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Rheology changes in oil-in-water emulsions stabilized by a complex system of animal and vegetable proteins induced by thermal processing

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ABSTRACT

Mixtures of animal and vegetable proteins were used to stabilize oil-in-water emulsions of a meat rich filling. Collagen and pea protein, combined in different proportions for a total protein of 9 g/100 g were used to prepare oil-water emulsions, to develop a new meat product. Texture and rheological parameters were measured to evaluate the behavior of the emulsions. Temperature sweeps from 20 to 90 °C and back (0.5 K/min) were applied and the impact on emulsions structure was monitored, in the rheometer, through the changes on the viscoelastic properties. All the mixtures studied exhibited a shear-thinning flow behavior and showed different viscoelastic properties. The tested systems, exhibited an increase of both viscoelastic moduli on cooling from 90 to 20 °C, where the storage modulus is always higher than the loss modulus. This increase in viscoelastic functions should result from intermolecular hydrophobic driven cross-linking as well as some hydrogen bonds and physical entanglements between proteins molecules on gel formation induced by the heating/cooling cycles. The 3:1 mixture of collagen and pea protein showed to be a potential formulation for the new meat-product development, as it shows texture values and rheological features according to product specifications.

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1. Introduction

In recent years, there has been a noticeable increase in the application of vegetable proteins in the food industry resulting from their low cost and reduced influence on the environment (Barac et al., 2010). The use of globular proteins from legumes can be an interesting alternative to enhance the nutritional and technological performance of animal based food (Bollinger, 2001). Consumers are increasingly interested in products with protein from a vegetable source and these are not only the vegetarians, but also those interested in a balanced ratio of vegetable/animal protein intake and consumers that associate specific health benefits with specific protein types (Russell, Drake, & Gerard, 2006).

Proteins from leguminous seeds have gained increasing importance since they are used to provide desired functional properties, including gelling, emulsifying, fat-absorbing and water binding properties (Nunes, Batista, Raymundo, Alves, & Sousa, 2003; Nunes, Raymundo, & Sousa, 2006a; Liang & Tang, 2014). The emulsifying properties of the proteins are an important functional feature for the food industry (Foegeding & Davis, 2011).

In addition to soybean seed, which has an overwhelming advantage in the market, but is shaded by the GMO label, pea seeds are one of the commercially available alternative plant protein sources (Tian, Kyle, & Small, 1999), and exhibit similar functional properties to soybean protein products (Boye, Zare, & Pletch, 2010).

Pea proteins, which are still evolving as new industrial proteins, show a well-balanced profile of amino acids, especially a high content in lysine (Schneider & Lacampagne, 2000), and contain two major globulin proteins: legumin (11S) and vicilin (7S) (Boye et al., 2010). Besides nutritional characteristics, their functional properties, such as gelling, emulsifying and foaming (Bacon, Noel, & Lambert, 1990; Bora, Brekke, & Powers, 1994; Raymundo, Empis, & Sousa, 1998; Tomoskozi, Lásztity, Haraszi, & Baticz, 2001), have led to a greater interest in this protein source as a promising food ingredient and as an alternative to the soybean protein. These proteins are also attractive to the food industry because of their low allergenicity, nutritional value and non-GMO status (Barac et al., 2010). The increasing knowledge about the emulsifying

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properties of these proteins can greatly extend their application in the food industry.

The interaction between animal and vegetable protein isolates is being now widely investigated to develop balanced animal and vegetable protein mixtures to produce derived meat products. However, the field of animal and vegetable protein interactions is one area that has to be deeply investigated.

Collagen fibrous protein is an animal protein responsible for structurally sustaining several animal tissues, being the main protein present in skin, bones, tendons, cartilages, and teeth. It is also the raw material for production of gelatin, cosmetics and foods, as well as an alternative for edible and/or biodegradable packaging film manufacture. Collagen and gelatin have been widely used in food industries as ingredients to improve the elasticity, consistency, and stability of foods, and can be used as emulsifiers in foods due to their ability to facilitate the formation of an emulsion, improve the stability, and produce desirable physicochemical properties in oil-in-water emulsions (Surh, Decker, & McClements, 2006). Since there is a lack of specific information on the rheological behavior of collagen in complex mixed systems (Olivo & Shimokomaki, 2002), the use of this protein was studied.

Over the past few years, there has been a growing interest in converting oil-in-water emulsions into gels, resulting from practical application in food formulations (Chen & Dickinson, 1999). Several workers have investigated the rheological properties of such emulsion-gels which can be used to create foods with improved sensorial, texture profile and interesting preservation properties. (Line, Remondetto, & Subirade, 2005; Manoi & Rizvi, 2009). Nonetheless, in many cases a thermal treatment is needed to produce these emulsion-gels, so their application in food formulations has to be more investigated.

The aim of this work is to study the interactions between pea protein (VP) and collagen (AP), on the development of stable oil-in-water emulsion-gels, with suitable texture profile and pleasant sensory properties, to be used as the basis of new meat products.

