Mechanical and Fracture Behavior of Gelatin Gels

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Abstract
Hydrogels were prepared from bovine and porcine gelatin powders of different Bloom values. Gelatin concentration and pH were selected to obtain self-supporting gels with stiffness in the range of those employed in structural applications in foodstuff, ballistic and pharmacy. Glycerol and sorbitol were also added in gel formulations. Deformation and fracture behaviors were assessed by means of gel strength measurements, uniaxial compression tests and wire cutting experiments. All gels showed non-linear elastic deformation with strain hardening and brittle fracture. Large deformation was described with a first order Ogden constitutive model. Strain hardening was attributed to protein chains renaturation capability under stress. It was found that neat porcine gelatin gels displayed higher gel strength, shear modulus, failure strain and stress, fracture toughness and water retention than bovine gelatin gels. The addition of sorbitol and glycerol caused an increase in water retention capability and a marked enhancement in gels´ fracture toughness. The major content of hydrophilic amino acids in the gelatin, the stronger the effect of glycerol and sorbitol on gelatin network morphology. The correlation between large deformation and fracture toughness parameters with the technological gel strength values was also investigated.

Keywords: hydrogels, gelatin, fracture toughness, depth sensing indentation

1. Introduction

Hydrogels consist of three-dimensional hydrophilic polymer network with the ability of swelling and retain large volumes of water without dissolution [1]. Hydrogels can be obtained from synthetic polymers, but those derived from biopolymers such as proteins and polysaccharides are being preferred due to their renewable nature, low toxicity, biodegradability and biocompatibility [2]. Among natural polymers gelatin is an attractive candidate as raw material for preparing hydrogels because its gelling ability. It is unique among hydrocolloids in forming thermo-reversible physical gels with a melting point close to body temperature [1].

The main gelatin hydrogels applications include their use as: *i)* ingredients to improve elasticity, consistency and stability of foods [3]; *ii)* human tissue simulants in ballistic studies [4]; *iii*) carriers for drug delivery systems [5]; iv) scaffolds for cell growth in tissue engineering [6]. Mechanical requirements for hydrogels in the aforementioned applications are large deformation capability together with proper toughness and stiffness level. Independent tuning of elastic modulus, deformation capability and fracture toughness becomes highly desirable to the development of hydrogels with tailored properties. So that, the assessment of mechanical properties and the knowledge of whether they are influenced by gelatin source and additives are of fundamental importance and constitute the aim of this research.

Determining reliable mechanical properties of soft materials like hydrogels is a challenged task due to its low modulus (of the order of kPa) and complex mechanical behavior. Hydrogels are soft but exhibit brittle failure and show dependence with strain rate, as polymers does (see for example in [7]).

The gel strength is the most popular property used to mechanically characterize gelatin gels [8]. It is determined using the so-called Bloom test, which consists on performing an indentation test under

specifically defined conditions -gelatin concentration (6.67 %), temperature (10 ºC), maturation time (17 h), indentation depth (4 mm), cylindrical plunger (12.5 mm diameter)- thus allowing gel strength to be expressed in the normalized Bloom value. It has been shown that the Bloom value is linked to the stiffness of the gel network [9].
In this work, deformation behavior and fracture toughness of gels based on two commercial mammalian gelatins differing in collagen source (*ie.* with different amino acid composition [10]) and Bloom value are assessed. Large deformation behavior is evaluated by uniaxial compression experiments and for fracture toughness, the Gc parameter is determined by the wire cutting technique [7]. The effect of adding sorbitol and glycerol, two common additives in gels formulations, on mechanical performance is also analyzed and discussed. The relationship between gel strength and shear modulus, fracture toughness and ultimate deformation properties is also investigated.

