Advances in structure formation of anisotropic protein-rich foods through novel processing concepts

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Development of protein-rich food products is currently limited by lack of scientific insights in structuring processes. The application of well-defined flow appears to be a good tool to create novel anisotropic food structures, on one hand, and to improve understanding of the behavior of protein-rich materials during processing, on the other hand. Concentrated protein dispersions show similarities with polymer systems under flow. Also in protein dispersions, the size of structural elements and interactions present account for structural changes due to flow. These insights can form a basis for the design of dedicated food structuring equipment.

Introduction

Protein-rich foods have recently gained increased interest due to health benefits involved in the consumption of proteins. Foods with high protein content induce fast satiation, which in turn has been shown to reduce people’s food intake (Antonio, 2006; El Khoury, Obeid, Azar, & Hwalla, 2006; Lee et al., 2006). Consumption of protein-rich foods can also prevent muscle loss (Maughan, 2002). Creating tasteful and palatable protein-rich products using proteins from various sources imposes technological challenges. The implementation, for example, of sustainable proteins derived from plants in novel foods is of high interest (Linnemann & Dijkstra, 2002). Anisotropic structures are often encountered in protein-rich foods, such as meat and fish. In the case of imitating meat, a key success factor appears to be the formation of an attractive fibrous structure (Aguilera & Stanley, 1993).

Proteins are versatile biomacromolecules that can have a globular (e.g., whey and soy protein) or random coil (e.g., casein) conformation. In contrast to synthetic polymers, proteins can be permanently altered as a result of a thermal treatment. During heating, globular proteins denature and this can lead to aggregation or even gelation. The use of a plasticizer, often water, is very common in protein systems, to reduce the viscosity and enhance mobility during processing. Proteins are sensitive to changes in pH and to the addition of ions, which both offer the possibility to fine tune the molecular forces present. The above-mentioned properties make proteins excellent structuring ingredients in foods (Aalbersberg et al., 2003).

In general, controlling the spatial organization of structural elements, such as proteins, using appropriate processing is an important aim of food structuring in order to create products with attractive textures (Aguilera, 2005). Due to the nature of the building blocks of foods, structuring at micro- and mesoscale is of high interest (Aguilera, 2006). Structure formation of proteins can be divided into a low and a high concentration regime, with a rough transition point around 10% protein where the viscosity increases rapidly due to close packing of the proteins (Panouille, Durand, & Nicolai, 2005). In the current paper, we will focus on structure formation of protein systems with concentrations exceeding 10%.

Traditionally, processes used in chemical engineering have been adapted for food structuring purposes even though there is a large difference in aims and applications (Aguilera, 2005). Equipment in the chemical industry is often focused on effective mixing of ingredients using low energy inputs. Relatively few innovations in dedicated food structuring processes have been reported over the last years, in spite of the advantages that can be attained when applying processes that maximize the potential use of ingredients without loosing nutritional quality. To illustrate this, patents for the creation of fibrous protein textures that have been filed or
Texturization of plant proteins comprises protein denaturation at relatively high temperatures (100°C), high pressures (17–60 atm) and moderate mechanical shear rates (120–180 s⁻¹) in order to obtain a molten mass that can be deformed (Aguilera & Stanley, 1993; Areas, 1992). In contrast to synthetic polymers, both reversible and non-reversible reactions occur in protein systems, which have large implications on the functional properties of the resulting products (Areas, 1992). After a typical residence time of several minutes in the extruder, the molten protein mass is pushed through a cooling die to obtain protein alignment (Aguilera & Stanley, 1993; Areas, 1992; Cheftel, Kitagawa, & Queguiner, 1992). Solidification results from cooling, non-covalent interactions and additional disulphide bond formation (Areas, 1992; Cheftel et al., 1992; Ledward & Tester, 1994).

Table 1 provides an overview of various ingredients, mostly mixtures of proteins and polysaccharides, and extrusion conditions that have been applied to texturize products. The formation of fibrous structures during extrusion has frequently been attributed to the deformation of these intrinsically phase separated systems, which are water-in-water emulsions that can form aligned structures (Tolstoguzov, 1993). Based on various products, ranging from extruded defatted soy flour (Noguchi, 1989), a salmon fish—soy protein blend (Cheftel et al., 1992) and a zein protein—wheat starch mixture (Buttermann-Azcona, Lawton, & Hamaker, 1999), we can deduce that elongated domains or fiber-like structures were found in the order of 0.1–100 μm in thickness (Table 1). No evidence of molecular orientation was found in extruded soy protein isolate and maltodextrin products (Yuryev et al., 1991). Cheftel et al. (1992) found that extrusion of soy protein with high protein purity did not result in a fibrous texture. It is argued that at the high temperatures used during extrusion, only a homogeneous phase can exist, even when proteins and polysaccharides are present (Ledward & Tester, 1994), therewith explaining the formation of structures that were not very fibrous. Table 1 shows that not all extruded products are assessed on their microstructural properties with microscopic techniques or on their degree of fibrousness using mechanical tests.

Besides the production of meat analogs, extrusion (80–120°C) has been applied to blend and emulsify dairy ingredients for the production of cheese analogs (Cheftel et al., 1992; Zuber, Megard, & Cheftel, 1987) and for the production of microparticles, which can be used as fat substi-tutes (Cheftel & Dumay, 1993; Onwulata, Konstance, Cooke, & Farrell, 2003). In general, homogeneous gelled materials were obtained as shown in Table 1. Fibrousness in a dairy protein—fat system is only reported for Mozarella cheese, which is produced by a cooking and stretching process (McMahon, Paulson, & Oberg, 2005). The fibrousness is caused by the presence of protein strands in the order of ~10 μm (O’Reilly et al., 2002), which are separated by fat globules (Paulson, McMahon, & Oberg, 1998).

