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Linear viscoelastic assessment of cold gel-like emulsions stabilized with bovine gelatin

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ABSTRACT

Gel-like oil-in-water emulsions with low lipid content, stabilized with bovine gelatin of different molecular weights were analyzed. Mean droplet size and droplet size distribution of the emulsions were determined by static light scattering. The emulsions were characterized for their rheological properties using dynamic oscillatory and creep-recovery tests in the linear viscoelastic region. Most of the systems exhibited gel-like properties even the samples were not submitted to thermal treatment and the temperature was maintained below 30 °C; this viscoelastic characteristic led to a delay of the phase-separation exceeding one month. Creep-compliance data were fitted to a four parameters Burgers model. The continuous relaxation spectra were estimated from the experimental values of G′ and G″ considering the Baumgaertel – Schausberger – Winter generalized model. Discrete relaxation moduli and relaxation times were derived from the continuous spectra. The effect of molecular weight of the protein and the stability during the storage at 20 °C was analyzed in order to interpret the structural characteristics of these emulsions.

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1. Introduction

Gelatin is a relatively high molecular weight protein derived from animal collagen (Ledward, 1986; Veis, 1964). Among commercial hydrocolloids used in the food industry, gelatin has been regarded as special and unique, serving multiple functions with a wide range of applications in various industries. Gelatin has long been used as a food ingredient (e.g., gelling and foaming agent), in the preparation of pharmaceutical products (e.g., soft and hard capsule, microspheres), in the biomedical field (wound dressing and three-dimensional tissue regeneration) and in the photographic industry, among other nonfood applications (Howe, 2000; Karim & Bhat, 2008). It is prepared by hydrolyzing collagen by boiling in the presence of either acid (Type A gelatin) or alkaline (Type B gelatin). The isoelectric point of Type A gelatin (~7–9) tends to be higher than that of Type B gelatin (~5).

Collagen is present in bovine hides and pig and fish skins. It acts as extracellular, structural protein in bone, tendon, skin, and the connective tissue of various organs. The characteristic features of collagen are the exceptional amino acid composition (33% glycine and 22% proline) and structure: the (rigid) triple extended helix. The triple-helix structure is characterized by three extended left-handed polyproline II-like helical chains that are supercoiled into a right-handed triple helix. The three chains are staggered by one residue with respect to each other, and are linked through inter-chain hydrogen bonds. The triple-helical conformation is associated with a distinctive amino acid sequence with glycine as every third residue and a high content of imino acids (Harrington & Rao, 1970; de Wolf, 2003).

Rheological properties of gelatin are also related to its production process. First, the hot water used for the extraction of collagen from conditioned tissue denatures the triple helical structure into individual soluble chains, or small polymers or fragments. During cooling, the chains can rewind into new triple-helical structures, but not necessarily identical as the native collagen structure, limiting the extent of re-formed triple helix (Karim & Bhat, 2008; Ledward, 1986).

The use of proteins in emulsified systems presents a growing trend in order to replace synthetic emulsifiers (Dickinson & Lopez, 2001; Garti, 1999). Proteins, and particularly gelatins, can be used as emulsifiers in foods because of their ability to facilitate the formation of an emulsion, improve the stability, and produce desirable physicochemical properties in oil-in-water (o/w) emulsions

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(Lobo, 2002; Surh, Decker, & McClements, 2006). A major potential advantage of proteins as emulsifiers in foods is their ability to protect polyunsaturated lipids from iron catalyzed oxidation (Hu, McClements, & Decker, 2003).

Over the past few years, there has been a growing interest in converting oil-in-water (o/w) emulsions into gels, because of their practical application in food formulations (Chen & Dickinson, 1999). Several workers have investigated the microstructure and rheological properties of such emulsion gels which can be used to create foods with improved organoleptic properties (Manoi & Rizvi, 2009; McClements, Monahan, & Kinsella, 1993; Sok Line, Remondetto, & Subirade, 2005). However, in many cases a thermal treatment is needed to produce these emulsion gels, so their application in formulations containing heat-sensitive components is restricted.

Rheological measurements are appropriate tools for obtaining information about the organization of macromolecules in the medium, thus the correlation of rheology with microstructure information is useful to understand the macroscopic behavior of the emulsions in terms of the microstructure organization (Barnes, 1994; Lorenzo, Zaritzky, & Califano, 2008a; Quintana, Lorenzo, Zaritzky, & Califano, 2007).

The objectives of the present work were: (a) to perform a rheological characterization (dynamic oscillatory and creep-recovery assays) of gel-like oil-in-water emulsions with low lipid content, stabilized with bovine gelatin; (b) to analyze the effect of molecular weight of the protein and storage time on the viscoelastic behavior of the emulsion; (c) to model the experimental data in order to interpret the structural modifications of the emulsions using the theory of rubber elasticity.

