



## Deformation and fracture behavior of physical gelatin gel systems



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### ABSTRACT

Food scientists usually used biopolymer physical gels as model systems because they are structurally and mechanically similar to many gel-like food products. In this paper, eight gelatin gel systems with different stiffness were prepared by varying gelatin concentration (10–30%w/w), collagen source (bovine/porcine) and solvent composition (0/40%w/w glycerol/buffer mixture). The swelling behavior was evaluated and the mechanical response was characterized through puncture tests, uniaxial compression experiments and wire cutting fracture tests. From these tests, apparent gel strength, first order Ogden constitutive parameters (shear modulus,  $\mu$ , and strain hardening capability,  $\alpha$ ) and fracture toughness ( $G_c$ ) were determined. Samples that display apparent gel strength and swelling behavior consistent with a more physically cross-linked structure exhibit larger  $\mu$  and  $G_c$  and lower  $\alpha$  values. It is shown that  $\alpha$  and  $G_c$  are related with  $\mu$  independently of gelatin concentration, collagen source and glycerol presence.  $\alpha$  decreases exponentially with increasing  $\mu$  whereas  $G_c$  increases linearly with  $\mu$ . The found experimental trends suggest that in the quasi-static range the overall mechanical behavior of gelatin gel systems is mainly controlled by the initial shear modulus, which is a direct measure of gel stiffness.

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## 1. Introduction

Determination of mechanical properties is of great importance for food scientists and technologists since the mechanical response affects food processing, handling and consumption. On one hand, during manufacturing, food products are subjected to large strains that may cause severe deformation or even final fracture, affecting their structural integrity. On the other hand, large deformations and fracture processes are involved in biting and mastication and therefore, they are linked to consumer's acceptance and preference. It has been shown that large deformation properties are associated to texture perception and display good correlation with sensory evaluation (Barrangou, Drake, Daubert, & Foegeding, 2006a; Foegeding, Brown, Drake, & Daubert, 2003; Foegeding, 2007; Foegeding et al., 2011; Takahashi, Hayakawa, Kumagai, Akiyama,

& Kohyama, 2009).

The structure of many foods, such as processed meats, cheese, gelatin desserts, cooked egg whites, frankfurters, surimi based seafood analogs, yogurt and confectionery products, is dependent on the formation of a gel network. Due to the high complexity of these foods, biopolymer gels are commonly adopted as model systems for mechanical investigations (Barrangou et al., 2006a, b; Foegeding & Daubert, 2008; Sala, 2007). Among biopolymer gels, physical gelatin gels are interesting materials since their stiffness can be easily manipulated. In these gels, the network is composed by ordered triple-helix (rigid) segments stabilized by intermolecular hydrogen bonds, which resembles the nature collagen state, interconnected by flexible protein chains that remain in the coil conformation (Joly-Duhamel, Hellio, Ajdari, & Djabourov, 2006a). The collagen-type triple helices act as the physical crosslinking points of the gel network. Gels containing large amounts of triple-helices are strongly cross-linked and become rigid (Bigi, Panzavolta, & Rubini, 2004; Joly-Duhamel et al., 2006a). The amount of triple helices in gelatin hydrogels depends on gelatin concentration,

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thermal treatment and amino-acid composition (Joly-Duhamel et al., 2006a). In addition, the presence of sugars and other polyols enhances the formation of triple helices due to a preferential hydration effect. It is known that low molecular weight carbohydrates bind water in their hydration shells enhancing protein–protein interaction and thus helix formation (Joly-Duhamel, Hellio, Ajdari, & Djabourov, 2006b; Sanwlani, Kumar, & Bohidar, 2011; Seishi & Matubayasi, 2014). Therefore, gelatin gel stiffness can be tailored through proper selection of these variables.

Determining true mechanical properties of biopolymer gels is not a simple task due to its low modulus (of the order of 1–100 kPa) and inherent complex mechanical behavior. Biopolymer gels are soft materials that support large deformations and show strain hardening, resembling the hyperelastic behavior of elastomers. However, these materials exhibit brittle fracture, which is highly dependent on strain rate (Bot, van Amerongen, Groot, Hoekstra, & Agterof, 1996; Czerner, Martucci, Fasce, Ruseckaite, & Frontini, 2013; Forte, D'Amico, Charalambides, Dini, & Williams, 2015; Gamonpilas, Charalambides, & Williams, 2009; Urayama, Taoka, Nakamura, & Takigawa, 2008). The fracture mechanism in biopolymer physical gels has been explained as a viscoplastic pull-out process of the chains that constitutes the network, which is different to the typical chain scission mechanism that occurs in chemical gels (Baumberger, Caroli, & Martina, 2006).

The puncture test is probably the most popular mechanical test used to measure textural properties of soft foods (Chen & Opara, 2013). As well, this test can be applied to determine the gel strength of gels (Chiou et al., 2006). A punch of defined geometry is pushed into the gel sample up to a selected depth, and the recorded load is the technical term gel strength. A particular case of this measurement is the so-called Bloom test extensively adopted to assess the grade and quality of a gelatin. It should be considered that this type of single point measurements is not completely representative of the large deformation behavior of gels. In an own previous work, it was shown that a wide variety of gelatin gels display strain hardening at deformations larger than those imposed in the puncture test (Sanchez Fellay, Fasce, Czerner, & Frontini, 2015).

A more complete description of the large deformation behavior of gels can be carried out by uniaxial compression tests (Christianson, Casiraghi, & Bagley, 1986; Forte et al., 2015; Gamonpilas et al., 2009; Miller, 2005; Takahashi et al., 2009). In this test, a cylindrical sample is compressed while the load and displacement are continuously registered. The stress–strain relationship can be directly obtained from the measured data and interpreted by proper constitutive models to obtain intrinsic material parameters. The Ogden constitutive model has been widely adopted to describe the hyperelastic behavior of soft materials including hydrogels and living tissues under uniaxial compression (Comley & Fleck, 2012; Gamonpilas et al., 2009; Rashid, Destrade, & Gilchrist, 2012; Sasson, Patchornik, Eliasy, Robinson, & Haj-Ali, 2012; Sparrey & Keaveny, 2011).

