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A combined rheology and time domain NMR approach for determining water distributions in protein blends

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ABSTRACT

We present a combined time domain NMR and rheology approach to quantify the water distribution in a phase separated protein blend. The approach forms the basis for a new tool to assess the microstructural properties of phase separated biopolymer blends, making it highly relevant for many food and non-food related applications. First, we determine the relaxation rate of absorbed water, and the viscoelastic properties of the separated phases as function of the water content. Next, the same properties are measured for the protein blends. Finally, predictions for water distribution obtained from rheological experiments are made via the polymer blending law, and compared to a more direct assessment of the water distribution with time-domain NMR relaxometry (TD-NMR). In this study, the protein blend consists of soy protein isolate (SPI) and vital wheat gluten (WG). We demonstrate that predictions for water distribution are similar for both TD-NMR and rheological measurements. It turns out that water does not distribute homogenously over the phases. Independent of the SPI and WG ratio, more water is absorbed by the SPI phase relative to the WG phase, which largely determines the resulting rheological properties of the blends.

1. Introduction

Microstructural assessment of biopolymer blends is highly relevant for many food and non-food applications. However, most analytical methods have been developed for more diluted dispersions and are not directly suitable for the characterization of denser materials (Kasapis, 2008). Unfortunately, the properties of a denser system cannot be simply predicted by analyzing the properties at lower concentration. For example, the interaction between water and protein is recently described as being concentration dependent (van der Sman, 2012), giving denser proteins blends properties, which cannot be derived from diluted mixtures. It explains the need for the development of new tools for dense-biopolymer materials.

Rheology is often used to characterize the properties of biopolymer blends (Song & Kim, 2004; van Riemsdijk, Snoeren, van der Goot, Boom, & Hamer, 2011). However, this method does not provide direct information about the characteristics of the individual phases and the blend microstructure. To obtain some information about the microstructure, the “polymer blending law” can be used to predict whether a protein blend reacts in an isostress, isostrain, or bicontinuous manner with respect to the applied forces. This empirical law was established by Takayanagi (Kasapis, Norton, & Johan, 2009; Takayanagi, 1963) and is expressed in its general form as:

$$M^n = \phi_X M_X^n + \phi_Y M_Y^n, \quad (1)$$

where ϕ denotes the volume fraction of the individual phases and M can be either Young's modulus (E), the shear modulus (G) or the storage modulus (G') from oscillatory measurements. The exponent

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equals $n = 1$ for isostrain, $n = -1$ for isostress in systems in which one component is dispersed in the other (Kasapis et al., 2009; Morris, 2009), and $n = 0.2$ for bi-continuous systems (Davies, 1971; Gilsenan, Richardson, & Morris, 2003; Piculell, Nilsson, & Muhrbeck, 1992). The isostress regime is suitable to describe a blend with a dispersed phase with a high viscosity, while the isostrain regime is suitable to describe the behavior of a dispersion in which the moduli of both phases are similar or the dispersed phase is weaker. In case of bi-continuous systems, it is assumed that both phases form a continuous phase and therefore contribute more equally to the final product properties.

The mechanical properties of a biopolymer blend are strongly influenced by the rheological properties of the phases, which depend on the water content in that phase, and therefore the water distribution in the blends. This fact has already been recognized by various authors (Clark, Richardson, Ross-Murphy, & Stubbs, 1983; Fitzsimons, Mulvihill, & Morris, 2008; Shrinivas, Kasapis, & Tongdang, 2009). In those studies, the water distribution, which can be expressed as avidity factor p, is taken as a fitted parameter when analyzing the rheological properties. Microscopy is then used as qualitative confirmation. Theoretically a quantitative interpretation of microscopy pictures is possible, provided that each phase can be clearly distinguished, for example by using different stains. However, this quantification requires a significant effort (taking many images of a sample to get a relevant overview, stacking of 2D pictures to get a 3D overview etc.), and in-depth image analysis, which relies heavily on computer algorithms to recognize, differentiate and quantify images (Aguilera, 2005).

It is clear that the interpretation of rheological data would benefit from the availability of a method for direct and accurate determination of the water distribution in the total sample. NMR relaxometry has been proposed as an adequate analytical tool for this determination (Clark et al., 1983).

