ratios produced droplets with a higher size, with a droplet population above 30 μm, reaching sizes up to 90 μm (as evidenced in the polarized micrographs). As discussed in section 3.2, the gel matrix density and viscoelastic properties are responsible for a lesser or more well-dispersed oleogel phase. Larger oleogel droplets are generally more susceptible to coalescence events and the lack of bigel structural homogeneity could lead to assorted mechanical properties. Major dissimilarities were encountered among bigels produced with 1 wt% of biopolymers as a consequence of the HG:OG ratio. Therefore, the macro-stability and properties of the bigels could be largely affected by this parameter when incorporated in a complex food matrix.

3.4. Rheological properties

The viscoelastic properties of the bigels produced with biopolymer concentrations of 1, 1.5 and 2 wt% were evaluated under the rheometer probe. Within this range, the registered storage modulus (*G'*) went up almost 9-fold (e.g., for 90:10 increased from 109 Pa to 982 Pa) suggesting a significant influence of the hydrogel polymeric content on bigels consistency (Fig. 5). The critical strain selected for the following oscillatory-temperature sweep tests (from the LVR determination) was 0.01%, guaranteeing that the region of irreversible deformations was not surpassed. Hydrogels produced with combinations of κ-car and LBG

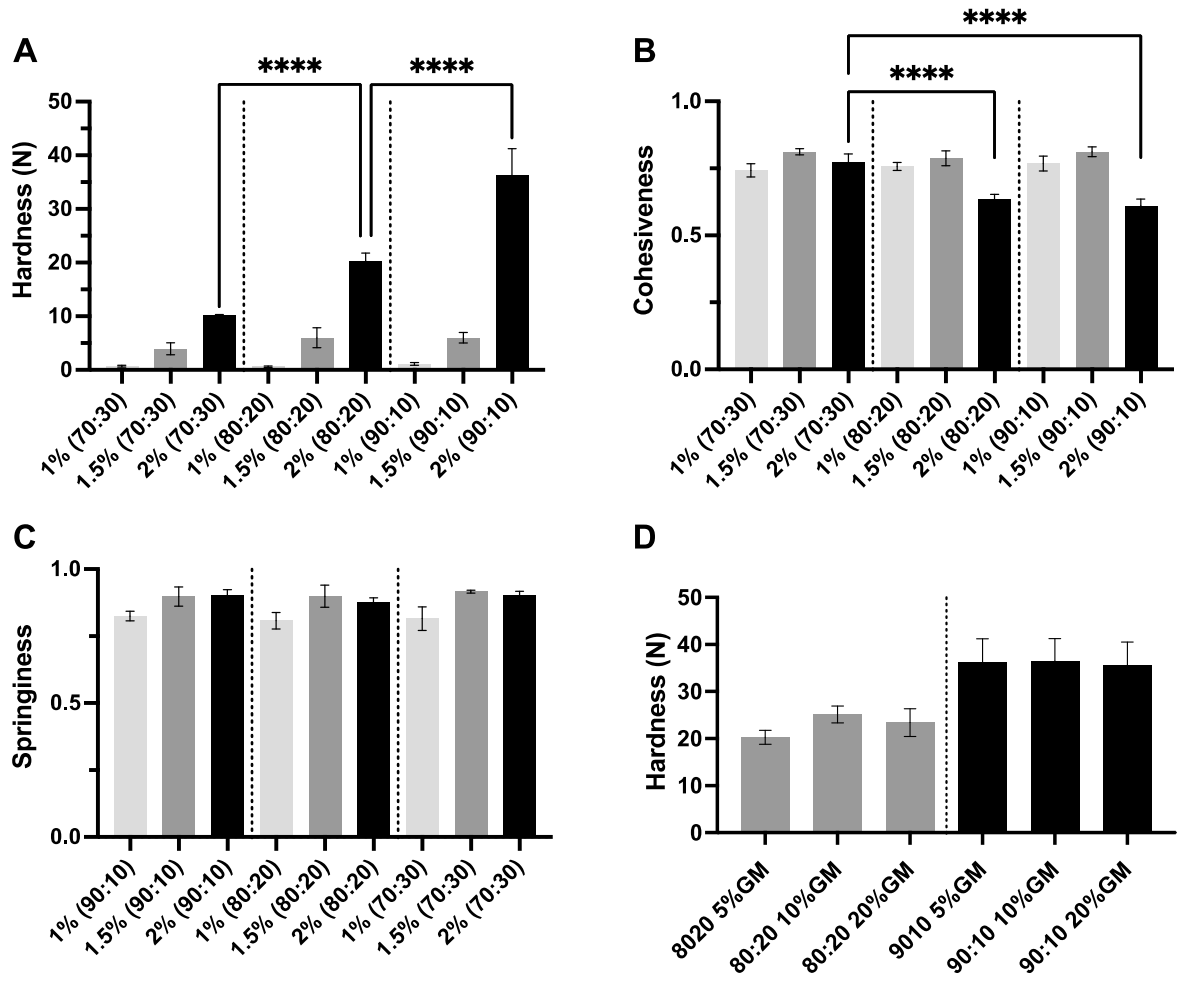

Fig. 7. A) Hardness, B) cohesiveness and C) springiness parameters gathered in TPA analysis for bigels produced with GM 5 wt%, increasing biopolymer concentration (i.e., 1, 1.5, 2 wt%) and different HG:OG ratios (i.e., 70:30, 80:20, 90:10). D) Hardness of bigels with 80:20 and 90:10 HG:OG ratio for increasing GM content with 2 wt% of biopolymer concentration.

are known to be thermo-reversible, however, the bigel structure can experience destabilization under high temperatures (Singh et al., 2014), as it is perceived here for some of the tested formulations. In such cases, no crossover point between G' (storage modulus) and G'' (viscous modulus) was detected. This gel-sol transition behaviour was equally confirmed through the phase angle (tan delta), which was gathered from the ratio of the viscous to elastic response of the material (Fig. 5 - dashed dots).