Regarding fracture characterization, different test configurations such as single edge bending, tear, constrained tension and wire cutting were proposed in the past to evaluate the fracture toughness of gels (Baumberger et al., 2006; Chen & Opara, 2013; Czerner, Fasce, & Frontini, 2014; Gamonpilas et al., 2009; Kamyab, Charalambides, & Williams, 1998; Luyten, Vanvliet, & Walstra, 1992). Among these test configurations, the so-called “Wire Cutting method” is very attractive due to its simplicity. It involves pushing wires of different diameters into a specimen to promote a steady state cutting process, while the load and the wire advance are recorded. The crack propagation rate is directly the imposed wire displacement rate, in contrast to other test configurations in which it needs to be measured by monitoring the crack length at

various time intervals. This method has been used to evaluate the energy release rate,  $G_c$ , of chesses (Goh, Charalambides, & Williams, 2005; Kamyab et al., 1998) and physical gels (Czerner et al., 2014; Forte et al., 2015; Gamonpilas et al., 2009). The arisen fracture toughness parameters were shown to be in agreement with those obtained by using more complex techniques. In particular for gelatin gels, consistent fracture toughness parameters have been determined provided that a flattened surface fracture pattern is developed during the test (Czerner et al., 2014).

In this work, gelatin gel systems displaying different stiffness were prepared. The investigation was conducted in order to analyze the relationships between stiffness and large strain and fracture parameters of physical gels. For this purpose, formulation variables, such as powder concentration, gelatin source and solvent composition, were intentionally varied to promote the formation of gels with different triple helix content and hence with different stiffness. Gel systems were subjected to puncture and uniaxial compression tests and to wire cutting fracture experiments. Mechanical parameters were determined and then interpreted in terms of the gel physical structure. This research pretends to provide deeper insight and knowledge on how gel stiffness controls the overall mechanical behavior of gel-like foods.

## 2. Materials and methods

### 2.1. Gelatin gels preparation

Two commercial lyophilized gelatins arisen from different sources were used to prepare gel samples: bovine hide gelatin (Type B, Bloom 200, isoelectric point 4.7–5.4) and pork skin gelatin (Type A, Bloom 250, isoelectric point 7–9), both kindly supplied by Rousselot Argentina. Phosphate buffer solution (pH 7) and borate buffer solution (pH 10) were used as solvents to obtain hydrogels. A 40%w/w glycerol/buffer solution was used to prepare mixed solvent gels. All chemicals were of analytical grade (Cicarelli).

Gel-forming solutions were obtained by dissolving gelatin in the solvent at different concentrations varying from 10 to 30%w/w. Solutions were homogenized under mild stirring for 15 min at 50 °C. For each gelatinsource type, the pH of the solvent was selected over the isoelectric point to obtain anionic polyelectrolytes gels. Gelatin solutions were poured into cylindrical Delrin® molds and cooled at room temperature to form the gels. Gel samples were wrapped in polyethylene film to minimize loss of water and stored at 4 °C during 48 h before testing.

Details of gels formulations are given in Table 1. Gel density values reported in Table 1 were measured by pycnometry and used to calculate the actual gelatin concentration ( $C_{gel}$ ) in the gels.

### 2.2. Swelling experiments

Gels used for swelling studies were first lyophilized for 48 h to obtain dried samples. After dehydration, samples were accurately weighed and immersed in 20 ml of distilled water at  $20 \pm 2$  °C until the swelling equilibrium was achieved (Qiao & Cao, 2014). At regular intervals of time, samples were taken out from the distilled water, blotted using absorbent paper and weighed. The Swelling Ratio (SW%) was calculated at each time point from the dried sample weight ( $W_d$ ) and the swollen sample weight at time  $t$  ( $W_t$ ), as:

$$SW\% = \frac{(W_t - W_d)}{W_d} \times 100 \quad (1)$$

The Equilibrium Swelling Ratio (ESR) was calculated as (Qiao & Cao, 2014):

**Table 1**  
Details of gelatin gel formulations and sample denomination.

Sample denomination	Gelatin source	Gelatin concentration (% w/w)	Solvent	Density (g/cm <sup>3</sup> )	C <sub>gel</sub> (g/cm <sup>3</sup> )	
B Gels	B10	Bovine	10	Phosphate buffer	1.046 ± 0.002	0.1046
	B10-G		10	Glycerol/phosphate buffer	1.033 ± 0.003	0.1033
	B20		20	Phosphate buffer	1.070 ± 0.001	0.2140
	B30		30	Phosphate buffer	1.103 ± 0.003	0.3309
P gels	P10	Porcine	10	Borate buffer	1.030 ± 0.001	0.1030
	P10-G		10	Glycerol/borate buffer	1.035 ± 0.001	0.1035
	P20		20	Borate buffer	1.070 ± 0.015	0.2140
	P30		30	Borate buffer	1.087 ± 0.005	0.3561

$$ESR = \frac{W_e}{W_d} \quad (2)$$

Where  $W_e$  is the average sample weight at equilibrium, calculated from the points after which SW% did not change with time.

### 2.3. Mechanical tests

All mechanical tests were conducted at  $21 \pm 1$  °C in an INSTRON 4469 universal testing machine equipped with proper grips (Fig. 1) and a 0.5 kN load cell. Previous to mechanical characterization, gel samples were conditioned at the testing temperature for 2 h and protective films were peeled off just before the experiment. Each test was replicated at least five times.

#### 2.3.1. Puncture test

Puncture tests (Fig. 1-a) were carried out on cylindrical gel samples of  $L_0$  (height) =  $D_0$  (diameter) = 25 mm at a crosshead speed of 5 mm/min. A flat-faced cylindrical punch (10 mm diameter) made of polymethylmethacrylate (PMMA) was used. Teflon spray was applied on the punch surface to diminish friction and adhesion effects.

Apparent gel strength was defined as the load registered when the punch penetrated 3 mm into the gel surface and it was expressed in N.

#### 2.3.2. Uniaxial compression tests

Uniaxial compression tests (Fig. 1-b) were performed on cylindrical samples ( $L_0 = D_0 = 25$  mm) at a crosshead speed of 25 mm/min. Teflon spray was applied onto the compression platens to diminish friction. Samples were loaded up to fracture. The recorded load–displacement data were converted to nominal stress–strain

( $\sigma$ – $\varepsilon$ ) and stress–stretch ratio ( $\sigma$ – $\lambda$ ), as:

$$\sigma = \frac{P}{A_0} \quad (3)$$

$$\varepsilon = \frac{L - L_0}{L_0} \quad (4)$$

$$\lambda = \frac{L}{L_0} \quad (5)$$

where  $P$  is the registered load,  $A_0$  is the original resistant area of the sample and  $L$  is the actual distance between compression platens.

