Rheological Studies on Glycerol Plasticized Gelatin and Its Blends with Epoxidized Soybean Oil

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Abstract: Blends of gelatin (Ge) plasticized with varying amounts of glycerol (Gly), buffer solution pH 10 and epoxidized soybean oil (ESO) to enhance hydrophobicity were prepared by mixing and injection-molding. Blends were characterized by rheological tests and microscopy to select optimal conditions for scaling up their processing. The effect of each component on rheological response was analyzed using parallel plate geometry. Coating of gelatin specimens with PDMS during rheological tests led to reliable and reproducible results since water evaporation was prevented. A gradual increment in ESO concentration led to blends with increased degree of phase separation, as evidenced by optical and confocal microscopy. Limited compatibility between ESO and Ge increased viscosity at high ESO levels, but up to 10% Gly could be replaced with ESO without a significant variation of rheological behavior.

Keywords: Rheology; renewable; plasticizers; gelatin blends; microstructure

1 Introduction

Gelatin is a water-soluble protein of animal origin that has found a wide range of industrial applications in fields as varied as pharmaceutical and food packaging [1,2]. The ability of this biopolymer to form stable films by different processing methods provides an ecologically and economically viable alternative for the potential replacement, at least in part, of petroleum-based polymers in the food packaging area [3-7].Traditionally, gelatin has been transformed into films by solution casting that is widely used at laboratory scale, but it is unfeasible at industrial level because it is slow and discontinuous [8-10]. Processes involving thermo-mechanical operations are more convenient for industrial applications than casting, since they allow much shorter times for the film obtaining and are easy to adapt to conventional pre-existing technologies [4,8,9,11]. Even though thermo-mechanical techniques have received less attention for proteins processing, some efforts have been made to produce compression molded [5,6,8,12], extruded [2,8-10,13] and blown [8-10,13] gelatin films.

Rheological behavior of gelatin in the melt state is a crucial step towards comprehending their flow behavior, structure and final properties. Gelatin must have sufficient melt flow under processing conditions for its thermal processing into films. Proteins normally involve high molecular interaction which results in high melt viscosity but low melt flowability. The addition of plasticizers such as glycerol and sorbitol enhances the melt flowability, facilitating the production of these films by thermal processing [5,7,10]. However, only few data are available concerning the rheology of protein melts during continuous processing, such as extrusion [8-10,14]. Moreover, there is little understanding about the relationship between plasticized-elatin formulations and shear flow properties, which is necessary to select the processing variables during extrusion or injection molding.

DOI: 10.32604/jrm.2019.00043

The main constrain in the experimental determination of rheological behavior of biopolymers is water evaporation upon measuring. This issue represents an obstacle to obtain a realistic flow behavior, being critical in cone-and-plate and parallel-plate geometries, where drying at the edge of the samples leads to large errors in the measured torque [15]. Consequently, associated errors or over-estimation in rheological results might have an impact on the determination of biopolymer processing parameters. Particularly, drying has a great influence on rheological measurement of gelatin based formulations. In this sense, Firoozmand et al. [16] used a *solvent trap* assembly by incorporating a moistened tissue to study the rheology of gelatin-oxidized starch gels. Meanwhile, Chatterjee and Bohidar [17] reported the use of a homemade sponge solvent trap in parallel plate geometry to prevent water evaporation from gelatin hydrogels. In other works, different techniques were employed to avoid the moisture loss, such as the combination of solvent trap and sample covering with dodecane [18] or the application of a thin layer of mineral oil to the exposed sample edges [19].

In this context, the aim of the present study was to analyze the flow properties of gelatin blends plasticized with glycerol and epoxidized soybean oil (ESO), as co-plasticizer and hydrophobizing agent, at varying levels. Previous studies have reported that ESO reduces hydrophilicity of wheat gluten thermoplastic starch and soybean protein isolate [8-10,20]. The optimal processing conditions of gelatin based formulations were determined and the relationship between rheological behavior and blend microstructure was analyzed. Besides, a simple alternative to prevent water evaporation from gelatin formulations during rheological measurements was presented, and the accuracy of the obtained results was analyzed.