2. Experimental

2.1. Gelatin Gels preparation

Bovine hide gelatin type B, Bloom 200, isoionic point (Ip) 4.7-5.4 and porkskin gelatin type A, Bloom 250, Ip 7-9 were kindly supplied by Rousselot (Argentina). Glycerol analytical grade were purchased from DEM Chemicals (Mar del Plata, Argentina). D-Sorbitol monhydrate (S) and buffer solution pH 7 and pH 10 were purchased from Anedra (Buenos Aires, Argentina).
Gelatin solutions were prepared by dissolving the gelatin powder (20wt%) in a buffer solution with continuous stirring at 50ºC. The buffer was selected over the gelatins' isoionic points (pH=7 for bovine and pH=10 for porcine gelatins). In addition, glycerol and sorbitol were incorporated at a concentration of 25wt% (based on dry gelatin powder). Glycerol and Sorbitol are non-solvents for gelatins so that the initial gelatin concentration in water was kept constant in each formulation. The designation of prepared gels is *B0*, *B1* and *B2* for bovine based gels containing neat gelatin, glycerol and sorbitol, respectively. Analogous, porcine gelatin gels are designated as *P0*, *P1* and *P2*.
The resulting homogeneous solutions were poured into specially design Delrin® molds and kept at room temperature during 15min to form the gels. Specimens were then wrapped in film in order to minimize drying out and stored at 4 ºC during 48 h. Before testing samples were conditioned at 21 ºC during 2 h.

2.2. Physical and mechanical characterization

2.2.1 Water Retention: For each formulation, three gel specimens were dried in an air circulating oven at 105ºC until constant mass. Samples were weighted with an accuracy of 0.0001g before and after drying to determine the initial mass (m_i) and the gelatin dry matter (m_0), which was normalized by the glycerol and sorbitol content. Gel water retention (WR) was then calculated as:

$$WR(\%) = \frac{m_i - m_0}{m_0}.100 \quad (1)$$

2.2.2 Transmission optical microscopy (TOM): Inspection was performed on gels samples employing a Leica DMLB microscope provided with a video camera Leica DC 100.
2.2.3 Scanning Electron Microscopy (SEM): Inspection was performed in a JEOL JSM-6460LV microscope in order to analyze the ultra-structure of gelatin gels. The prepared samples were frozen, lyophilized, cryofractured and coated with a thin film of Au/Pd before inspection.
2.2.4 Gel Strength: Measurements were carried out on prepared gels using an INSTRON 3369 universal testing machine. Samples were cylindrical blocks having 25 mm of diameter and height. Gel strength was determined as the maximum load measured after a steel cylindrical plunger penetrated 4mm into the sample surface [11]. The plunger was flat-faced and had a diameter of 10 mm. A Load cell of 0.1 kN was used and the penetration speed was 25mm/min. The gel strength was taken as the average value of four measurements performed on different samples.
2.2.5 Uniaxial compression: Tests were performed on cylindrical samples with a diameter and height of 25mm in an INSTRON 4469 universal testing machine. Experiments were conducted at two loading rates (5 and 25 mm/min). A 0.5 kN load cell was used. A Teflon spray was applied at the interface between sample and compression platens in order to diminish friction. At least five replicates were tested at each loading rate. Samples were loaded up to fracture. The obtained load (P)- displacement (v) data were converted to true stress (σ) vs. true strain (ε) curves assuming that the material was incompressible. Data were also expressed in terms of and true stress (σ) – stretch ratio ($\lambda = e^{\varepsilon}$).
2.2.6 Wire Cutting tests: Experiments were carried out in an INSTRON 4469 universal testing machine equipped with a specially designed grip and a 0.5 kN load cell. The grip allows the wire to be tight and perfectly aligned with the sample surface. Seven steel wires of different diameters (d) ranging from 0.2 to 0.8 mm were used and the cutting rate was 25 mm/min. The samples were rectangular blocks of length 30 mm, width 20 mm and height 20 mm. For each wire diameter at least five cuts were performed. The steady-state cutting energy was obtained by dividing the cutting force, F_c, by the specimen width, b. A linear relationship between the normalized cutting force F_c/b and wire diameter d is postulated [12]:

$$\frac{F_c}{b} = G_c + \sigma_y \left(1 + \mu_k\right) d \tag{2}$$

where σ_y is a characteristic stress and μ_k is the kinetic friction coefficient. By extrapolating the steady state cutting energy to zero wire diameter, G_c is obtained (Eq. 2) since the work input is consumed solely by the propagating crack.
2.2.7 Statistical Analysis: Analysis of variance (ANOVA) was carried out to find differences in the measured properties of gelatin gels due to the addition of glycerol and sorbitol. The bovine gelatin gel series (*B0, B1* and *B2*) and the porcine gelatin gel series (*P0, P1, P2*) were analysed by an independent ANOVA. Differences between means were analysed using the Tukey´s test for post hoc comparison ($p < 0.01$). Analysis was performed using STATISTICA 6.0 (Statsoft, Inc., Tulsa, USA).