The first order Ogden constitutive model was used to describe the uniaxial compression hyperelastic response of the gels (Bower, 2010). The  $\sigma$ – $\lambda$  relationship of an incompressible material is given by:

$$\sigma = \frac{2\mu}{\alpha} \left( \lambda^{(\alpha-1)} - \lambda^{\left(-\frac{\alpha}{2}-1\right)} \right) \quad (6)$$

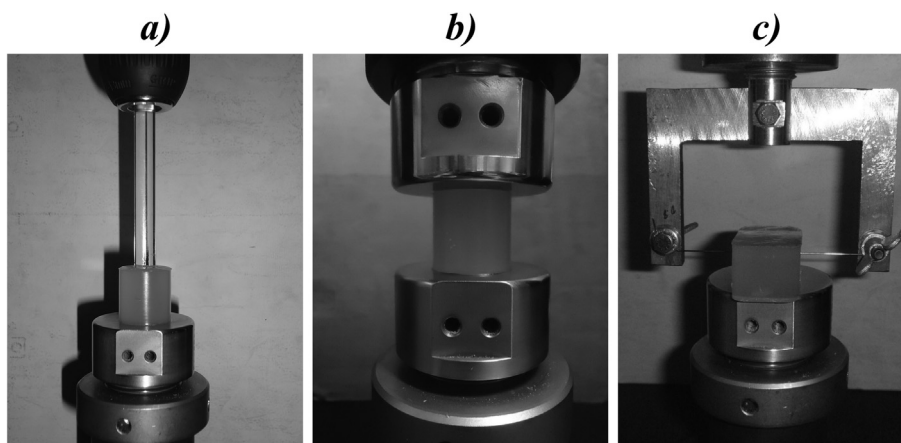
where  $\mu$  is the initial shear modulus and  $\alpha$ , a strain hardening capability parameter.

$\mu$  is a measure of the ability of a material to resist transverse deformations (shear stresses), it is also known as rigidity and it is a parameter that quantifies the material stiffness.  $\alpha$  is representative of the non-linear behavior due to strain hardening of the material.

The Ogden model parameters were determined by Least Squares method using as initial guesses  $\mu = 10$  kPa and  $\alpha = 2$ .

#### 2.3.3. Wire cutting tests

Wire cutting experiments were conducted at 100 mm/min using an on purpose designed device (Fig. 1-c). The grip allowed the wires



**Fig. 1.** Mechanical tests configurations: a) Puncture, b) Uniaxial Compression, c) Wire Cutting.

to be tight and aligned with the sample surface. The selected test rate was the lowest at which a flattened surface morphology was induced by the cutting action for all of the gels assayed (Czerner et al., 2014). Gel samples were rectangular blocks of length 30 mm, width 20 mm and height 20 mm. The test consisted on pressing steel wires of different diameters ( $d_w$ ) ranging from 0.2 to 0.6 mm into the gel sample while the force ( $F$ ) and displacement ( $v$ ) were continuously registered. At least six cuts were effectuated per each gel sample. The fracture toughness ( $G_c$ ) values were obtained by linear fit, as follows (Kamyab et al., 1998):

$$\frac{F_c}{B} = G_c + \sigma_c(1 + \mu_k)d_w \quad (7)$$

where  $F_c$  is the steady state cutting force,  $B$  the sample width,  $\sigma_c$  a characteristic stress and  $\mu_k$  the kinetic friction coefficient.

For fitting, the Least Squares method with instrumental weighting was used. Data lying outside the 95% confidence limits from the first best-fit line were eliminated from the analysis.  $G_c$  value was obtained extrapolating to zero wire diameter a second linear fit of valid data (Czerner et al., 2014).

### 3. Results and discussion

#### 3.1. Swelling behavior and gel strength measurements

The prepared gels were first characterized by swelling experiments and apparent gel strength measurements to verify that the chosen formulations effectively lead to gels with different triple-helix content and hence with different physical cross-linking degree. It has been previously shown that the equilibrium swelling ratio and the gel strength are both related to the triple-helix content in gelatin gels. The relationships have been found to be inversely proportional for the first parameter (Klepko & Mel'nichenko, 1995) and directly proportional for the second parameter (Bigi et al., 2004; Eysturskarð, Haug, Ulset, & Draget, 2009). Results of swelling studies are shown in Fig. 2 while equilibrium swelling ratio and gel strength values are presented in Fig. 3.

All of the gel samples exhibit the typical swelling behavior (Klepko & Mel'nichenko, 1995; Quiao & Cao, 2014): during the first few hours SW% rapidly increases and then reaches a plateau value (Fig. 2). This plateau corresponds to the gel saturation level ( $W_e$ ). Gels containing glycerol as co-solvent show lower SW% and ESR values than the hydrogels prepared only with buffer solutions and also reach the equilibrium at shorter times. Bovine gelatin gels display higher SW% and ESR values than porcine gelatin gels. For both collagen sources, SW% and ESR decrease with increasing gelatin concentration. As can be seen in Figs. 2 and 3, swelling behavior is more affected by collagen source and solvent composition than by gelatin concentration.

Apparent gel strength markedly increases with increasing gelatin concentration (Fig. 3) and with the incorporation of glycerol. In addition, porcine gels exhibit higher apparent gel strength values than their analogous bovine ones. It is evidenced in Fig. 3 that apparent gel strength is more influenced by gelatin concentration than by the other formulation variables.

The variations observed in swelling behavior and apparent gel strength with formulation variables can be both associated to the amount of triple helices formed in the gels. It has been shown that: i) triple-helix content increases with increasing gelatin concentration for a given thermal history (Joly-Duhamel et al., 2006b); ii) porcine gelatin contains higher amounts of Glycine-proline-hydroxyproline sequences than bovine gelatin, which are responsible of forming and stabilizing triple-helices (Courty, Gornall, &