2 MAterials and Methods

2.1 Materials

Bovine gelatin type B (Ge; Bloom number 150 and an isoionic point (Ip) of 5.1) was kindly provided by Rousselot (Argentina). Glycerol (Gly, p.a.) and buffer solution (pH = 10) were supplied by Cicarelli (Argentina). In order to enhance the hydrophobicity of gelatin-based materials [21,22], glycerol was partially replaced by Epoxidized Soybean Oil (ESO; oxirane functionality of 3.5; provided by Unipox S.A, Argentina), but keeping constant the total weight percentage of these additives (40 % w/w, Ge dry basis). Polydimethylsiloxane (PDMS, Petrarch Systems Inc) was used as coating material during rheological testing.

2.2 Preparation of Injection-Molded Samples

Pre-mixtures were obtained by mixing manually appropriate amounts of Ge, Gly, ESO and buffer solution (pH = 10) at room temperature for 15 min. Then, pre-mixtures were intensively mixed in a laboratory mixer (LMM, Laboratory mixing molder, Atlas), and subsequently they were injected into a stainless-steel circular (diameter = 25 mm, thickness = 3 mm) or rectangular (70 mm × 10 mm × 3 mm) molds. Different blends were prepared varying component ratio (Tab. 1). Blending conditions were fixed at 60°C, 100 rpm and 45 min, according to preliminary studies. To minimize possible aging effects on gelatin blends after injection, samples were stored under controlled temperature (4 ± 1°C) in sealed polyethylene bags prior to further testing, up to 48 h. Samples were named Ge-*X*Gly-*Y*ESO-*Z*B, where *X*, *Y* and *Z* refers to Gly, ESO and buffer solution (B) concentrations (% w/w, dry Ge basis), respectively.

Table 1: Gelatin based blends containing Gly, ESO and buffer solution

Sample	Gly	ESO	Buffer
		(% w/w, dry Ge basis)	
1	30	10	20
2	30	10	30
3	30	10	40

4	0	0	30
5	20	0	30
6	30	0	30
7	40	0	30
8	0	5	30
9	0	10	30
10	0	20	30
11	35	5	30
12	25	15	30
13	20	20	30

2.3 Characterization

Apparent density of injected gelatin specimens was calculated by the mass/volume ratio. Mass was determined gravimetrically using an analytical balance (0.0001 g; Ohaus, USA), while volume was calculated from sample dimensions measured with a micrometer (± 0.01 mm, Bta. China). Five specimens were considered and mean density values were reported. Experimental data were statistically analyzed by one-way analysis of variance (ANOVA) along with Tukey’s tests at 95% confidence interval (α=0.05).

Thin sections of injected specimens were examined by using an optical microscope Karl Zeiss (Germany) at different magnifications (2.5, 10 and 25x). The possible presence of air bubbles and/or oil droplets was evidenced by examining thin sections of each specimen by Confocal Laser Scanning Microscopy (CLSM) using a Leica TCS SP2 Confocal Laser Scanning head mounted on a Leica IRE2 (SDK) upright microscope (Leica Microsystems Inc., Heidelberg, Germany) equipped with a 20 × HC PL APO CS/0.70NA dry immersion objective lens. Previously, samples were stained with an appropriate amount of a fluorescent dye solution containing Nile red (0.5 µL/mL). The stained specimens were placed on concave confocal microscope slides, covered with glycerol-coated cover slips, and examined with a 100 magnification lens and an argon/krypton laser having an excitation line of 514 nm.

Flow behavior of gelatin blends was studied on an AR-G2 rheometer (TA Instruments) using a parallel plate geometry (diameter = 25 mm; gap = 2800 µm). In order to avoid sample moisture loss during flow behavior determination, tests were performed coating the gelatin specimens with a thin layer (~1 mm) of PDMS. The possible influence of PDMS layer on the rheological behavior of gelatin samples was analyzed. PDMS was selected based on the immiscibility of this silicon-based polymer with gelatin and its Newtonian behavior in the studied strain range. To establish the linear viscoelastic region, dynamic strain sweep tests at 60°C were performed at different constant frequencies (0.1 Hz; 0.32 Hz and 100 Hz) with strain values ranging from 0.01-10%. An injected circular gelatin sample containing intermediate Gly, ESO and buffer concentrations (30% w/w Gly, 10% w/w ESO and 30% w/w Buffer) was chosen to set the viscoelastic region. It was verified that, in the analyzed frequency range, the rest of the formulations were also in the linear viscoelastic regime. Once this regime was determined, dynamic frequency sweep assays were carried out at 60°C. Storage (G') and loss (G'') moduli, as well as, complex viscosity ($|\eta^*|$) were recorded. All rheological studies were performed in triplicate.