In this work, we therefore focus on the characterization of concentrated, phase separated protein blends. We use an aqueous dispersion consisting of soy protein isolate (SPI) and vital wheat gluten (WG). SPI and WG are assumed to be immiscible at high concentrations and to form separate phases with sizes in the ~100 μm range upon shearing and heating. This assumption is based on the fact that especially gluten is unable to completely dissolve in water. It absorbs twice its weight in water at most, giving a soft-solid material with the characteristic visco-elastic (dough-like) behavior (Grabowska, Tekidou, Boom, & van der Goot, 2014; Peighambardoust, Hamer, Boom, & Van der Goot, 2008). Such swollen gluten material cannot be mixed at molecular level with other biopolymer materials, which was confirmed by microscopic analysis of a structured SPI-gluten blend that revealed clear phase separation (Grabowska et al., 2014; Krintiras, Diaz, van der Goot, Stankiewicz, & Stefanidis, 2016; Krintiras, Gobel, Bouwman, van der Goot, & Stefanidis, 2014). In a phase separated SPI-WG blend, the protein concentration in each phase is related to the water distribution over the two phases. Since the water holding capacities of SPI and WG differ largely, we expect that water will not distribute evenly over the SPI and WG phases, which implies that the volume fractions of the individual components in the SPI-WG blend will not be equal to the mass fractions after equilibration. If water does not distribute homogeneously, one phase would be more diluted, whereas the other would be more concentrated. The actual protein concentration in each phase can be calculated using mass balances as explained in Appendix A.

Here, we develop an approach based on time domain nuclear magnetic resonance relaxometry (TD-NMR) to assess water content and relaxation rate of absorbed water in the separate phases (Bosmans et al., 2012; Mariette, 2009; Van As & van Duynhoven, 2013; Van Duynhoven, Voda, Witek, & Van As, 2010). Since our model system is phase separated into a WG and a SPI phase, we measure proton transverse relaxation times T_2 of water in dispersions of the individual SPI and WG proteins and use this as input to obtain estimates for the water distribution (and phase volume fractions) in the SPI-WG blends. Diffusive exchange is expected to be negligible. This is based on the fact that SPI and WG are assumed to be immiscible and to form relatively large separate phases with high viscosity given the density of the blend. Recently, a similar conclusion was drawn for dispersions containing dense whey protein particles (Peters et al., 2016). The objective of this study is to obtain better insight in the rheological behavior of SPI-WG dispersions by considering the distribution of water over both phases and to understand the effect of water content on the rheological properties of the individual phases. The results are used to hypothesize on the microstructure in the blends.

2. Materials and methods

2.1. Materials

Soy protein isolate (SPI) and vital wheat gluten (WG) were donated by Barentz (Hoofddorp, The Netherlands). Soy protein isolate (Supro EX 37) is a powder containing at least 90 wt% protein (N × 6.25), <5 wt% ash and <6 wt% water, as indicated by the manufacturer (Solae, St. Louis, Missouri, USA). Vital WG, produced by Roquette (Lestrem, France), contains >83 wt% (N × 6.25) protein based on the dry matter and 8 wt% water, as indicated by the manufacturer. Water binding capacity of soy protein isolate is reported to be 8.9 g water/g dry matter and the water binding capacity of wheat gluten is 1.9 g water/g dry matter (Grabowska et al., 2014). Food-grade sodium chloride (NaCl) was obtained from Sigma–Aldrich (Zwijndrecht, The Netherlands).

2.2. Sample preparation

Dispersions of SPI (11.0–50.0 wt%) and WG (20.0–60.0 wt%), and SPI-WG blends (25.0–40.0 wt%) were prepared at different ratios SPI and WG (20/80, 35/65, 50/50, 65/35, 80/20). All samples contained 1.0 wt% NaCl. The salt was dissolved in water before adding the solution to the protein powders. NaCl was added because it is often used in SPI-WG blends, which are structured into fibrous materials for the meat analog application (Grabowska et al., 2014). In case of the SPI-WG blend, the protein powders were mixed thoroughly before adding the salt solution. The dispersions were then mixed with a spatula. All samples were allowed to equilibrate at room temperature for 10 min before actual measurement. It must be noted that there was no difference for the rheology and TD-NMR measurements in case protein powders were added to water or water was added to the protein powders or kept for longer times.

2.3. ^{1}H time domain NMR relaxometry

Samples were transferred into 7-mm NMR tubes that were sealed to prevent water loss during the experiment. All experiments were repeated four to eight times to allow adequate estimation of the experimental error.

^{1}H time-domain NMR (TD-NMR) relaxometry was performed at 0.72 T magnetic field strength (30.7 MHz ^{1}H resonance frequency)