Apart from the major influence (on the transition temperature range) registered for increased polymeric concentration in the hydrogel fraction, oleogel also had an additional influence on this behaviour (associated with the oleogelator melting temperature). The oleogel increase and its particle arrangements played an important role in the overall rheological response of these materials. As seen in Fig. 5, bigels produced with 2 wt% of biopolymers, revealed a decrease in gel strength without any registered gel-sol transition (observed for lesser polymer strength). These results are in agreement with the observations made by Patel et al. (2015), where a similar thermal response was detected for a structured emulsion-based system, also using LBG and carrageenan. Upon cooling, the gel structure was recovered for all the prepared bigels, in the vicinity of the 60 °C mark. A solid-like behaviour was observed during the entire oscillation-temperature ramps, where the G' (storage modulus) recorded higher average values when compared to G'' within the LVR region.

An increase in GM allowed us to understand the impact of the differences induced by the oleogel strength. Overall, higher storage and loss modulus values were recorded as positively correlated with the GM content Fig. 6. Oleogel strength increase, through the rise of the oleogelator content, showed no major differences during heating (no crossover was identified). However, upon cooling, the viscoelastic parameters reached superior values. The values at the end of the temperature sweep recorded a G' of 18758, 27709 and 40184 Pa, for 5, 10 and 20 wt% of GM, respectively. These values were greater than the ones amassed at the beginning of the heating stage, evidencing a more pronounced structuring stage immediately after the cooling stage.

3.5. Mechanical properties

The texture profile analysis provided information on properties such as hardness, cohesiveness, and springiness, which translate to different micro and macro-structural insights about the bigels behaviour under chewing. These results confirmed the influence of hydrogel strength (biopolymer content) and HG:OG ratio on bigels' texture profile parameters. Results showed that for concentrations above 2 wt% of biopolymer there is a critical point related to the structural arrangement of bigels. The differences ($p < 0.05$) among bigels' hardness were only registered for samples produced with 2 wt% of biopolymers. This same trend was not verified for bigels produced with 1 and 1.5 wt%, as the increase of the hydrogel content (starting at 70:30) shaped gels with similar hardness, as seen in Fig. 7A.

Similarly, for the cohesiveness parameter, the recorded values were equal for almost all the tested samples, and only the ones discussed