3 Results and Discussion

3.1 Assessment of Rheological Measurement Conditions

Linear viscoelastic regime of gelatin blends was detected in the strain range between 0.01-0.09%, at the studied frequencies (0.1 Hz; 0.32 Hz and 100 Hz). For that reason, an intermediate strain value (0.05%) was fixed for further rheological determinations. To study the flow behavior of gelatin mixtures, dynamic frequency sweep assays were carried out at 60°C and 0.05% strain. A thin layer (~1 mm) of

PDMS was used to cover gelatin sample after being placed between the rheometer plates, in order to prevent the loss of water upon testing. Complex viscosity of uncoated samples (Fig. 1) increased significantly after repeating the assay with the same specimen due to the expected water loss from gelatin blends upon rheological measurements. Water evaporation induced changes in sample composition, which in turn might lead to erroneous and over-estimated results. Contrarily, complex viscosity of PDMS-coated specimens kept invariant after assaying the same specimen repeatedly (Fig. 1). Coated samples exhibited lower viscosity values compared to uncoated ones, 2.3×10^3 Pa.s and 8.4×10^4 Pa.s, respectively, both determined at 0.123 Hz after the third scan. The absence of a rigid crust at gelatin-air interface in coated samples after testing demonstrated that the hydrophobic nature of PDMS hindered the water evaporation during testing. These results provide an insight into the effect of water evaporation on the precision in the flow behavior determination of gelatin-based formulations. It was demonstrated that the lack of control on water loss might lead to significant overestimation of the rheological properties. Accordingly, PDMS coating was used to study the flow behavior of gelatin-based formulations and determine the optimal processing conditions.

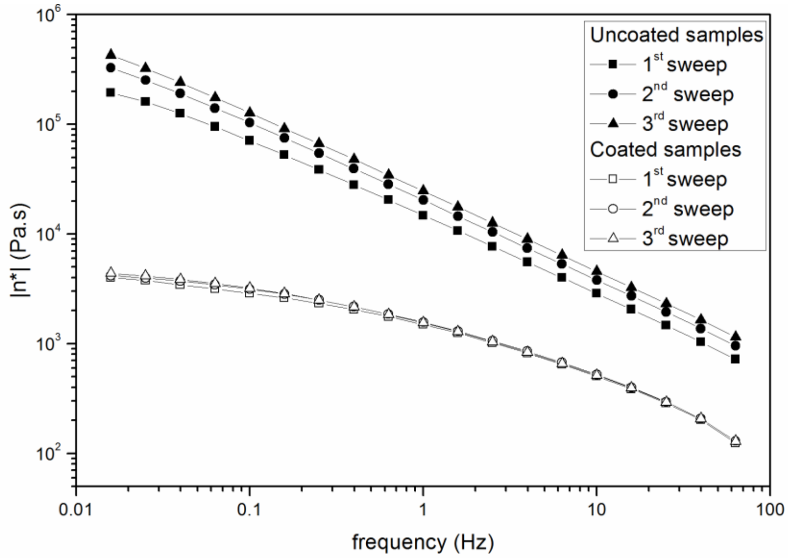


Figure 1: Complex viscosity (|η*|) *vs.* frequency for uncoated and PDMS-coated gelatin blends (Ge-30Gly-10ESO-30B)