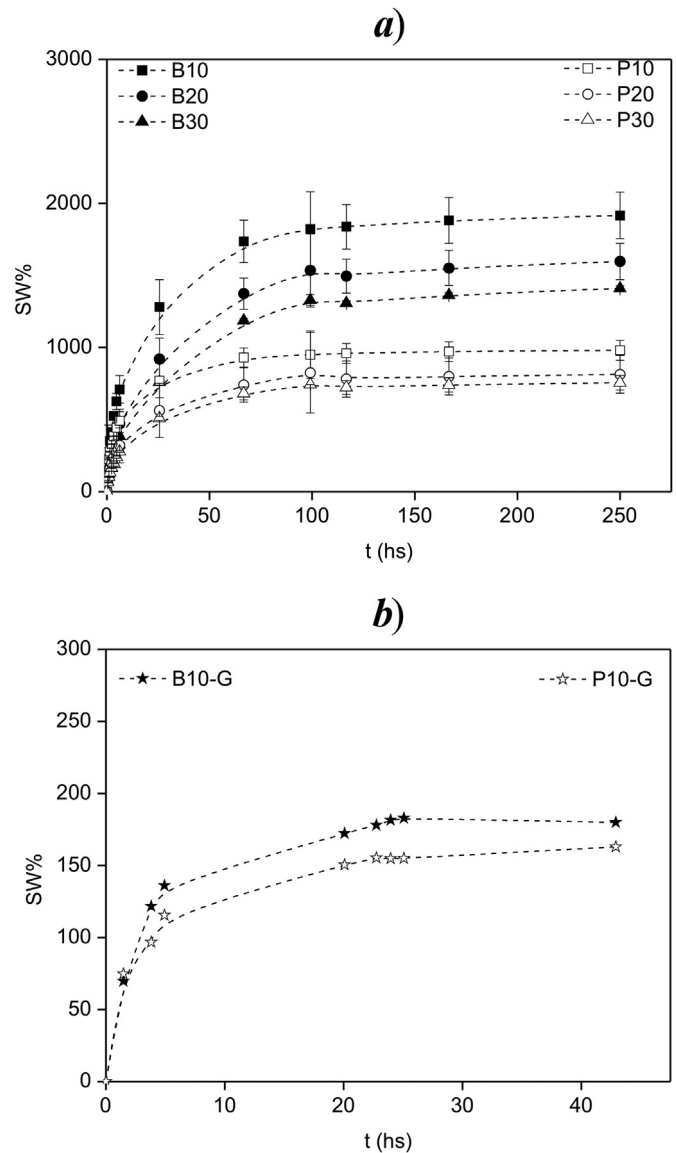


Fig. 2. Swelling Ratio (Eq. (1)) as a function of immersion time for gelatin hydrogels (a) and mixed solvent gels (b).

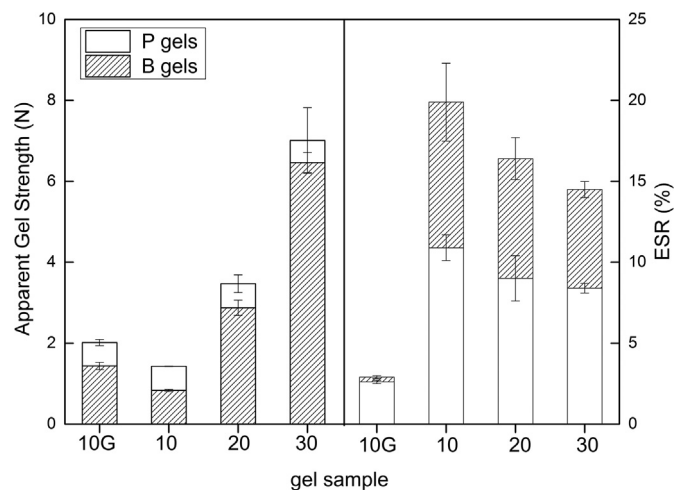


Fig. 3. Apparent gel strength and Equilibrium swelling ratio (ESR) of gelatin gels.

Terentjev, 2006; Gómez-Guillén, Giménez, López-Caballero, & Montero, 2011; Raja MohdHafidz, Yaakob, Amin, & Noorfaizan, 2011) and *iii*) the presence of glycerol as co-solvent enhances the formation of triple-helices due to a preferential hydration effect (Joly-Duhamel et al., 2006b; Sanwlani et al. 2011), i.e. it behaves as  $\alpha$ -helicogenic agent.

3.2. Uniaxial compression response

In previous works, we verified the reversibility of the deformation behavior (Czerner, Fasce, & Frontini, 2012) and the strain rate independency of the stress–strain curves shape of gelatin gels in the quasi-static range (Czerner, Fasce, Martucci, Ruseckaite, & Frontini, 2011).

Typical stress–strain curves obtained in uniaxial compression experiments are shown in Fig. 4-a) and b) for bovine and porcine gels, respectively. All of the gels exhibit a non-linear elastic response with strain hardening, i.e. gel samples behave as hyperelastic solids. Stress–stretch ratio curves were then fitted according to Eq. (6). The First order Ogden model accurately describe the uniaxial compression response of all gelatin gel samples as judged

by the large regression coefficients obtained ( $R^2 > 0.99$ ). The fitted constitutive parameters,  $\mu$  and  $\alpha$ , are plotted as a function of gelatin concentration in Fig. 5.

Gelatin gels exhibit  $\mu$  values ranging from 5 to 90 kPa. Porcine gelatin gels display larger  $\mu$  values than their analogous bovine gels. For both collagen sources,  $\mu$  increases with increasing gelatin concentration following a power law relationship:  $\mu \sim C_{gel}^{1.79}$  for bovine and  $\mu \sim C_{gel}^{1.63}$  for porcine gelatin hydrogels. The observed power law functionalities are in agreement with those reported in literature for other gelatin gel systems in a lower stiffness range (see for example in Bot et al., 1996 and Baumberger et al., 2006 and references there in). Therefore, our results show that an exponent of  $\sim 1.7 \pm 0.1$  describes the stiffness–concentration relationship for self-supporting gelatin gels in a wide range of stiffness.

The addition of glycerol as co-solvent enhances 2.3 and 1.6 times the  $\mu$  values of bovine and porcine gelatin gels, respectively (Fig. 5-a). An increment of  $\sim 1.6$  times in the shear modulus with the addition of glycerol (40%w/w) has been reported by Baumberger

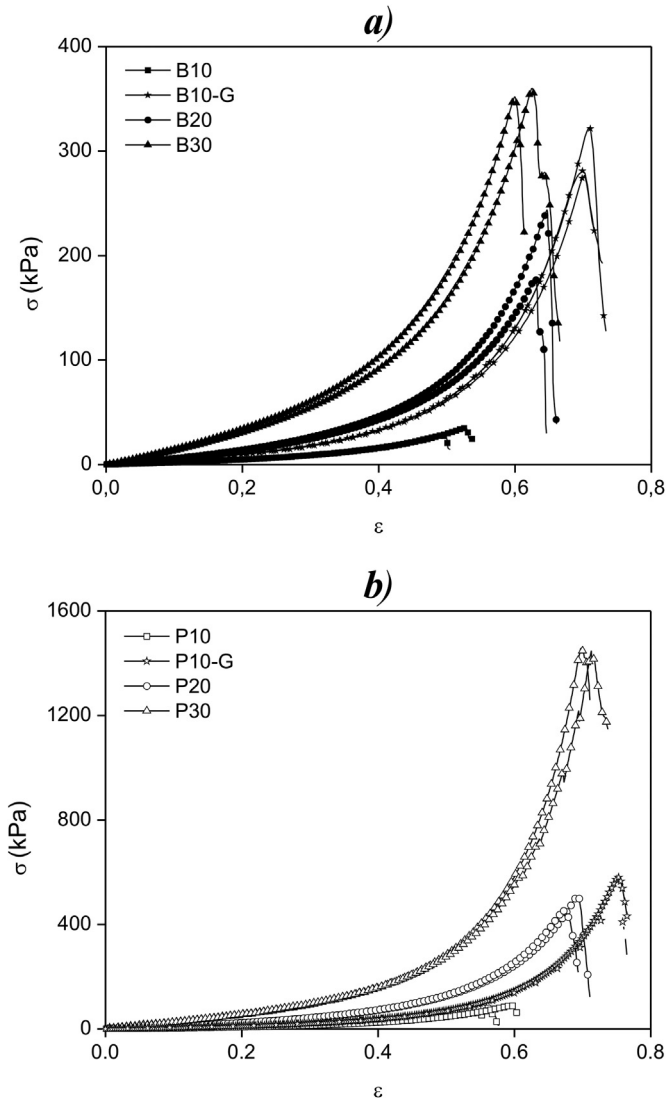


Fig. 4. Uniaxial compression behavior of gelatin gels. Representative stress–strain curves of bovine (a) and porcine (b) gelatin gels.