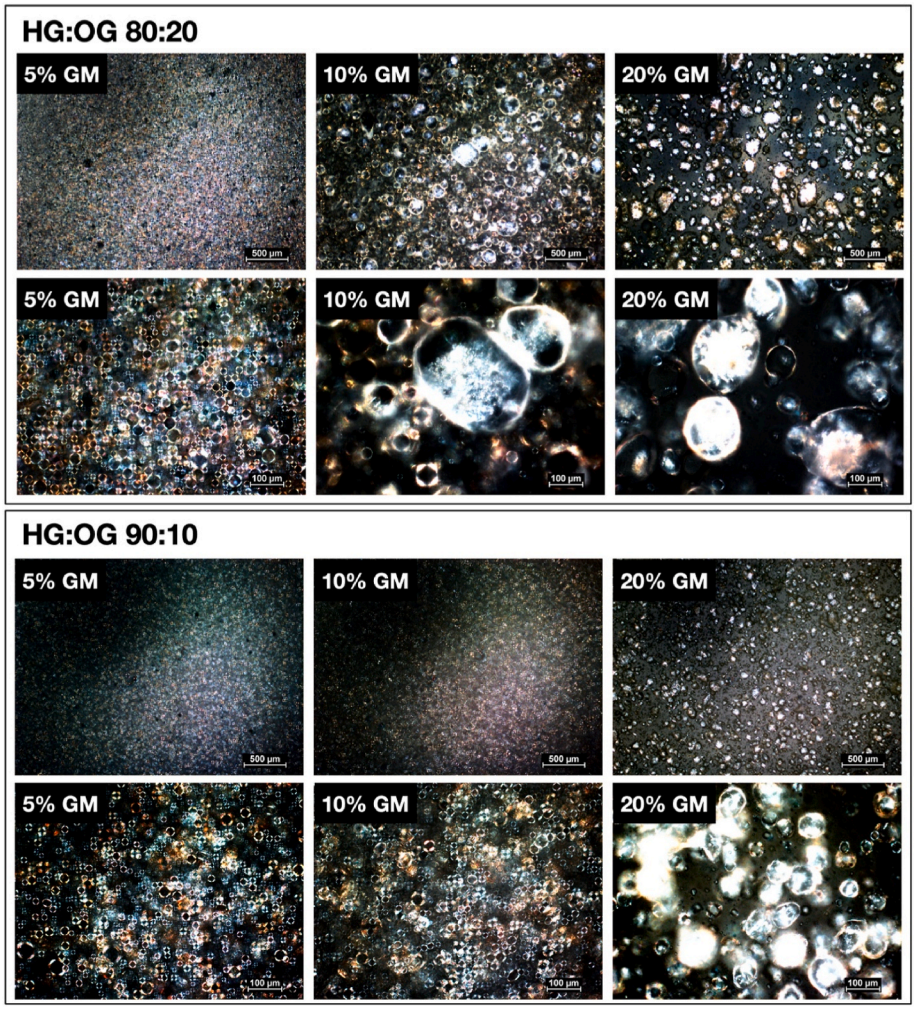

Fig. 8. Polarized micrographs (50 and 200X magnification represented above and below, respectively for each HG:OG ratio) of 80:20 and 90:10 HG:OG bigels produced with 2 wt% of biopolymer concentration and increasing GM content (from left to right).

above registered significant differences. In this case, the compression strength for 2 wt% bigels decreased for bigels with higher hydrogel fractions, not allowing them to equally retain their initial shape after bearing the first compression. We hypothesize that this behaviour is a consequence of modifications within the internal network resulting from the first deformation. Consequently, oleogel particles/droplets were not able to display higher mobility (due to increased gel viscosity), thus significantly affecting this parameter. Even with this internal structural behaviour, no variations were recorded for the springiness parameter. This means that even the exerted compression force during the first bite, bear no differences in the height of the samples between the end of the first compression and the beginning of the second one. Still, the springiness response of the bigels for every sample, registered values above 80%, and the ones produced with polymer concentrations above 1.5 wt% recorded values above 90% (Fig. 7C). This is an important feature, meaning that bigels were capable of exhibiting a high recovery capability. The increase of oleogel strength (through the use of higher GM content on the production of the oleogel) did not produce an active (internal) filling particle effect. This role was not verified even when concentrations progressively higher than the critical gelation concentration (10 and 20 wt% of GM) were used. For this case, the oleogel particles acted as inactive fillers, assumed from their shortage of interaction with the hydrogel matrix and oleogel low viscosity (Wijarnpreecha et al., 2021). Overall, the lack of interfacial stabilization did not endorse any other improvement or enhancement of the mechanical properties, regarding the overall bigels' texture response (Fig. 7D). As we can observe from the polarized micrographs (Fig. 8) important microstructural modifications happened due to GM content increase.

From the micrographs (Fig. 8) and considering 80:20 HG:OG formulations, it was observed that the crystalline material (provided by GM) was not only present in the interfacial space but also visible within the internal oleogel space. This is indicative of an active filling effect that would possibly generate a superior bigel hardness due to the creation of a stronger (inner) oleogel phase due to an increase of the solid mass within the overall internal structure. However, due to the already hypothesized lack of synergy at the interfacial space between the composites, associated with the well-known mechanical limitation of GM oleogels, which are highly prone to shear movements (Fasolin et al., 2021), an increase in the bigel hardness was not registered.