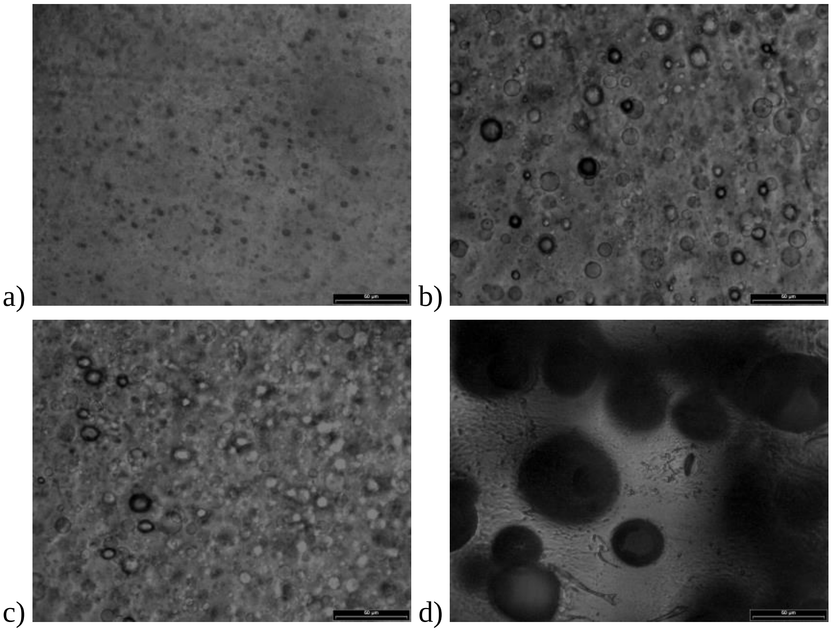
3.2 Influence of Buffer Solution Concentration

Even though water is essential to process gelatin, since it increases protein chains mobility and blend flowability, an excessive water concentration can lead to undesirable foam formation inside extruders [7,10]. Accordingly, different gelatin formulations were processed, modifying only the amount of aqueous solution (20-40% w/w), while keeping constant Gly and ESO concentration at intermediate levels (30% w/w Gly-10% w/w ESO). Gelatin blends with buffer concentrations higher than 20% w/w showed lower apparent density (Table 2), attributed to a major presence of air bubbles, as it was observed in optical micrographs (Figs. 2(a)-2(c)). Viscosity decreased with buffer concentration being more relevant in the range of 20% to 30% w/w (Fig. 3), due to the plasticizing effect of water. According to these results, the optimal buffer solution concentration was set at 30% w/w. This percentage allowed obtaining a proper processing of gelatin-based materials, minimizing water incorporation in their formulations.

Table 2: Apparent density of blends based on Ge, Gly, ESO and buffer solution

Gly	ESO	Buffer	Apparent density (g/cm^3)
% w/w dry Ge basis			
30	10	20	1.43 ± 0.00 ^{a}
30	10	30	1.29 ± 0.01 ^{b,c}
30	10	40	1.33 ± 0.05 ^{b,c}
0	0	30	1.24 ± 0.02 ^{b}
20	0	30	1.32 ± 0.01 ^{b,c}
30	0	30	1.32 ± 0.05 ^{b,c}
40	0	30	1.28 ± 0.04 ^{b,c}
0	5	30	1.32 ± 0.04 ^{b,c}
0	10	30	1.30 ± 0.00 ^{b,c}
0	20	30	1.35 ± 0.02 ^{a,c}
35	5	30	1.30 ± 0.01 ^{b,c}
25	15	30	1.29 ± 0.01 ^{b,c}
20	20	30	1.30 ± 0.01 ^{b,c}

Mean values ± standard deviations. Mean values within the same column followed by the same letter are not significantly different ($p > 0.05$, Tukey test).