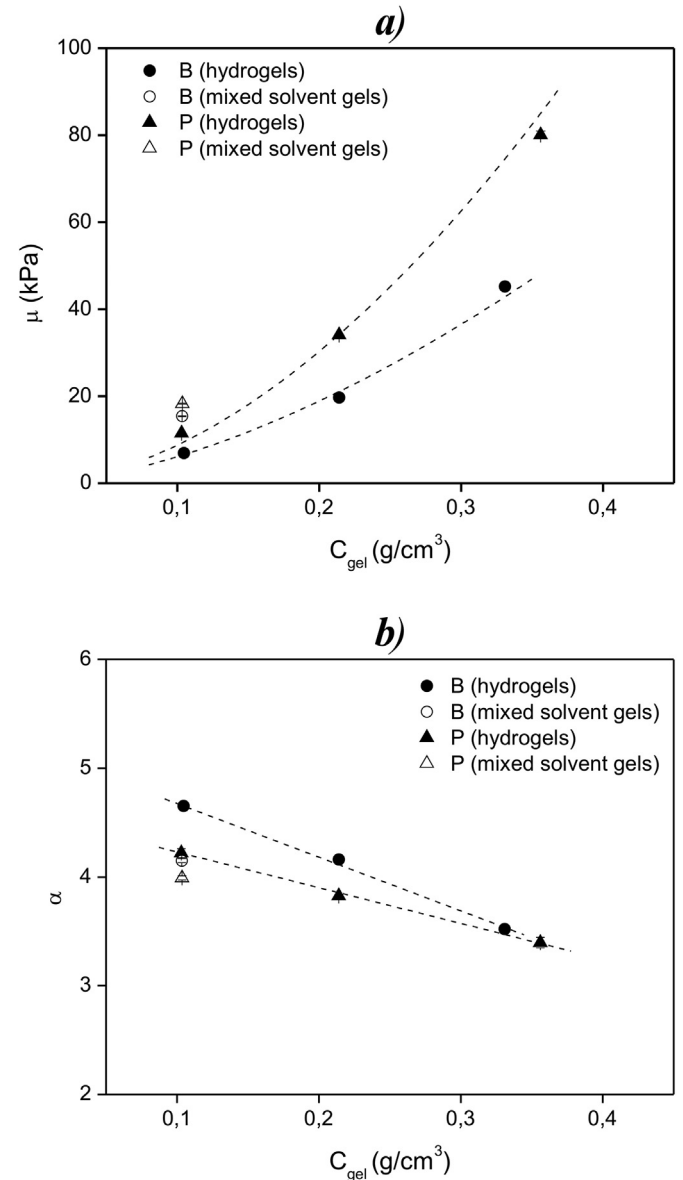


Fig. 5. First order Ogden model parameters as a function of gelatin concentration: a) shear modulus ( $\mu$ ) and b) strain hardening capability ( $\alpha$ ).

et al. (2006) for a 5%w/w porcine gelatin gel. According to these results, the  $\alpha$ -helicogenic effect of glycerol appears to depend on the gelatin source in a larger extent than on gelatin concentration.

The trends observed in  $\mu$  values (Fig. 5-a) can be directly linked with the triple helix content of the gels according to Joly-Duhamel et al. (2006a). These authors have shown that regardless of the gelatin origin, thermal history or solvent composition, there is a universal relation between the shear modulus -measured through low strain oscillatory experiments- and the triple-helical content. The shear modulus vs. helix concentration plot yields a power law master curve with a positive exponent (Joly-Duhamel et al., 2006a). Therefore, the enhancement in gel stiffness due to the addition of glycerol, the increase in gelatin concentration and in the amount of glycine-proline-hydroxyproline segments indicate that, as expected, the gel network structure is effectively modified.

The strain hardening capability ( $\alpha$ ) shows a slight decreasing trend with increasing gelatin concentration for all of the hydrogels. Bovine gelatin gels display  $\alpha$  values somewhat larger than porcine ones (Fig. 5-b). The presence of glycerol causes a reduction in  $\alpha$ , which is more pronounced in bovine gelatin gels than in porcine ones. These suggest that gels displaying a stiffer network structure have less strain hardening capability. This statement is also supported by Bot et al. (1996) studies, who reported that the elasticity parameter of the BST model,  $n$ , (similar to  $\alpha$ ), diminishes with increasing gelatin concentration in other gelatin gel systems. Bot et al. (1996) and Courty et al. (2006) attributed the strain hardening capability to the growth of physical cross-links due to a deformation induced coil-helix transition.

As shown in Fig. 6, ultimate properties determined from uniaxial compression tests depend on gel formulation variables, mainly on collagen source. Porcine gelatin gels exhibit larger values of failure stress and strain than bovine gelatin gels. The addition of glycerol contributes to the enhancement of both ultimate properties. Gelatin concentration markedly affects the stress at break although it has less effect on failure strain. The variation in stress at break resembles the trend observed for apparent gel strength (Fig. 3) and initial shear modulus (Fig. 5-a). Bot et al. (1996) and Zúñiga and Aguilera (2009) also observed a straight relationship between the failure stress and the shear modulus for lower concentrated gelatin gel systems. These results confirm that, contrary to the typical behavior of chemical gels (Kong, Wong, & Mooney, 2003; Tanaka et al., 2005), failure stress increases with increasing the amount of cross-links in physical gels.