3. Results and Discussion

Gelatin powder concentration, pH and temperature values used in gels formulation were selected to obtain self-supporting gels with stiffness in the middle range of those employed in structural applications in foodstuff, ballistic and pharmacy (E = 1 to 10^3 kPa) [13,14]. Gelatin concentration

was kept constant in all materials, but owing to the different amino acid composition [10] and molecular weight of both types of gelatins and due to the presence of additives, different gel mechanical properties are expected.
All prepared gelatin gels show optically clear appearance and transparency. The incorporation of additives- glycerol and sorbitol- seems to not alter their physical integrity or optical appearance.

3.1 Water Retention Capability

As can be seen in Table 1, *P0* gel shows higher water retention capability than *B0*. The incorporation of glycerol and sorbitol increases the amount of retained water in both types of gels. In comparison, the increase in water retention capability is larger in gels containing glycerol than in those with sorbitol because glycerol molecules are more hygroscopic than sorbitol ones due to its smaller size.

3.2 Gel Strength

Gel strength values of gelatin gels are also given in Table 1. Gel strength of *P0* is higher than *B0* in agreement with the larger Bloom value of porcine gelatin. As well, all porcine gelatin based gels are stronger than bovine based ones, being *P2* the strongest gel of the series.
Gel strength values do not decrease with increasing water content in modified gels, indicating a strong impact of glycerol and sorbitol on gelatin gels network structure. The increase in gel strength is consistent with the explanation that the addition of polyhydric alcohols may create a shorter mean distance between junction zones by increasing the number of crosslinking points [15] and that co-solvent do not participate in the interlinking of protein molecules but they reduce the available water by preferential hydrogen bonding with water molecules [16].

Table 1. Water retention and gel strength of prepared gelatin gels.

Gel	Water Retention (%)	Gel Strength (N)
B0	355.6 ± 1.5^{a}	4.09 ± 0.12^{a}
B1	447.7 ± 1.9^{b}	3.61 ± 0.24^{b}
B2	365.7 ± 3.1^{c}	3.88 ± 0.14^{ab}
P0	429.3 ± 6.4^{A}	4.40 ± 0.08^{A}
P1	448.3 ± 2.9^{B}	4.12 ± 0.01^{B}
P2	437.4 ± 1.8^{B}	4.71 ± 0.10^{C}

Different letters in the same column indicate significant differences between means ($p<0.05$).

3.2 Large Deformation Properties

Previous investigations [17, 18] demonstrated that the uniaxial compression response of the studied

gelatin gels is independent of strain rate within a range from 0.1 to 10min^{-1} and that it is completely reversible.

Figure 1 shows the stress-strain curves obtained for bovine (Fig. 1-a) and porcine (Fig. 1-b) based gelatin gels in uniaxial compression experiments at a strain rate of 1/min. The compression response of all gels is non-linear elastic with strain hardening up to catastrophic fracture. The scattering in uniaxial compression curves is wider in porcine gels than in bovine gels. This maybe related to the higher content of trapped air microbubbles in the porcine gels as thrown by TOM inspection of gel samples.