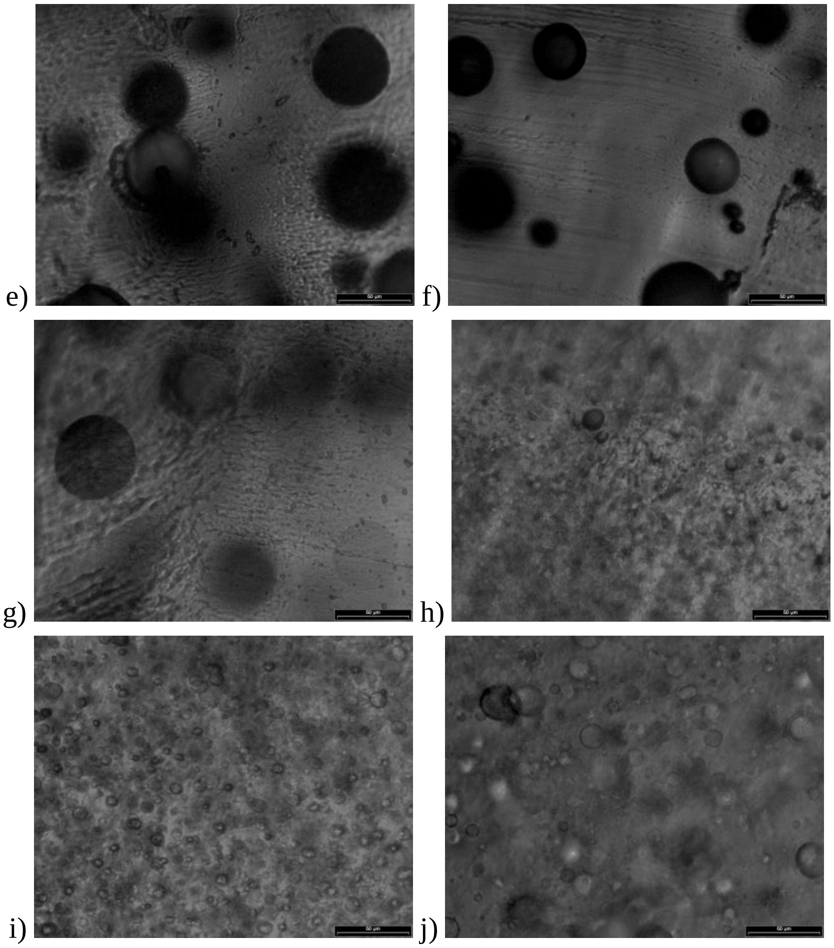
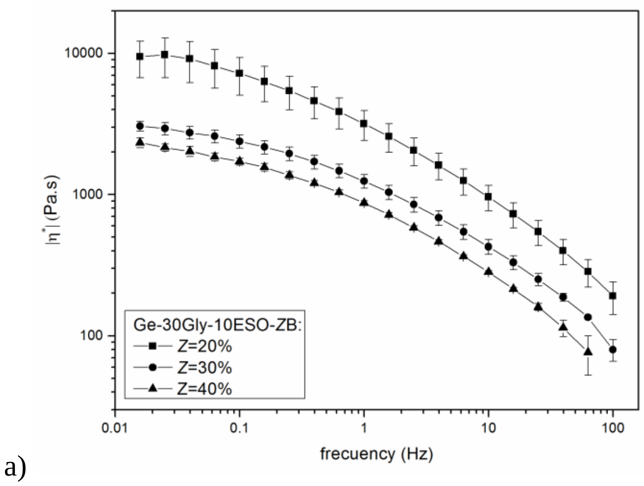


Figure 2: Optical micrographs (25X) of gelatin blends with different components concentration: a) Ge-30Gly-10ESO-20B; b) Ge-30Gly-10ESO-30B; c) Ge-30Gly-10ESO-40B; d) Ge-0Gly-0ESO-30B; e) Ge-20Gly-0ESO-30B; f) Ge-30Gly-0ESO-30B; g) Ge-40Gly-0ESO-30B; h) Ge-0Gly-5ESO-30B; i) Ge-0Gly-10ESO-30B; j) Ge-0Gly-20ESO-30B