The results of the perforation tests (Fig. 9) comprise a positive peak evolution, which is related to the strength required to penetrate the bigels' outer layer. The penetration profile along the selected displacement length demonstrated the progressive increase of the surface resistance, which resulted from the increase in polymer concentration. Also, the presence of air, which was internalized during the mixing procedure at high speed, was likewise identified in the structure and, as a result, clear patterns (up-and-down force-related outlines) were recognised during the probe displacement. This profile was easily seen for stronger (highly viscous) gels since the reduced oleogel dispersibility generated larger air gaps, influencing the textural response during probe travelling. Less viscous and increasingly fluid bigels (e.g., 1 and 1.5 wt% of biopolymer concentration) produced smaller up-and-down outlines

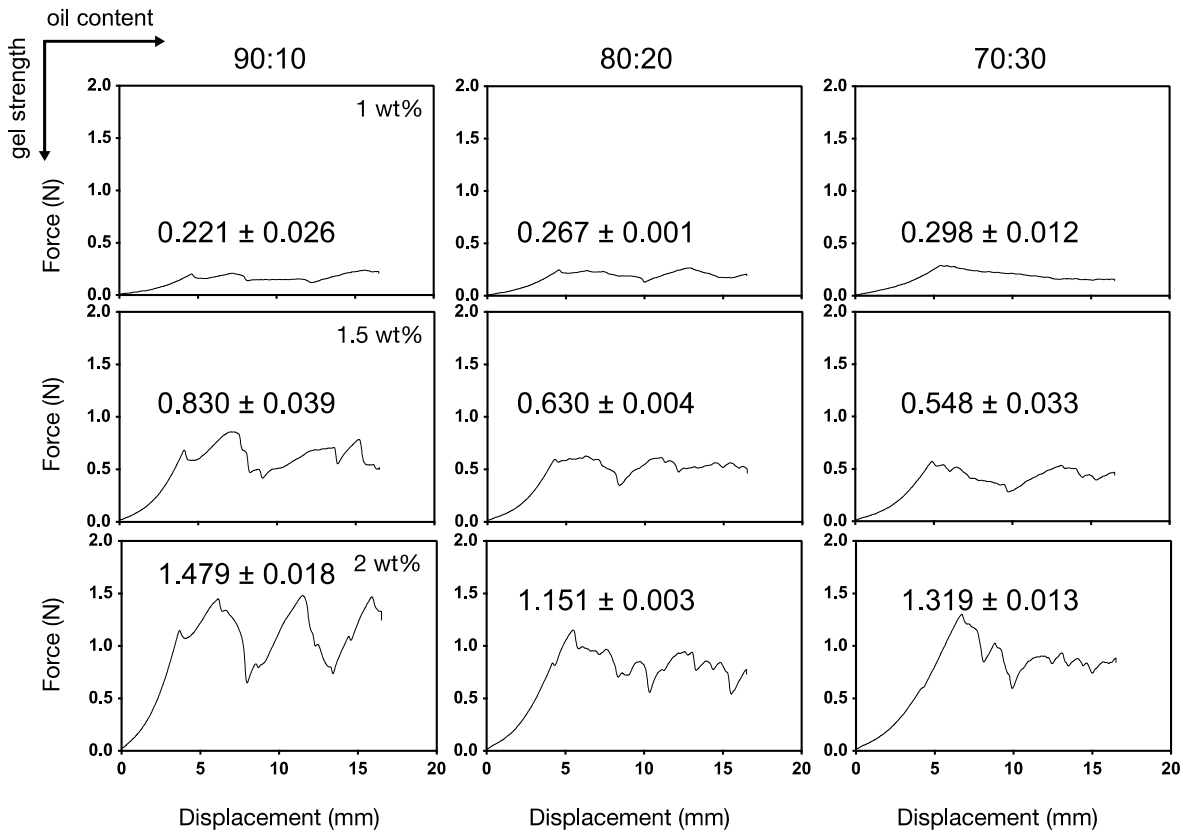

Fig. 9. Perforation profile of bigels (produced with 5 wt% of GM) with different HG:OG ratios and increasing biopolymeric content.

and registered lower overall forces as a consequence of a weaker inner strength. Bigels produced with equal hydrogel strength showed a clear tendency on the perforation results for different HG:OG ratios. Here, the increase of oleogel content induced the arrangement of a more "continuous" internal matrix with less air (or at least not so larger air gaps/bubbles). The measured overrun, during the bigel development stage, was not significant among samples produced, therefore it was not further discussed here.

3.6. Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD)

FTIR was used to evaluate the potential interactions among the used components of all phases; sunflower oil, oleogel, gelator components (both from oleogel and hydrogel phases), and selected bigel samples. Apart from the gelators, the bigel samples tested were formulated within the selected range of HG:OG ratios with increased biopolymer content as well. Additionally, bigel formulations with a GM concentration of 10 and 20 wt% were studied.

The oxidative grade of oils can be examined through the frequency and absorbance registered for the bands related to the formation of first (hydroperoxides) and secondary (ketones and aldehydes) oxidation products, namely ranges 3100–3600 cm^{-1} and 1730–1750 cm^{-1}. As seen in Fig. 10A, no major differences were identified between pure oil and oleogel samples, a slight decrease in relative intensity (around 1750 cm^{-1}) was observed for bigel samples, with minor visibility for 90:10 samples (Fig. 10C), probably as a consequence of minor oleogel content. Hydrogen bonding, N–H and O–H stretching are associated with the broad peak (~3300 cm^{-1}), which is mainly related to the water content and hydroxyl groups from the hydrogel (Sagiri et al., 2015).