Even the shape of stress–strain curve of gelatin gels is rate-

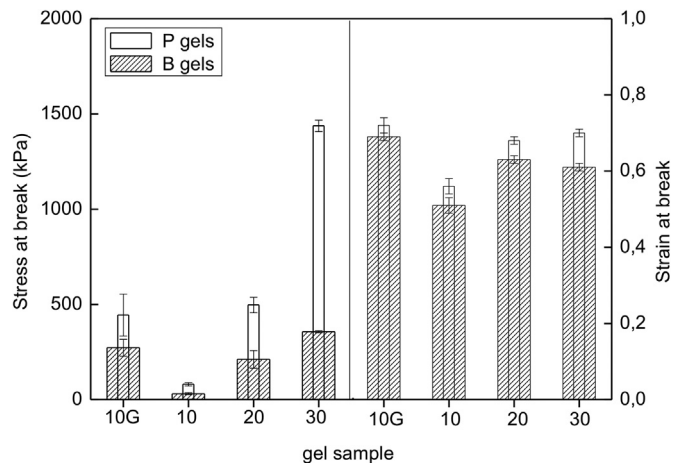


Fig. 6. Ultimate properties of gelatin gels determined from uniaxial compression experiments.

independent in the quasi-static range, the failure strain does increase with increasing strain rate. This dependency was recently modeled by Forte et al. (2015) for a 10%w/w bovine gelatin gel and explained by the role that the liquid contained within the pores exerts on deformation.

### 3.3. Fracture toughness measurements

As previously mentioned, the rate at which Wire Cutting experiments were carried out was selected in order to allow the direct comparison of  $G_c$  for the different formulations under the same propagation regime and rate. It is known that for gelatin gels and other elastomeric materials there exists a critical crack propagation rate above which the fracture pattern changes from rhombus-like to flat. The apparent energy consumption in the rhombus-like regime is larger than in the flat one due to crack path deflection (Baumberger, Caroli, Martina, & Ronsin, 2008; Czerner et al., 2014). In addition, fracture toughness in gelatin gels has been shown to increase with increasing wire cutting rate under the flat propagation regime (Czerner et al., 2014; Forte et al., 2015).

Typical normalized force–displacement curves ( $F/B$  vs.  $v$ ) obtained in Wire Cutting experiments are shown in Fig. 7. At the beginning of the test, the wire indents the gel and the load increases with increasing wire penetration. Once fracture is initiated, the load abruptly falls down and reaches the steady state cutting value ( $F_c$ ). The differences in the peak values are due to the fact that fracture initiates at random preexisting flaws (Forte et al., 2015). The value of  $F_c$  increases with increasing the wire diameter (Fig. 7). Trustable force values could be measured for all of the gels with the sole exception of P30 samples. In this latter case, a parabolic propagation path instead of a straight one was developed due to the wire deflection induced by the high sample stiffness. As a consequence, the cutting energy ( $F_c/B$ ) was overestimated and P30 data were discarded.

Fig. 8-a) and b) show the averaged  $F_c/B$  values plotted against  $d_w$  for bovine and porcine gelatin gels, respectively. For every gel system,  $F_c/B$  vs.  $d_w$  data display a linear relationship, which was fitted to Eq. (7) with  $R^2 > 0.95$ . The ordinate of the linear fit,  $G_c$ , and the slope,  $\sigma_c(1 + \mu_k)$ , are affected by gelatin concentration, solvent composition and collagen source.

Fig. 9-a) clearly shows the variation of  $G_c$  with formulation variables. Porcine gels exhibit larger  $G_c$  values than bovine gels. For

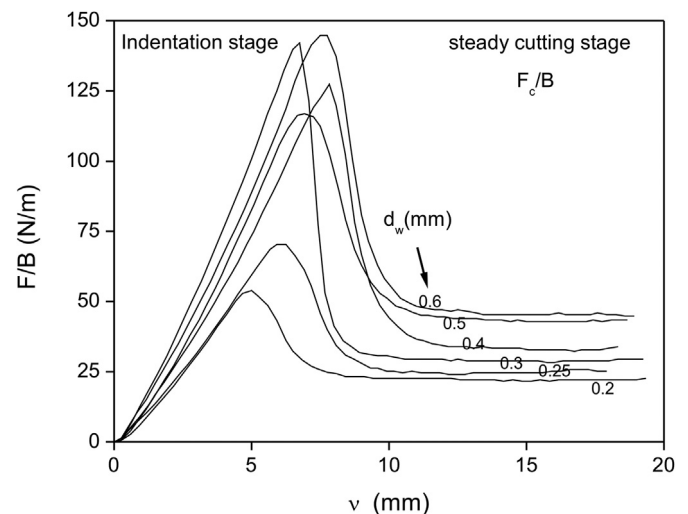
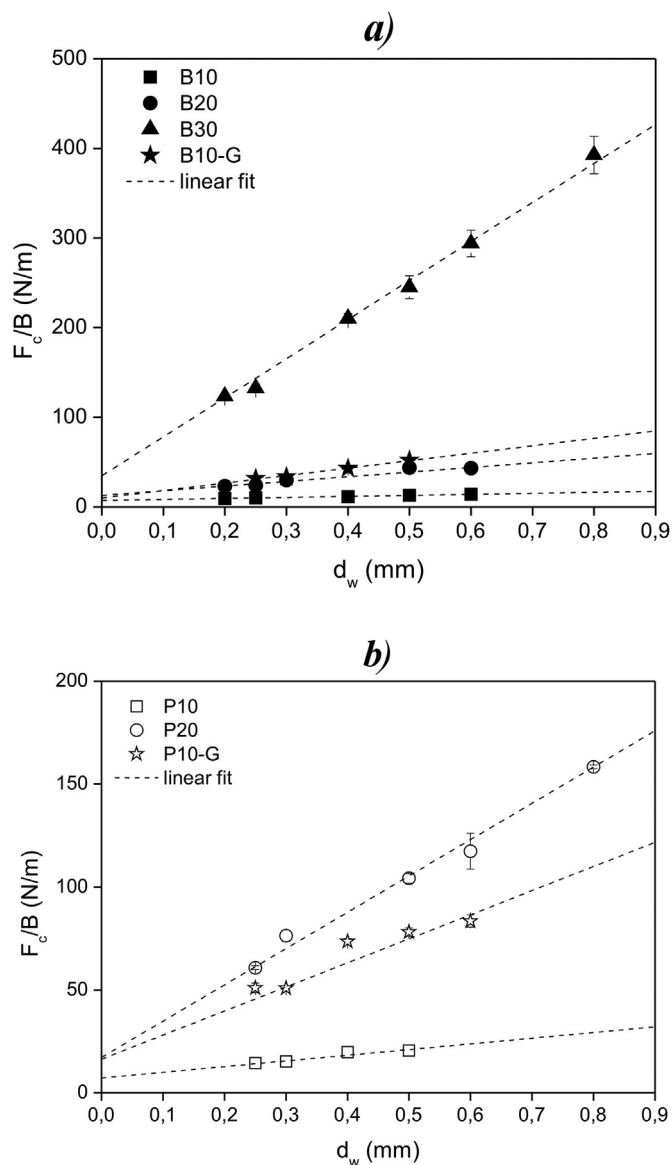


