Food Hydrocolloids 26 (2012) 421–426

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

From peptides and proteins to micro-structure mechanics and rheological properties of fibril systems

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ARTICLE INFO

Article history:
Received 30 September 2010
Accepted 24 November 2010

Keywords:
Peptide
Protein
Micro-mechanics
Fibril
Rheology

ABSTRACT

This article provides a summary of an example of how relationships between molecular scale properties and macroscopic properties are formulated, in this case with a focus on fibril microstructures and according system elasticity. Entropy plays a dominant role on all length scales. The elasticity is reviewed in terms of the micro-mechanical properties of the fibrils over a wide range of concentrations. The elasticity is concluded to be of an entropic nature. The micro-mechanical fibril property is the persistence length. For fibrils based on globular proteins the response to temperature change and flow has been reported to be either reversible or non-reversible, dependent on the age of the fibril. The reversibility is argued to be related to the fact that the fibril consists of peptides originating from hydrolysis of the globular proteins, and that the initial step of fibrillar peptide assembly is entropic of nature. The longer term irreversibility is speculated to be caused by a secondary assembly step being a collective transition into inter-beta-sheets.

1. Introduction

Food science faces considerable challenges in the next few decades. The choice for an ingredient will become more and more dependant on its availability and according costs, in addition to the current deliberate choices based on functional requirements on deliciousness, nutrition, and/or health. A more recently formulated requirement is sustainability along the food chain. This multitude of requirements relates to controlling any one of the transitions the food experiences during processing (large or small scale), transport, storage, consumption, and digestion. For optimal control of these transitions along the entire food chain one strives for a simultaneous optimization of this multitude of requirements, all as a function of ingredient choice. This in turn demands insight in the non-linear relationships between properties of the food that are manifest along the entire food chain. An underlying framework for obtaining insights in these relationships is provided by a physical and chemical description of the properties of the foods as a function of its ingredients. As for the physics much can be formulated in terms of heat and mass transport (molecular based), interaction with sound and EM radiation, and response upon deformation.

Because every ingredient, whether it is chemically pure or a mixture, is characterized by its molecules, the ultimate scientific goal is to relate molecular properties to food properties, along the entire food chain. The amount of information that has to be linked together is enormous. The strategy is to reduce this amount to the level that is relevant for the specific problem at hand. Most foods have some intermediate characteristic length scale, the micro-structural scale or meso-scale. The food properties will depend on the properties and interactions of the micro-structural elements. Their properties and interactions subsequently depend on the molecular properties of the ingredients. It is the description on the level of the micro-structural length scale that is enabling to diminish the amount of information necessary to describe food properties in terms of molecular properties of its ingredients. The micro-structural information is therefore key for relating molecular to macroscopic properties.

In order to systematically address the food properties and their responses in terms of molecular properties one needs to consider principle parameters. This is usually done by considering pressure p, volume V, and temperature, T, i.e. a p–V–T phase diagram. However, during processing, storage, transport, consumption and digestion, the food experiences additional forces, and the response of the food to these forces is also an important subject. As examples for the forces one may think of electric forces (pulsed electric field), forces due to temperature gradients, or forces of mechanical origin (hydrostatic, shear, elongation, torsion). For including a mechanical force and its response, it was suggested by Liu and Nagel to treat the mechanical force as a pressure contribution and come to a jamming state diagram (Liu & Nagel, 1998). This idea was used later on by Trappe et al. (Trappe, Prasad, Cipelletti, Segre, & Weitz, 2001) in order to quantitatively distinguish between the state of systems being either jammed or flowing. Since volume can be expressed as

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doi:10.1016/j.foodhyd.2010.11.019

an inverse of ingredient concentration, and temperature as a relative measure of the interaction strength, one may define three alternative principal axes for describing the state of a micro-structured system, i.e. exerted force strength, interaction strength between the micro-structural elements, and their concentration (Trappe et al., 2001). In the many non-equilibrium situations also time matters, forming the fourth axis. The micro-structure itself may be expressed in terms of the number of disconnected pieces, mean curvature, so-called Gaussian curvature, and the interfacial area with the dispersing phase (Nelson, Piran, & Weinberg, 1989). Shape is usually distinguished as a 1-dimensional (fibril), 2-dimensional (platelet) or 3-dimensional form (sphere).

Following this general scheme, we will address in this article aspects of the mechanical (shear-) response of several fibril containing systems. Specifically, the macro-mechanical response will be related to the mechanical response of the micro-structure (micro-mechanics), in terms of concentration and interaction strength of the micro-structural elements. The micro-structure and its properties are in the end also related to the chemical composition of the system and to aging of the sample. In this respect, this article provides insight in how relationships between molecular scale and macroscopic scale actually are formulated. In this particular case on peptides from globular proteins and for gelatin forming fibrillar structures, and how these structures give rise to elasticity of the macroscopic system.