C–H stretching-related peaks are visible at 2920 and 2850 cm^{-1} (slightly shifted depending on the samples) and specific peaks at around 2920, 2850, and 1460 cm^{-1} presented the stretching vibration of the C–H or C double bond. The increase in peak intensity is certainly related to the ratio differences between oleogel and the hydrogel, translated to differences among oleogel fraction displacement. The existence of peaks at around 1745 and 1160 cm^{-1} are associated with the presence of C–O and C═O stretching vibration of triglycerides in sunflower oil (Cakmak-Arslan, 2022), thus the clear peak intensity changes observed between HG:OG 80:20 and 90:10 within this region are related to the increase of the oleogel fraction and no new chemical bonds were identified, pointing towards a physical arrangement of the bigels.

The XRD analysis was conducted to further study the crystal morphology of bigels, GM oleogel and its constituents in powder form. XRD patterns for the gelator compounds (powders) are visible in Fig. 11A, and the diffraction of oleogel samples in Fig. 11B demonstrates the intensification of crystallinity due to the increase of GM in oleogel formulation. The almost complete loss of crystallinity was observed for all bigel samples under XRD (Fig. 11C), with the broad peak at 4.41 Å in the diffraction pattern indicative of the main amorphous structure.

4. Conclusions

The different known oil structuring routes are indeed highly promising techniques for fabricating novel healthier edible solutions for the food industry. In this work, we fabricated food-grade bigels, based on the combination of a biopolymer-based hydrogel with glyceryl monostearate oleogel. Results showed that the increase of the oleogel fraction in the bigel constitution induced lower hardness, while evidencing at the same time a more resolved gel-sol (rheological) transition. The microstructure was also actively influenced by the HG:OG ratio, and depending on the biopolymer concentration, larger oleogel particles became substantially more difficult to get stabilized within the hydrogel matrix, thus producing bigels with lower solvent holding capacity. Likewise, the increase of biopolymers affected viscosity levels, greatly influencing the self-emulsifying capacity of the system, thus reaching a