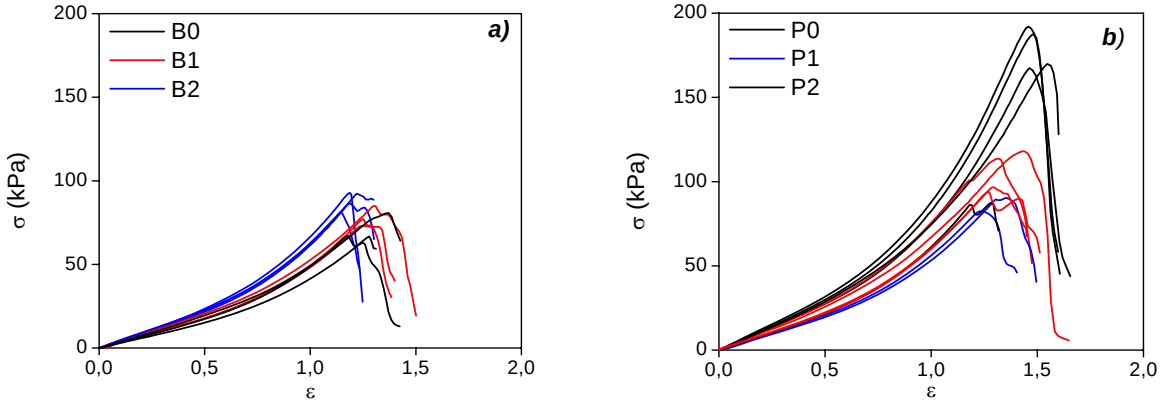

Figure 1. Stress-strain curves obtained in uniaxial compression tests for a) bovine and b) porcine based gelatin gels.

The simplest law used to model materials that behave elastically and show strain hardening when subjected to large strains is the first order Ogden constitutive equation. It can be expressed in terms of true stress σ and stretch ratio λ, as:

$$\sigma = \frac{2\mu}{\alpha}\left(\lambda^{\alpha} - \lambda^{-\alpha/2}\right) \tag{3}$$

where μ is equal to the material shear modulus and α is a parameter related with strain hardening capability [19]. More negative α values indicate large strain hardening, while less negative ones stands for more linear elastic behavior.

For each experimental set of curves, a least square fit was performed taking all stress-stretch ratio data up to λ=0.35. The obtained model parameters are given in Table 2. This simple model (Eq. 3) is able to well describe the behavior of gelatin gels as judged by the values of the regression coefficients (R^2) and the errors in the model parameters. The source of strain hardening capability of gelatin gels may rely on the ability of the protein molecules to recover the native collagen triple helix configuration under stress. This behavior has already been suggested to explain improvements in mechanical properties of gelatin films [20]. As protein renaturation is a completely reversible process, gelatin gels are capable to recover its original configuration after load removal, displaying non-linear elastic behavior. *P0* is more able to strain harden than *B0* (see the more negative α value in Table 2).

The shear modulus of *P0* gel is larger than the one determined for *B0* gel (Table 2), consistently with its higher Bloom value. The addition of sorbitol and glycerol slightly modifies the Ogden constitutive parameters except for the *P2* gel in which α becomes less negative and μ decreases. The change in α is consistent with a larger amount of physical crosslinking points in the gelatin network, which

constrains renaturation of protein molecules under stress. The decrease in μ maybe related to the higher water content in the gel.

Table 2. Mechanical parameters obtained from uniaxial compression tests.

Gel sample	μ (kPa)	α	R^2	Fracture stress (kPa)	Fracture strain
B0	9.29 ± 0.09^{a}	-1.40 ± 0.02^{a}	0.9826	69.2 ± 8.1^{a}	1.23±0.12
B1	12.07 ± 0.06^{b}	-1.44 ± 0.01^{b}	0.9954	90.6 ± 3.5^{b}	1.20±0.02
B2	9.57 ± 0.04^{c}	-1.37 ± 0.01^{c}	0.9983	79.1 ± 5.1^{ab}	1.23±0.06
P0	14.59 ± 0.15^{A}	-1.53 ± 0.01^{A}	0.9747	176.6 ± 35.4^{A}	1.42±0.14
P1	13.52 ± 0.19^{B}	-1.38 ± 0.04^{B}	0.9599	102.8 ± 32.9^{B}	1.31±0.04
P2	10.88 ± 0.19^{C}	-1.14 ± 0.05^{C}	0.9507	83.1 ± 7.4^{B}	1.37±0.10

Different letters in the same column indicate significant differences between means. Differences in fracture strain were not significative for both B and P series ($p<0.05$)

The ultimate properties obtained from the failure point in the stress-strain curves are also reported in Table 2. Fracture stress and strain are related to crosslinking density and network strength but also depend on microstructural defects. *B0* gel displays lower ultimate properties than *P0* gels despite the presence of microbubbles. In brief, porcine gelatin gels structure is more resistant and has a larger deformation capability than bovine gelatin one. Surprisingly, the addition of sorbitol and glycerol has a marked impact in the ultimate properties of porcine based gels but slightly influences the bovine ones. The detriment in fracture strain can be explained by an increase in protein-protein interactions, which leads to a more physically crosslinked network.