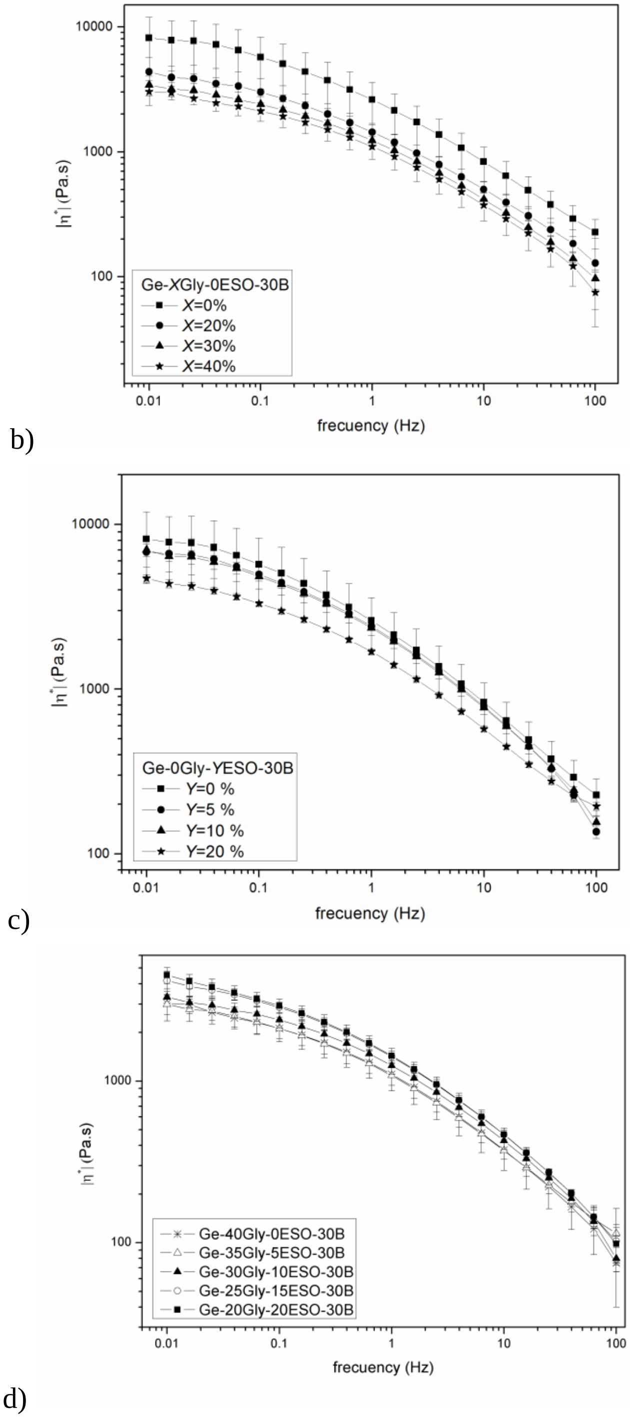


Figure 3: Complex viscosity ($|\eta^*|$) *vs.* frequency for gelatin blends with variable concentration of: a) buffer solution; b) Gly; c) ESO; d) Gly and ESO combinations

3.3 Influence of Glycerol Concentration

Blends containing 20-40% w/w Gly (in absence of ESO, Samples 5-7, Tab. 1) contained a considerable amount of bubbles. Glycerol content has no effect on bubble size and distribution (Figs.

2(d)-2(g)). Apparent density increased with the addition of Gly (Tab. 2), but values varied slightly with increasing plasticizer level ($p < 0.05$).

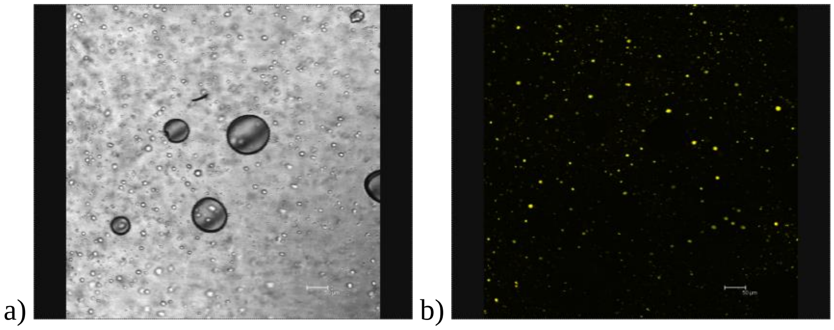
Large dispersion in viscosity values was observed for control samples (0 % w/w Gly, Fig. 3(b)). The incorporation of 20% w/w of Gly reduced significantly the viscosity of the blend, attributed to an increment of protein chain mobility due to the plasticizing effect of Gly [23]. Slightly lower viscosity values were observed for higher Gly concentrations (30-40% w/w). From these results, it can be concluded that there were no restrictions about Gly concentration, ranged from 20% to 40% w/w, for the processing of gelatin blends.

3.4 Effect of Epoxidized Soybean Oil Concentration

The incorporation of ESO (in absence of Gly, Tab. 2) induced an increment in blend density respect to control sample (Ge-0Gly-0ESO-30B), being the mean differences statistical significant ($p < 0.05$) only for the highest ESO concentration (20 % w/w). The presence of air bubbles of smaller diameter in ESO-added formulations (Figs. 2(d), 2(h)-2(j)), compared to control one, could be the main reason of the increase in apparent density. Gelatin blends with 5% and 10% w/w ESO showed similar viscosity curves (Fig. 3(c)), mainly in the intermediate range of frequencies, having slightly lower values than that of the control blend (Ge-0Gly-0ESO-30B). The drop of viscosity for 20% w/w ESO evidenced the plasticizing capability of ESO, particularly at low oscillation frequencies. Similar results were observed for ESO-ethyl cellulose being assigned to the ability of ESO in reducing the intermolecular interactions between ethyl cellulose and polymer chains [7,24].