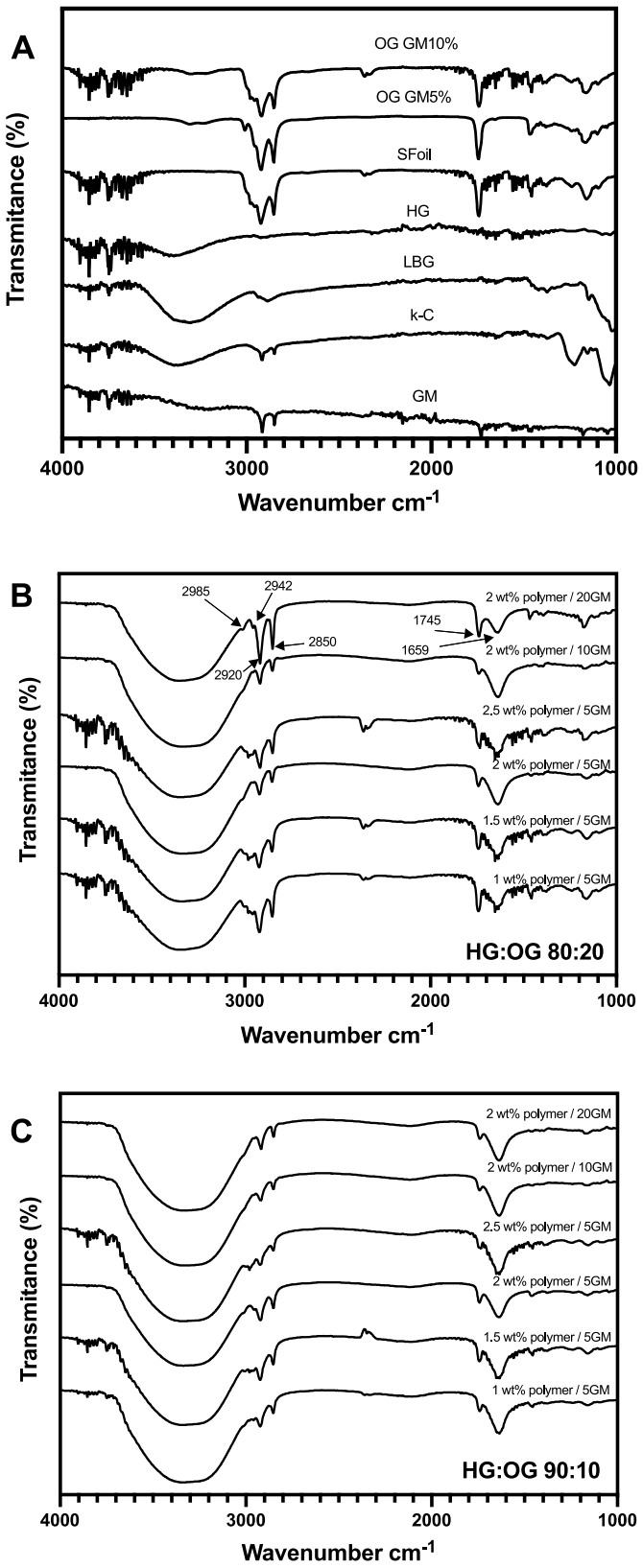

Fig. 10. FTIR-ATR spectra for components (κ-car - κ-carrageenan; LBG – locust bean gum; SFoil – sunflower oil; HG – hydrogel; OG – oleogel; GM – glyceryl monostearate) and bigels with different gelator concentrations (5, 10, 20 wt% of GM and 1, 1.5, 2 and 2.2 wt% of biopolymers).

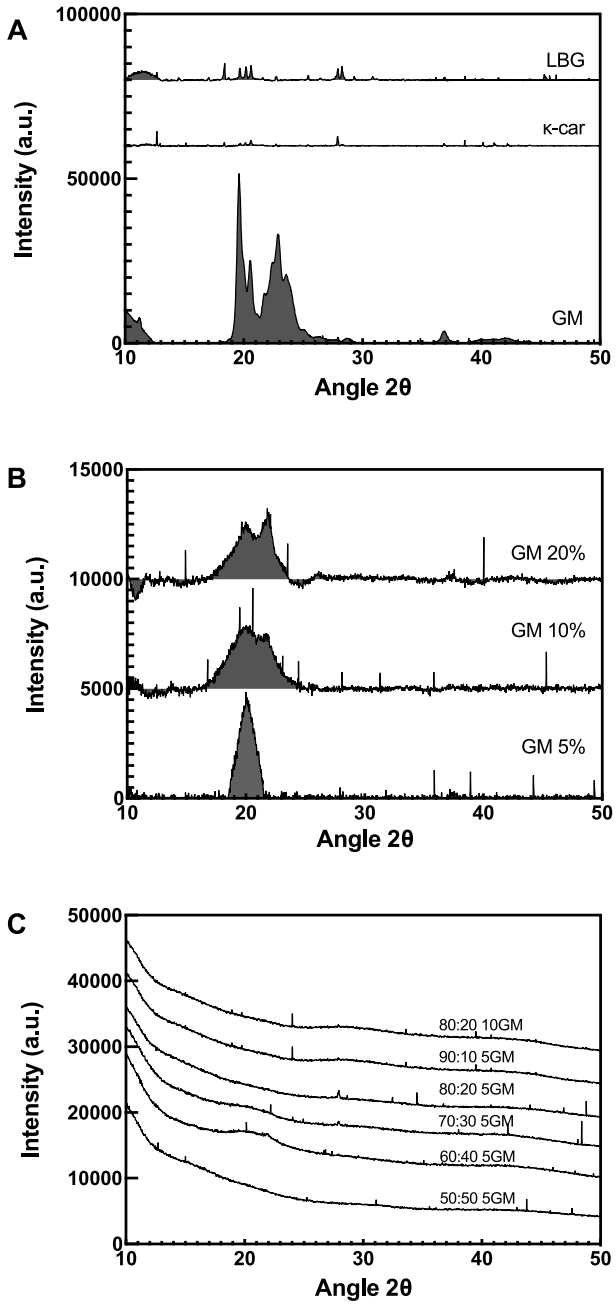

Fig. 11. XRD spectra for A) gelators in powder form; B) oleogels produced with increasing GM concentration (5, 10 and 20 wt%); C) bigels with different HG: OG ratios and oleogelator concentration.

point where phase separation occurred. The non-chemical arrangements presented for these bigels constitute an important feature, that is relevant towards their applicability in different food products. The synergy between GM and an additional surfactant, or a co-gelator with crystallization modification activity, could be enough to promote such an effect on polymeric bigels such as the ones tested here. The texture and thermal properties of bigels make these structures an interesting healthier solution that can be suitable to be used as substitutes of saturated fats in food products that require such improvements. Both oleogel and/or hydrogel compositional properties widen their potential applicability towards different food systems with distinct needs (e.g., texture, compositional stability, delivery of bioactives).

Author contributions

Conceptualization, A.J.M., P.F., L.M.P. and M.A.C.; methodology, A.J.M.; investigation, A.J.M.; data curation, A.J.M. and M.A.C.; writing—original draft preparation, A.J.M.; writing—review and editing, A.J.M., A.G., P.F., P.S., A.V., L.M.P. and M.A.C.; All authors have read and agreed to the published version of the manuscript.

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

No data was used for the research described in the article.