The ultra-structures of *P0*, *P2*, *B0* and *B2* gels were analyzed by SEM. The images of the morphologies are shown in Figure 2. All gels exhibit a network structure, but the addition of sorbitol in gel formulations appears to promote a large number of bridging sites in the gel main network (Fig. 2 *b* and *d*). This effect is more pronounced in P2 gel (Fig. 2 *b*), consistently with the major change in ultimate stress and strain hardening capability. It can also be observed that the network is more opened in *P2* gel even it is more interconnected. These features can explain the decrease in gel stiffness and are in agreement with the higher water retention capability of *P2* gel.

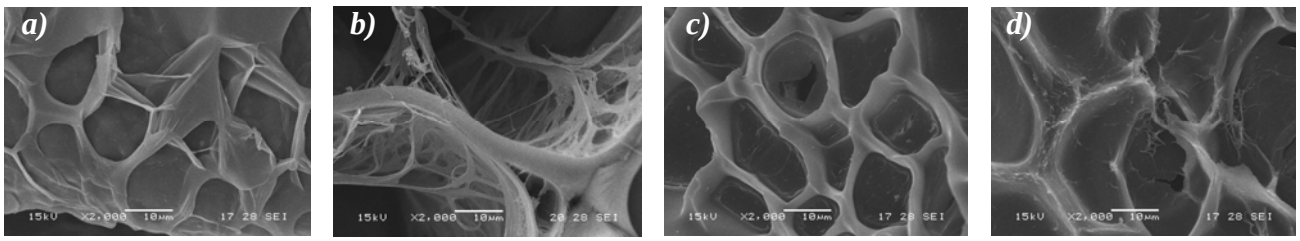

Figure 2. SEM images of gelatin gels revealing the detailed morphology of gelatin network ultra-structure a) P0, b) P2, c) B0 and d) B2.

3.3 Fracture Toughness

For all samples, wire cutting curves show two phases: an indentation phase and a steady- state cutting phase. First, the wire indented into the gel up to a given point at which the material starts to fracture. From such point, the load decreased suddenly reaching an almost constant force that corresponds to the steady-state cutting phase, F_c. In every case, the cutting force increases with increasing wire diameter. Figure 3 shows typical force-displacement curves obtained at different wire diameters for one of the tested gels (*B0*). The same wire cutting behavior was described for other soft materials [see for example in [7, 12, 21].

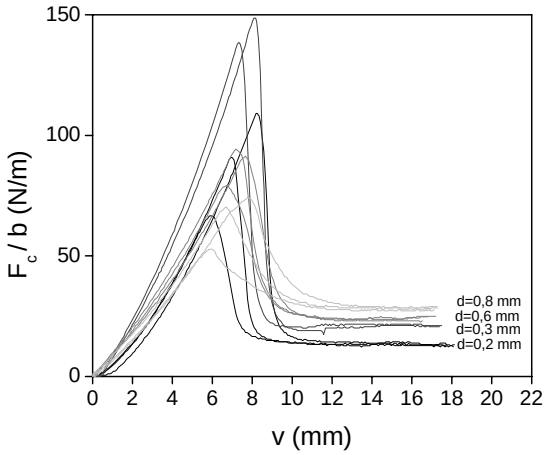

Figure 3. *Typical normalized load-displacement data measured in wire cutting tests of B0 gel using different wire diameters.*

The cutting energy versus wire diameter plots are shown in Figure 4. All data obtained for each wire diameter were averaged and plotted with its error. Data were linearly fitted according to Eq. 2 using Least Squares. Data lying outside the 95% confidence limits from the first best fit line were eliminated from the analysis. The arisen fitted parameters are listed in Table 3.