3.5 Combined Effect of Glycerol and Eso

Previous studies have reported that ESO reduces hydrophilicity of wheat gluten [7,21], thermoplastic starch [7,22] and soybean protein isolate [7,20]. In order to enhance the hydrophobicity of gelatin based materials [20,21], glycerol was partially replaced by ESO, but keeping constant the total weight percentage of these additives (40% w/w, Ge dry basis). The partial replacement of Gly by ESO did not affect blend density (Tab. 2) and induced a more homogeneous appearance. However, further replacement of Gly by ESO led to an undesirable occlusion of little bubbles and/or oil droplets. In order to clearly differentiate air and oil phases, CLSM was employed, staining the hydrophobic phase (ESO) with Nile red (Fig. 4). Microscopy evidenced the homogeneous distribution of ESO, with mean particle size around 10 µm (bright yellow spots in Figs. 4(b) and 4(d)). Regarding air bubbles, there was a bimodal distribution with mean diameter near to 100 µm and those similar to oil droplets (Fig. 4(a)), except for Ge-20Gly-20ESO-30B blend, where a homogeneous bubble size distribution near to 10 µm was detected (Fig. 4(c)).



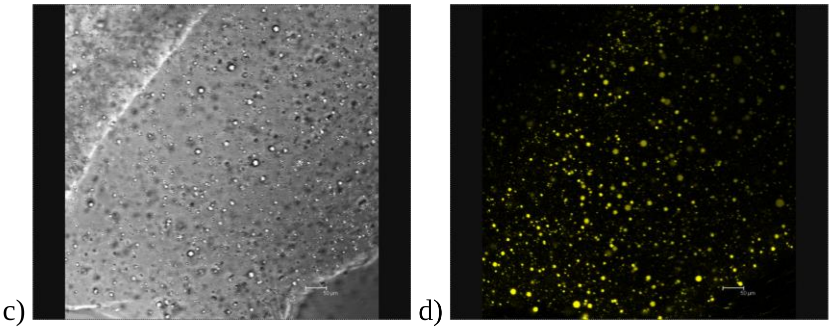


Figure 4: CLSM images (20X) of gelatin blends: a-b) Ge-30Gly-10ESO-30B, c-d) Ge-20Gly-20ESO-30B (scale bar: 50 µm)

Gelatin formulations containing 40%, 355% and 30 % w/w of Gly (Ge-40Gly-0ESO-30B, Ge-35Gly-5ESO-30B and Ge-30Gly-10ESO-30B, respectively) presented the lowest viscosities, mainly at low frequencies, among all studied formulations (Fig. 3(d)). On the other hand, mixtures having lower Gly concentration (Ge-25Gly-15ESO-30B and Ge-20Gly-20ESO-30B) behaved similarly between themselves. By CLSM, it was demonstrated that ESO segregated as droplets at high concentrations. The presence of ESO droplets was significant at 15% and 20% w/w ESO, evidencing a limited compatibility between Ge and ESO. This is the main fact responsible for the increased viscosity at high glycerol replacement. So, it could be inferred that at least 10 % w/w Gly can be replaced with ESO without altering the rheological behavior.

4 Conclusions

This study analyzed the morphology and rheological properties of injection-molded gelatin blends with glycerol, epoxidized soybean oil and buffer solution at different concentrations. Rheological studies and microscopy allowed analyze the relationship between morphology and flow properties of gelatin formulations. The use of hydrophobic PDMS as coating material during rheological measurements gave reliable and reproducible results by preventing water evaporation upon testing. The viscosity was noticeably decreased by increasing Gly in the formulation meanwhile the plasticizing effect of ESO was evident at 20% w/w. The partial replacement of Gly by ESO evidenced phase separation, associated with the restricted compatibility between ESO and the plasticized-gelatin matrix, increasing the blend viscosity at high oil concentration. However, at least 10% w/w Gly could be replaced with ESO without a significant variation of blend rheological behavior, in order to develop gelatin-based materials with enhanced hydrophobicity which could be processed at conventional pre-existing technologies.

Acknowledgments: Authors want to express their gratitude to the National Research Council (CONICET), the National Agency for Scientific and Technological Promotion of Argentina, the National University of the South (UNS) and the National University of Mar del Plata (UNMdP).

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