Acknowledgements

Work developed under project BetterFat4Meat (POCI-01-0247-FEDER-039718) co-financed by Compete 2020, Portugal 2020 and the European Union, through the European Regional Development Fund (ERDF). This study was also supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UIDB/04469/2020 unit, and by LABBELS – Associate Laboratory in Biotechnology, Bioengineering and Microelectromechanical Systems, LA/P/0029/2020.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodhyd.2023.108893.

References

Authority, E. F. S. (2018). Scientific and technical assistance on trans fatty acids. *EFSA Supporting Publications, 15*(6). https://doi.org/10.2903/sp.efsa.2018.en-1433

Behera, B., Sagiri, S. S., Pal, K., Pramanik, K., Rana, U. A., Shakir, I., & Anis, A. (2015). Sunflower oil and protein-based novel bigels as matrices for drug delivery applications—characterization and in vitro antimicrobial efficiency. *Polymer - Plastics Technology and Engineering, 54*(8), 837–850. https://doi.org/10.1080/03602559.2014.974268

Bhandari, S. D., Delmonte, P., Honigfort, M., Yan, W., Dionisi, F., Fleith, M., Iassonova, D., & Bergeson, L. L. (2020). Regulatory changes affecting the production and use of fats and oils: Focus on partially hydrogenated oils. *Journal of the American Oil Chemists' Society, 97*(8), 797–815. https://doi.org/10.1002/aocs.12366

Blach, C., Gravelle, A. J., Peyronel, F., Weiss, J., Barbut, S., & Marangoni, A. G. (2016). Revisiting the crystallization behavior of stearyl alcohol : Stearic acid (SO : SA) mixtures in edible oil. *RSC Advances, 6*(84), 81151–81163. https://doi.org/10.1039/c6ra15142f

Cakmak-Arslan, G. (2022). Monitoring of Hazelnut oil quality during thermal processing in comparison with extra virgin olive oil by using ATR-FTIR spectroscopy combined with chemometrics. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 266*, Article 120461. https://doi.org/10.1016/j.saa.2021.120461

Cerqueira, M. A., Fasolin, L. H., Picone, C. S. F., Pastrana, L. M., Cunha, R. L., & Vicente, A. A. (2017). Structural and mechanical properties of organogels: Role of oil and gelator molecular structure. *Food Research International, 96*. https://doi.org/10.1016/j.foodres.2017.03.021

Chen, Z., Bian, F., Cao, X., Shi, Z., & Meng, Z. (2023). Novel bigels constructed from oleogels and hydrogels with contrary thermal characteristics: Phase inversion and 3D printing applications. *Food Hydrocolloids, 134*, Article 108063. https://doi.org/10.1016/j.foodhyd.2022.108063

Fasolin, L. H., Martins, A. J., Cerqueira, M. A., & Vicente, A. A. (2021). Modulating process parameters to change physical properties of bigels for food applications. *Food Structure, 28*, Article 100173. https://doi.org/10.1016/j.foostr.2020.100173

Golodnizky, D., & Davidovich-Pinhas, M. (2020). The effect of the hlb value of sucrose ester on physiochemical properties of bigel systems. *Foods, 9*(12). https://doi.org/10.3390/foods9121857

He, H., Ye, J., Zhang, X., Huang, Y., Li, X., & Xiao, M. (2017). κ-Carrageenan/locust bean gum as hard capsule gelling agents. *Carbohydrate Polymers, 175*, 417–424. https://doi.org/10.1016/j.carbpol.2017.07.049

López-Pedrouso, M., Lorenzo, J. M., Gullón, B., Campagnol, P. C. B., & Franco, D. (2021). Novel strategy for developing healthy meat products replacing saturated fat with oleogels. In *Current opinion in food science* (Vol. 40, pp. 40–45). Elsevier Ltd. https://doi.org/10.1016/j.cofs.2020.06.003.

Lupi, F. R., Gentile, L., Gabriele, D., Mazzulla, S., Baldino, N., & de Cindio, B. (2015). Olive oil and hyperthermal water bigels for cosmetic uses. *Journal of Colloid and Interface Science, 459*, 70–78. https://doi.org/10.1016/j.jcis.2015.08.013

Manzoor, S., Masoodi, F. A., Naqash, F., & Rashid, R. (2022). Oleogels: Promising alternatives to solid fats for food applications. *Food Hydrocolloids for Health, 2*, Article 100058. https://doi.org/10.1016/j.fhfh.2022.100058

Martín-Illana, A., Notario-Pérez, F., Cazorla-Luna, R., Ruiz-Caro, R., Bonferoni, M. C., Tamayo, A., & Veiga, M. D. (2022). Bigels as drug delivery systems: From their components to their applications. *Drug Discovery Today, 27*(4), 1008–1026. https://doi.org/10.1016/j.drudis.2021.12.011