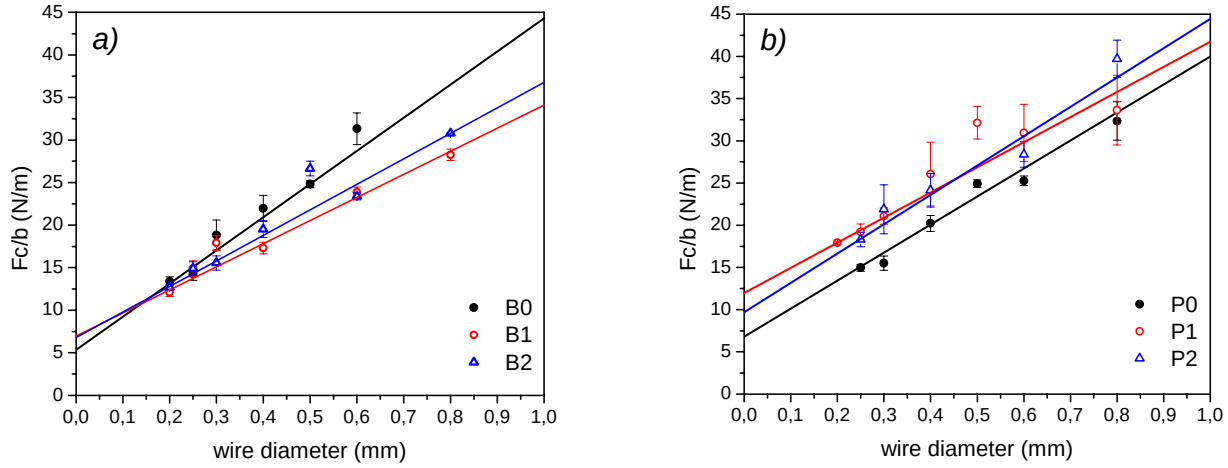

Figure 4. *Wire cutting data measured for a) bovine and b) porcine gelatin gels.*

The resulting experimental trend agreed with the wire cutting method proposal (Eq. 3) as judged by the obtained R^2 values greater than 0.93. Better regression coefficients values yield from bovine gelatin gels than for porcine gelatin gels. This can be attributed to the larger content of

microbubbles in the porcine gels.

The fracture toughness values are in the range of those required in pharmacy and tissue engineering applications (1 to 10 N/m) [13]. *P0* gels exhibit higher fracture toughness than *B0* gels, despite its larger amount of retained water. This is also true for the whole bovine and porcine series. Moreover, the addition of sorbitol and glycerol in gel formulation has a beneficial effect on the fracture toughness of both bovine and porcine gelatin gels. The enhancement in fracture toughness is more pronounced in porcine gels according to the development of a more interconnected network (Figure 2).

Table 4. *Fracture toughness of gelatin gels, G_c*

Gel sample	Gc (N/m)	$\sigma_0(1+\mu)$ (kPa)	R^2
B0	5.35± 0.78	38.95± 1.98	0.99222
B1	6.96± 0.71	27.15± 1.44	0.98887
B2	6.81± 0.25	29.96± 0.33	0.99951
P0	6.81± 1.66^{A}	33.16± 3.81	0.93736
P1	11.98± 0.42^{B}	29.75± 1.99	0.97802
P2	9.70± 1.58^{B}	34.44± 3.76	0.95393

Different letters in the same column indicate significant differences between means. Differences in Gc were not significative in B serie ($p<0.05$).

4. CONCLUSIONS

Gelatin hydrogels with stiffness values in the range required for applications in ballistic, food, pharmaceutical and tissue engineering were prepared from commercial powders and their large deformation and fracture behaviors were investigated. The main findings of this research are summarized as follows:

- Under uniaxial compression, bovine and porcine gelatin gels behave as non-linear elastic materials with strain hardening, which can be attributed to partial protein renaturation under stress. Gelatin gels were able to support deformation levels over 120% before failure.
- Gelatin gels show brittle fracture behavior with fracture toughness values in the range of those required in pharmacy and tissue engineering.
- Neat porcine gelatin gels exhibit higher gel strength, shear modulus, failure strain and stress, fracture toughness and water retention capability than bovine gels.
- The addition of sorbitol and glycerol causes an increase in water retention capability of both types of gelatin gels as well as a marked enhancement in fracture toughness.
- The ultra-structure of modified gelatin gels presents a more opened network but contains a higher amount of interconnection sites than the neat gelatin gels.
- The effect of additives on elastic modulus and ultimate properties is detrimental in porcine gelatin gels and negligible in bovine gelatin gels.
- A correlation between the technological gel strength measurement with fracture and mechanical parameters was not found for gelatin gels containing additives.

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