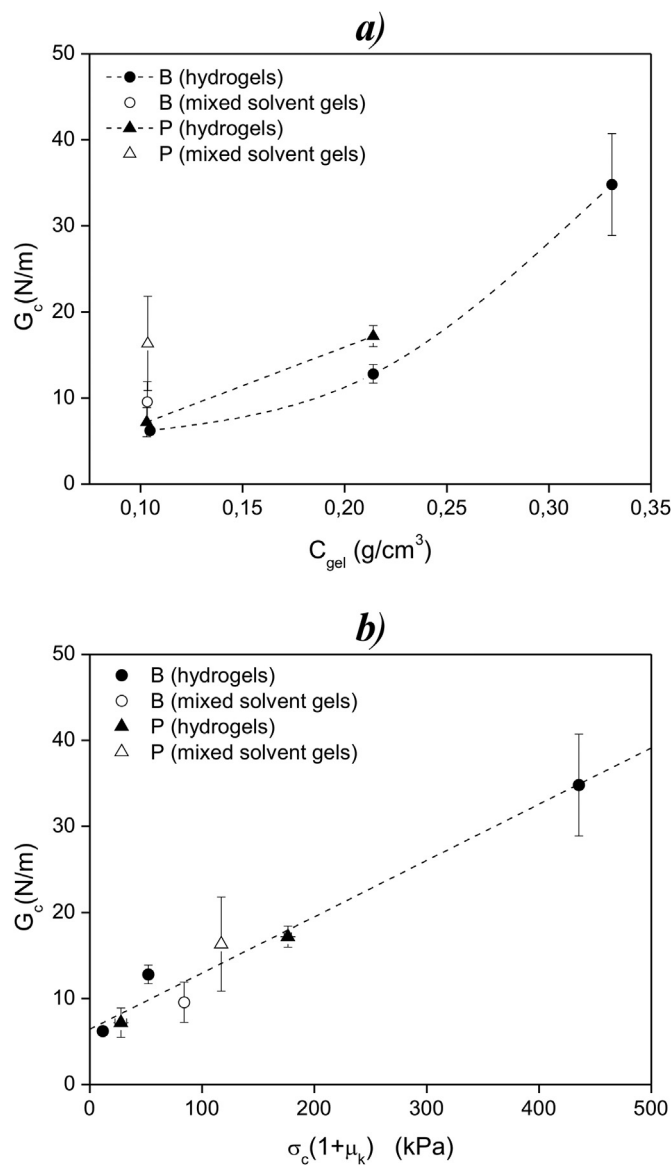
Fig. 7. Typical wire cutting measurements in gelatin gels. Normalized load versus wire displacement obtained for B20 samples using wires of different diameters.



**Fig. 8.** Wire Cutting plots of bovine gelatin gels (a) and porcine gelatin gels (b): Linear fits of normalized steady cutting load versus wire diameter data.

gels of both collagen sources, the increase in gelatin concentration and the incorporation of glycerol causes an enhancement of  $G_c$ . The fracture toughness parameter of bovine hydrogels appears to follow a power law relationship with gelatin concentration ( $G_c \sim C_{gel}^{1.89}$ ). Similar effects of gelatin concentration and glycerol addition on fracture toughness has been previously reported by Baumberger et al. (2006) for other gelatin gel systems. These authors have attributed the effects to two facts: *i*) the increase in solvent viscosity which influences the viscous disentanglement fracture process and *ii*) the increase in the shear modulus.

As can be seen in Fig. 8, the slope of the Wire Cutting plots,  $\sigma_c(1 + \mu_k)$ , increases with increasing gelatin concentration and with the presence of glycerol. Fig. 9-b) demonstrates that  $G_c$  is linearly proportional to  $\sigma_c(1 + \mu_k)$ , since parameters arisen from gels with different formulations lies on the same straight line. Forte et al. (2015) have shown that, for a 10%w/w bovine gelatin gel tested at different cutting rates,  $G_c$  is directly proportional to  $\sigma_c$ , if friction effects are neglected. The combined analysis of our results and those of Forte et al., indicates that the fracture toughness scales



**Fig. 9.** Fracture toughness parameters ( $G_c$ ) arisen from Wire Cutting Experiments as a function of gelatin concentration (a) and  $\sigma_c(1 + \mu_k)$  slope (b).

with the cohesive stress of the gel network,  $\sigma_c$ , for a wider range of gelatin gel systems. If the previous statement is assumed and as  $\sigma_c$  is supposed to be proportional to the Young modulus (Forte et al., 2015), it is expected that  $G_c$  will scale with the gel stiffness.

### 3.4. Final discussion

In the previous sections, results arisen from the characterization of eight gelatin gels differing in gelatin concentration (10–30%w/w), gelatin source (bovine/porcine) and solvent composition (0/40% w/w glycerol/buffer mixture) were presented. In this section the experimental trends found for the apparent gel strength, the mechanical parameter that describes the strain hardening capability ( $\alpha$ ), the fracture toughness ( $G_c$ ) and the shear modulus ( $\mu$ ) are analyzed.

Fig. 10 shows that overall apparent gel strength increases with increasing  $\mu$ . It was previously demonstrated by FEM simulations (Sanchez Fellay et al., 2015) that for a first order Ogden material, the gel strength scales with  $\mu$  and it is almost unaffected by  $\alpha$ , provided

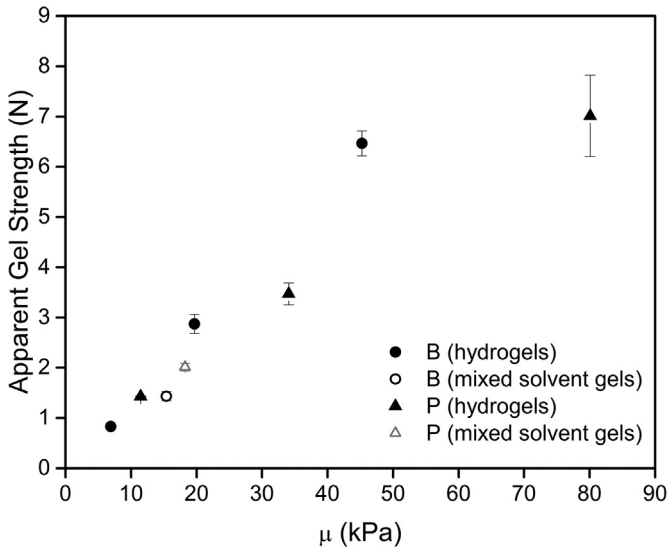


Fig. 10. Apparent gel strength versus initial shear modulus ( $\mu$ ) for all gelatin gels.

that  $\alpha > 2$ . The deviations observed in Fig. 10 from the expected straight line may arise from differences in the stress states used to determine apparent gel strength and  $\mu$  parameters as well as spurious friction and adhesion effects between the indenter and the sample.