Oliveira, S. M., Martins, A. J., Fuciños, P., Cerqueira, M. A., & Pastrana, L. M. (2022). Food additive manufacturing with lipid-based inks: Evaluation of phytosterol-lecithin oleogels. *Journal of Food Engineering, 111317*. https://doi.org/10.1016/j.jfoodeng.2022.111317

Patel, A. R., Dumlu, P., Vermeir, L., Lewille, B., Lesaffer, A., & Dewettinck, K. (2015). Rheological characterization of gel-in-oil-in-gel type structured emulsions. *Food Hydrocolloids, 46*, 84–92. https://doi.org/10.1016/j.foodhyd.2014.12.029

Pinto, T. C., Martins, A. J., Pastrana, L., Pereira, M. C., & Cerqueira, M. A. (2021). Oleogel-based systems for the delivery of bioactive compounds in foods. *Gels, 7*(3), 1–24. https://doi.org/10.3390/gels7030086

Qiu, R., Wang, K., Tian, H., Liu, X., Liu, G., Hu, Z., & Zhao, L. (2022). *Analysis on the printability and rheological characteristics of bigel inks: Potential in 3D food printing.* Food Hydrocolloids, Article 107675.

Rehman, K., Mohd Amin, M. C. I., & Zulfakar, M. H. (2014). Development and physical characterization of polymer-fish oil bigel (Hydrogel/Oleogel) system as a transdermal drug delivery vehicle. *Journal of Oleo Science, 63*(10), 961–970. https://doi.org/10.5650/jos.ess14101

Roche, H. M. (2005). Fatty acids and the metabolic syndrome. *Proceedings of the Nutrition Society, 64*(1), 23–29. https://doi.org/10.1079/PNS2004405

Saffold, A. C., & Acevedo, N. C. (2022). The effect of mono-diglycerides on the mechanical properties, microstructure, and physical stability of an edible rice bran wax–gelatin biphasic gel system. *Journal of the American Oil Chemists' Society, 99*(11), 1033–1043. https://doi.org/10.1002/aocs.12640

Sagiri, S. S., Singh, V. K., Kulanthaivel, S., Banerjee, I., Basak, P., Battachrya, M. K., & Pal, K. (2015). Stearate organogel-gelatin hydrogel based bigels: Physicochemical, thermal, mechanical characterizations and in vitro drug delivery applications. *Journal of the Mechanical Behavior of Biomedical Materials, 43*, 1–17. https://doi.org/10.1016/j.jmbbm.2014.11.026

Singh, V. K., Banerjee, I., Agarwal, T., Pramanik, K., Bhattacharya, M. K., & Pal, K. (2014). Guar gum and sesame oil based novel bigels for controlled drug delivery. *Colloids and Surfaces B: Biointerfaces, 123*, 582–592. https://doi.org/10.1016/j.colsurfb.2014.09.056

Wakhet, S., Singh, V. K., Sahoo, S., Sagiri, S. S., Kulanthaivel, S., Bhattacharya, M. K., Kumar, N., Banerjee, I., & Pal, K. (2015). Characterization of gelatin–agar based phase separated hydrogel, emulgel and bigel: A comparative study. *Journal of Materials Science: Materials in Medicine, 26*(2), 118. https://doi.org/10.1007/s10856-015-5434-2

Wang, L., Wen, Y., Su, C., Gao, Y., Li, Q., Du, S., & Yu, X. (2022). Effect of water content on the physical properties and structure of walnut oleogels. *RSC Advances, 12*(15), 8987–8995. https://doi.org/10.1039/d2ra00920j

Wijarnprecha, K., de Vries, A., Sonwai, S., & Rousseau, D. (2021). Water-in-Oleogel emulsions—from structure design to functionality. *Frontiers in Sustainable Food Systems, 4*. https://doi.org/10.3389/fsufs.2020.566445

Zhai, X., Sun, Y., Cen, S., Wang, X., Zhang, J., Yang, Z., Li, Y., Wang, X., Zhou, C., Arslan, M., Li, Z., Shi, J., Huang, X., Zou, X., Gong, Y., Holmes, M., & Povey, M. (2022). Anthocyanins-encapsulated 3D-printable bigels: A colorimetric and leaching-resistant volatile amines sensor for intelligent food packaging. *Food Hydrocolloids, 133*, Article 107989. https://doi.org/10.1016/j.foodhyd.2022.107989

Zheng, H., Mao, L., Cui, M., Liu, J., & Gao, Y. (2020). Development of food-grade bigels based on κ-carrageenan hydrogel and monoglyceride oleogels as carriers for β-carotene: Roles of oleogel fraction. *Food Hydrocolloids, 105*, Article 105855. https://doi.org/10.1016/j.foodhyd.2020.105855