Fibrillar structures occur in many different types of foods. One may think e.g. of single chain polysaccharides, gelatin helices, and fine stranded protein networks. The rheology obviously depends on the properties of the fibrillar structures themselves, their mutual interactions, and interactions with other surrounding molecules and structures. One of the reasons we are interested in the rheology of fibril containing systems is that fibrils provide a weight effective way to thicken a liquid. For example one is able to prepare extremely low weight fraction gels. The weight effectiveness turns out to be related to the thickness to length ratio of the fibril. Moreover, the stiffness of the fibril itself also plays a major role in the weight effectiveness, as well as the ionic strength if the fibrils are charged. Therefore we first address the gel point, i.e. the minimal concentration at which a gel is being formed. Apart from the weight efficiency for gelation, one is also interested in the concentration dependence of the elasticity of the gel. For gelatin gels, a strikingly simple picture arises where one parameter determines the elasticity, both at low and high concentrations. This turns out to be the flexibility or persistence length of the triple helix, in turn determining the triple helix fluctuations. The fluctuations are a non-linear function of the triple helix concentration and the persistence length, leading to another characteristic length scale, the deflection length. During the fibril formation, as well as after the fibril formation, the length distribution can be affected by flow. As such the results illustrate how fibrillar structures connect non-linearly to rheological properties. Effects of aging are shown to provide some insight into routes for fibrillisation, which later on have been more firmly established by means of chemical analysis of fibril composition.

2. Elasticity of fibril systems near the gel point: fibril stiffness and temperature

Knowing the fact that fibrils are weight efficient, the questions arises how one may create fibrillar structures apart from the usual existing food structures such as the triple helix formed by gelatin. It is known that many proteins can form fine stranded networks, and the extreme case would then be the fibrillar case. Indeed, many different proteins exhibit this ability under specific circumstances, such as beta-lactoglobulin, BSA and ovalbumin (Veerman, Sagis, & van der Linden, 2003b). The minimum volume fraction for gelation by rigid rods has been first formulated by Philipse et al. (Philipse, 1996; Philipse & Kluijtmans, 1999; Philipse & Verberkmoes, 1997; Philipse & Wierenga, 1998). The main idea is that the minimum volume fraction at which gelation takes place is determined by a minimum amount of contacts per rod between neighbouring rods. This leads to a minimum gel fraction $c_p = \alpha D_0/L$, where α is a constant of order unity (related to the number of contacts per rod), D_0 is the thickness of the rod and L is its length. For semi-flexible fibrils, the result of Philipse et al. must be modified to take into account the finite value of the persistence length, L_p, of the fibril. For that case, Veerman et al. (Veerman, Ruis, Sagis, & van der Linden, 2002) have reported $c_p = \alpha D_0/L_p$. This expression has described the gelation of three different classes of semi-flexible protein-based fibrils, i.e. beta-lactoglobulin, BSA and ovalbumin (Veerman et al., 2003b). This expression is applicable when one considers rod like fibrils that feel each other only by means of their excluded volume interaction. The number of contact points per rod is in this picture solely related to the excluded volume per rod, i.e. no additional attractive interaction. This excluded volume interaction is in fact an interaction that exists due to the finite temperature of the system.

If one does introduce an extra attractive interaction between the fibrils one is able to reduce the minimal gelation concentration even further, cf. a publication by Veerman et al. (Veerman et al., 2003b). Extremely low weight fraction gels have been realised in this case, down to 0.07%. This is an order of magnitude lower than using the conventional cold gelation method, or heat-induced gelation. The way to obtain these extremely low weight fraction gels is to first form long linear fibrils, for instance by using the protein beta-lactoglobulin (β-lg) at pH 2. These fibrils exhibit stability even when, after their formation, the pH is changed towards 7 or 8 (Veerman, Baptist, Sagis, & van der Linden, 2003a; Veerman et al., 2002, 2003b). We will see later on why this is possible. Second, if, after changing the pH to 7 or 8, one adds $CaCl_2$, one obtains Ca-bridges, which are stronger than kT (resembling the excluded volume interactions), and therefore these lead to a more efficient network formation.

It should be noted that beta-lactoglobulin fibrils under certain conditions are recently reported to show also multi-fibrillar arrangements into ribbons (Adamcik et al., 2010), which under these circumstances will give rise to additional contributions to the elasticity. The extent to which this is dominant over the other contributions is unclear at the moment but should be subject to further studies.

3. Elasticity of fibril systems: fibril stiffness, confinement, and temperature

One example of a fibril system is gelatin. Another example is fibrils formed by β-lac at low pH. For gelatin, recent data of Joly-Duhamel et al. (Joly-Duhamel, Hellio, Ajdari, & Djabourov, 2002) summarise a concise and very thoroughly conducted experimental investigation on gelatin gelation. They reported the modulus of gelatin gels in terms of the concentration of triple helices. Their data convincingly show that the elastic modulus, G′, was found to scale according to $G' \sim (c - c_p)^t$, with c the concentration of helices in the system and $t = 1.9$, the exponent for so-called isotropic force percolation and c_p the concentration of gelation. They report $c_p = 0.0028$, which is equal to a gel point of D_0/L_p for $\alpha = 1/3$, and experimental parameters $D_0 = 1.5$ nm and $L_p = 170$ nm. The scaling relation was also found to explain the data by Veerman et al. for the β-lac fibril systems (Veerman et al., 2002). The exponent $t = 1.9$ expresses the relative efficiency by which the interconnections of rods contribute to the elasticity of the network. Not all interconnections contribute to the elasticity, there are also loose branches of interconnection threads. The efficiency of interconnections contributing to elasticity is predicted by percolation theory, including the exponent reported