2. Materials and methods

2.1. Materials

Food grade commercial gelatins of different molecular weights were tested: 60 kDa, 80 kDa and 120 kDa. All tested gelatins were from bovine skin and were gently provided by PB Leiner (Argentina). Main specifications of these products were kindly supplied by the producers and they are summarized in Table 1. Commercial 100% sunflower oil (Molinos Río de La Plata SACIFI, Buenos Aires) was purchased from a local supermarket and used without any further treatment. Analytical grade NaH_2PO_4 and Na_2HPO_4 were used to buffer the continuous phases (Anedra, Argentina). Distilled and deionized water was used in all solutions and emulsions.

2.2. Methods

2.2.1. Preparation of aqueous phases

For each sample, continuous phases were prepared by dispersing 15 g of gelatin (3 g/100 g) in 500 g of an aqueous solution buffered at pH 7.0 (0.005 mol/L), containing 0.1 g/100 g of sodium azide to avoid microbial growth in the samples (total ionic strength: 0.093 mol/L). Dispersions were prepared at a controlled temperature of 25 °C and stirred overnight to ensure a complete hydration of the protein.

Table 1
Main specifications of the tested gelatin samples as provided by the manufacturer.

Gelatin sample	A	B	C
Weight-Average Molecular weight (M_W, kDa)	60	80	120
Bloom (g)	182	217	265
Viscosity of a 6.66 g/100 g solution (mPa)	28	30	41
Moisture (g/100 g)	10.7	10.0	9.8
Ashes (g/100 g)	≤2.0	≤2.0	≤2.0
pH of a 1 g/100 g solution	5.5	5.8	5.3
Granulometry (sieve size, mm)	0.6	0.6	0.6

2.2.2. Preparation of oil-in-water emulsions

Low-in-fat o/w emulsions (15 g/100 g) were prepared by adding sunflower oil to aqueous phases containing different emulsifiers (i.e. bovine gelatin with different molecular weights). Final concentration of gelatins in the studied emulsions was 2.55 g/100 g. A two step emulsification process was used to prepare the o/w final emulsion. First, the whole sample was pre-emulsified for 4 min at 11,500 rpm with the Ultra-Turrax T25 (IKA Labortechnik, Germany) homogenizer (rotor stator principle), equipped with a dispersing tool (520-25-NK196). Then, the pre-emulsified system was passed through a high pressure valve homogenizer (Stansted Fluid Power FPG 7400, Essex, UK) to perform the final model emulsions. The pressure was set at 40 MPa and 4 MPa for the first and second valve, respectively, and the emulsion was recycled four times through the homogenizer to achieve a monodispersed system. The temperature during the high pressure homogenization was monitored to ensure that the emulsions never exceed 30 °C.

2.2.3. Droplet size distribution (DSD) and emulsion stability

Mean droplet size and droplet size distribution of emulsions were determined by static light scattering using a Mastersizer 2000 (Malvern Instruments Ltd., Malvern, Worcester, UK). The fundamental size distribution derived by this technique is volume based, which uses the Mie theory. The refractive index was 1.449 for the emulsion particle and 1.33 for the dispersant medium. The absorbance value of emulsion particle was 0.001. Three aliquots of each emulsion were carefully dispersed step-by-step, applying gentle agitation in order to disrupt droplet flocs. Values of the Sauter mean diameter, $d_{3,2}$, which is inversely proportional to the specific surface area of droplets, were obtained as follows:

$$d_{3,2} = \sum_{i=1}^{N}\left(n_i d_i^3\right) \Big/ \sum_{i=1}^{N}\left(n_i d_i^2\right) \quad (1)$$

where d_i is the droplet diameter, N is the total number of droplets and n_i is the number of droplets having a diameter d_i.

In order to determine emulsion stability, four (50 ml) aliquots of each emulsion were carefully poured in 50 ml glass graduated cylinders and quiescently stored at 20 °C in a temperature-controlled room. These samples were periodically observed and the time at which an oil–water interface appeared in each case determined the stability period.

2.2.4. Creep-recovery experiments

Creep-recovery studies were conducted in a controlled stress Haake RS600 rheometer (ThermoGap, Germany). In the creep-compliance test the undeformed samples of emulsions were suddenly subjected to a constant shear within the linear viscoelastic region (LVR). The deformation of the viscoelastic materials increases with time and approaches a steady state where the deformation rate remains constant, in this point the stress can be suddenly removed and can be analyzed for recoverable shear. All the emulsions were tested under a shear stress for 1200 s and then released to allow sample recovery for 600 s.

The LVR was determined through stress sweep tests (from 0.3 to 60 Pa) at a fixed frequency (6.28 rad/s–1 Hz).

During the measurements the temperature was maintained constant at 25 °C by thermostatically controlling the temperature of the plates (35 mm diameter). The samples were loaded, a 1 mm gap between plates was fixed and a resting time of 600 s was adopted to relax any normal stresses induced during sample loading.

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