Fig. 11 shows that  $\alpha$  decreases almost exponentially with increasing  $\mu$ . Parameters determined for gels of different collagen sources, solvent compositions and gelatin concentrations appear to collapse onto a single curve. As proved by Joly-Duhamel et al. (2006a), there exists a single correlation between the shear modulus and the triple helix content in gelatin gels. So, from Fig. 11, it can be inferred that  $\alpha$  could be also affected by the gel triple helix content. Following the interpretation of the strain hardening capability given by Bot et al. (1996) and Courty et al. (2006), the trend observed between  $\alpha$  and  $\mu$  is consistent with the fact that in a more physically cross-linked gel, the triple-helical zones have lower possibilities to grow because the movement of protein molecules is more restricted.

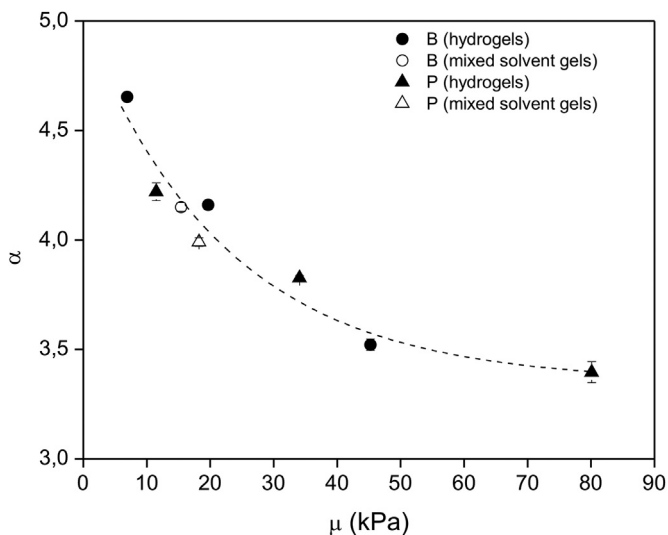


Fig. 11. Strain hardening parameter ( $\alpha$ ) versus initial shear modulus ( $\mu$ ) for all gelatin gels.

Fig. 12 shows that  $G_c$  appears to increase linearly with increasing  $\mu$ . Interestingly, values determined for gels of different collagen sources, solvent compositions and gelatin concentrations collapse onto a single line. Moreover,  $G_c$ - $\mu$  data taken from literature for a 10%w/w bovine gelatin gel (Forte et al., 2015) and for a 5%w/w porcine gelatin gel (Baumberger et al., 2006) at the same crack propagation rate lie on the observed trend (see cross points in Fig. 12). Considering that the shear modulus is directly governed by the triple helix content of the gel network (Joly-Duhamel et al., 2006b),  $G_c$  also appears to be straightly related with this structural parameter. Results shown in Fig. 12 supports the fact that for physical gels, contrary to chemical gels, fracture toughness increases with increasing stiffness.

However, it has to be pointed out that fracture toughness is not solely governed by  $\mu$ . A clear evidence is that  $G_c$  is rate-dependent whereas  $\mu$  is rate-independent. This fact was shown by Forte et al. (2015) for a 10%w/w bovine gelatin gel, by Baumberger et al. (2006) for a wide range of gelatin gel compositions and by ourselves (Czerner et al. 2014). According to the models proposed by Forte et al. (2015) ( $G_c \sim V^{0.5}$ ) and by Baumberger et al. (2006) ( $G_c \sim V$ ), we would expect that increasing the cutting rate ( $V$ ) will shift  $G_c$  to higher values.

#### 4. Conclusions

In this work, several formulation variables were intentionally varied to obtain self-supporting gelatin gels with stiffness values lying within the range of many food products (~5–90 kPa). Stiffer gels were obtained when the gelatin concentration was increased, glycerol was added as co-solvent or porcine gelatin was used. Stiffer gels exhibited more physically cross-linked networks, i.e. higher triple helix content, as inferred from apparent gel strength measurements and swelling experiments results. Gels were mechanically characterized by uniaxial compression tests and novel wire cutting fracture experiments. Shear modulus and strain hardening capability parameters were determined from the stress–stretch ratio curves according to the First order Ogden constitutive model. An appropriate cutting rate had to be selected to properly evaluate the fracture toughness of all samples under the same propagation pattern in the quasi-static range (100 mm/min).

Based on the experimental results from the present investigation, we can conclude that:

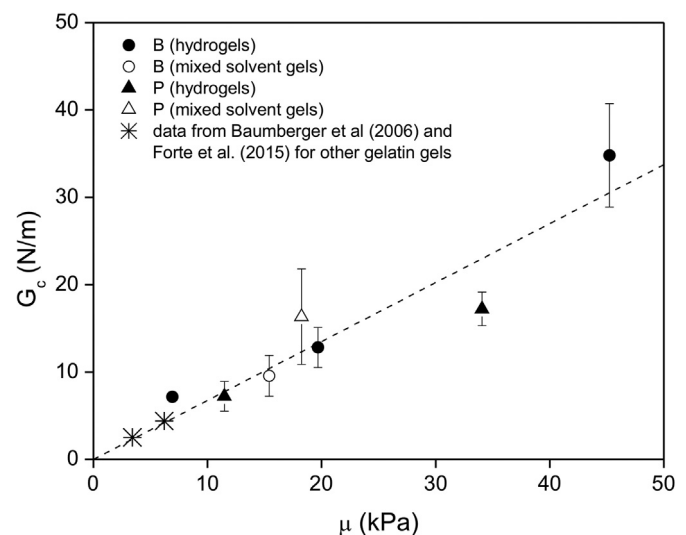


Fig. 12. Fracture toughness ( $G_c$ ) as a function of initial shear modulus ( $\mu$ ).



- Gelatin concentration is the main formulation variable that affects gel stiffness. A power law relationship between shear modulus and gelatin concentration is observed. This result is in agreement with similar findings for diluted systems and extends the functionality to high gelatin contents.
- The stiffening effect of glycerol is more pronounced in bovine than in porcine gelatin gels.
- More physically cross-linked gels display larger shear modulus and fracture toughness and lower strain hardening capability than less cross-linked gels.
- Strain hardening capability and quasi-static fracture toughness are closely related to shear modulus, independently of gel formulation.
- Strain hardening capability decreases with the increase in shear modulus. This is because the molecular movement required for growing triple-helix structures is more restricted in more physically cross-linked networks.
- Quasi-static fracture toughness increases linearly with the increase in shear modulus, indicating a strong elastic contribution to the fracture toughness.

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