2. Materials and methods

2.1. Materials

The materials used to develop this work are commercial grade and were provided by the company Nobre, Alimentação, Lda.; pea protein isolate – PEA PROTEIN 85 by Agridient (Amsterdam), collagen protein isolate COLPROPUR D (Protein Ingredientes naturales, S.A., Spain). The olive oil as in dispersed phases, a blend of refined olive oil and extra virgin olive oil, was provided by JCCoimbra; Sodium ascorbate (E-301) by Helm Iberica SA, and glucose syrup SFINC GLUCO 21 by Pelicula Food Ingredients.

2.2. Methods

2.2.1. Preparation of mixtures

Based on preliminary testing, targeting the characteristics of a filling product designed by the industry (marketing department), oil-in-water emulsions were prepared with 9.0 g/100 g of total protein, from a mixture of collagen protein isolate (AP) and pea protein isolate (VP). Five mixtures were prepared at varying AP-to-VP ratios of 100:0, 75:25, 50:50, 25:75 and 0:100 (w/w), which are denoted as 100AP:0VP, 75AP:25VP, 50AP:50VP, 25AP:75VP and 0AP:100VP, respectively. All samples included fixed amounts of olive oil (39 g/100 g) (dispersed or lipid phase), deionized water (24.5 g/100 g) (continuous or aqueous phase), sodium ascorbate (2.0 g/100 g) and glucose syrup (3.0 g/100 g). The sodium ascorbate and glucose syrup were added to control oxidation and reduce water activity, respectively.

For each sample the proteins mixtures were hydrated in the deionized water for approximately 12 h at room temperature. The emulsion preparation was performed in a thermo-processor (Bimby-Worwerk) at room temperature for about 10 min at positions 6, the lipid phase was added gradually into aqueous phase for all mixtures tested. After the emulsification, all samples were placed into a glass flask and kept at 4 °C during 24 h to achieve equilibrium, and only after that time the stability determinations, as rheological behavior and texture profile, were done.

2.2.2. Rheological measurements

All rheological measurements were carried out in a controlled stress rheometer (Haake Mars III - Thermo Scientific), with an UTC - Peltier system to control temperature, using a serrated parallel-plate sensor system (PP20 and 1 mm gap), to overcome the slip effect (Franco, Raymundo, Sousa, & Gallegos, 1998).

The shear steady flow curves were obtained ranging the shear rate from 1.00×10^{-5} to 5.00×10^{2} 1/s and applying different temperature conditions - 20 and 40 °C. The characterization of the emulsions flow at 40 °C was investigated to predict the behavior of the emulsions at a crucial processing step of pumping, at this temperature, in the manufacturing sequence at industry. Therefore, it was important to understand the impact of this middle range temperature on the flow parameters during pumping, as these are complex materials with proteins of different sources.

The comparison of the flow curves was performed from the adjustment of the Carreau model:

$$\eta = \eta_0 \Big/ \left[1 + (\gamma \cdot / \gamma \cdot_c)^2\right]^s \quad (1)$$

where η_0 is the zero-shear rate limiting-viscosity (Pa.s), $\boldsymbol{\gamma \cdot_c}$ is the critical shear rate for the onset of the shear-thinning behavior (1/s) and **s** is a parameter related to the slope of this region.

Heating/cooling curves were applied to all samples to study the structural changes resulting from the variation of temperature, at the rheometer plate, heating from 20 °C to 90 °C, at 0.5 K/min heating rate, kept for 5 min at 90 °C, and cooling down from of 90 °C back to 20 °C, at the same heating rate, and kept at 20 °C for 5 min. This temperature profile was designed to reproduce the temperatures applied, after pumping, during this specific industrial processing sequence for these systems.

During the heating profile mentioned, carried out at 1 Hz of frequency and constant stress, within the linear viscoelastic region, previously determined by a stress sweep test, the viscoelastic functions (G'- storage modulus and G" – loss modulus) were registered.

To evaluate the impact of the thermal treatment on the emulsions structure a frequency sweep test was carried out, i.e. the mechanical spectrum before and after the thermal treatment at 20 °C was determined.

Mechanical spectra at 20 °C were obtained varying the frequency between 0.001 Hz and 100.0 Hz, at a constant shear stress within the linear viscoelastic region of the samples. Each test was repeated at least two times and reproducible results were obtained.

2.2.3. Texture and colour characterization

Emulsions macrostructure was evaluated using the texture profile analysis (TPA), as previously described by Raymundo, Franco, Partal, Sousa, and Gallegos (1999). Texture parameters were determined with a TA-XTplus (Stable MicroSystems, UK) texturometer using a 5 kg load cell. Penetration tests were performed with a cylindrical probe (25 mm diameter, 15 mm of penetration, 5 s of waiting time and 1 mm/s of crosshead speed), and the samples were placed in cylindrical glass flask (45 mm of

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