Overall, it seems that most studies concerning the effect of process parameters on the structure formation of protein-rich extrudates are rather empirical. Often ingredient composition, moisture content, screw speed and temperature along the extruder barrel are varied (Table 1), therewith making comparisons between processes and products complicated, let alone providing insight into structure formation.
mechanisms. The concept of solidification and deformation explains the limitations of extrusion to produce fibrous products. Due to sequential locally high and low shear rates in the extruder barrel, random re-orientation of the proteins takes place, undoing any orientation and yielding an isotropic material. The fact that a certain degree of alignment is achieved in the cooling die can be attributed to the better defined flow in the die (i.e., laminar flow), and often the presence of a phase separated system.

### Spinning

Spinning is another technique employed to induce alignment in biopolymer solutions based on deformation and solidification. Similarly to extrusion, alignment during...
spinning is promoted by the use of intrinsically phase separated systems (Ledward, 1993; Tolstoguzov, 1993). While pushing the viscous biopolymer solution through a spinneret, the water-in-water emulsion is aligned, and the resulting fibers are stretched (Gallant, Bouchet, & Culioli, 1984). Solidification is achieved using coagulation agents, such as salts, acid or alkali solutions. After coagulation, the spun fibers are washed. The resulting fibers have a thickness in the order of the size of the spinneret holes, usually hundreds of micrometers (Gallant et al., 1984; Tolstoguzov, 1993).

Non-spinneret spinning has also been applied to produce protein fibers. In this case, the biopolymer solution is extruded through shaping nozzles, resulting in many fibers (Tolstoguzov, 1993). Various milk proteins, plant proteins and polysaccharides have been used for spinning, which is summarized in Table 1. The use of protein—polysaccharide mixtures was found to reduce the water-solubility of the produced fibers (Antonov, Zhuravskaya, & Tolstoguzov, 1985; Downey & Burgess, 1979a, 1979b). Spun pea protein fibers were reported to have an aligned cortex and a granular core (Aguilera, Kosikowski, & Hood, 1975; Gallant et al., 1984). Similarly to extrusion, no evidence of molecular orientation was found after spinning of soy protein isolate (Rampon, Robert, Nicolas, & Dufour, 1999).

The use of spinning as a process to produce food fibers involves several disadvantages, such as generating large waste streams from the coagulation and washing baths, the use of chemical coagulants, the difficulty of guaranteeing microbiological safety due to high moisture contents, and the complexity of assembling single fibers into a food product on an industrial scale. The fact that research on spinning for food applications has been scarce since 1999 probably indicates that the drawbacks of the process may outweigh the benefits.

Need for a new approach for food structuring processes

The previous sections indicate that developments, especially in extrusion, in the past have been spectacular. But, it seems that the extreme complexity of the conditions during processing hinders further developments. We think that advances can be attained by increased control over flow patterns in structuring processes.

With respect to deformation, both shear flow and elongational flow are present in the traditional protein structuring processes leading to complex flow patterns. Focusing on one type of deformation would help in acquiring knowledge about the fundamentals of these processes. The shear component of the deformation does not allow for changes in the structure of the protein fibers. Subsequent (re-)design of dedicated equipment for structuring based on this knowledge can then lead to a next step in structuring of concentrated protein foods. In addition, the trend towards product diversity will probably induce a new interest for batch processes. Deformation applied in a batch process offers the advantage of uncoupling flow (shear) rate and process time, which may prove more useful for food structuring.

The fact that properties of protein foods are mainly determined by the presence of a polymeric component (here proteins) suggests that studies of simpler (synthetic) polymer solutions may indicate the direction for developments for new structuring processes. Formation of anisotropic structures is often encountered in polymeric systems, ranging from polymer melts to liquid crystals. Relevant parameters for structure formation as well as tools to study structure formation can be deduced from these model studies. The following section will focus on these aspects.

Structure formation in polymer model systems

In contrast to the processes described in the previous section, structure formation of complex polymeric fluids has been studied extensively with respect to deformation, usually under well-defined flow conditions using rheometers. Flow-induced structures encountered in complex fluids are often anisotropic in nature, although these are not fixed. We will focus on the flow-induced formation of anisotropic (i.e., lamellar and bundle-like) structures in various systems, which have the common features that they are phase separated on a microscopic or macroscopic scale, and that they comprise structural elements that can be affected by flow. The relevance of the phenomena involved for structure formation of anisotropic protein-rich foods in terms of process conditions and mechanistic insight will be highlighted.

Anisotropic structures

Table 2 provides examples of wormlike micelle solutions, polymer solutions, colloidal and non-colloidal suspensions where anisotropic structures as a result of shearing have been observed.

<table>
<thead>
<tr>
<th>System</th>
<th>Properties</th>
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<tbody>
<tr>
<td>Weak surfactant solution</td>
<td>Formed lamellar structures</td>
</tr>
<tr>
<td>Micellar surfactant solution</td>
<td>Formed lamellar structures</td>
</tr>
<tr>
<td>Colloidal solution</td>
<td>Formed lamellar structures</td>
</tr>
<tr>
<td>Non-colloidal solution</td>
<td>Formed lamellar structures</td>
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</table>

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Table 2. Selection of various model systems where anisotropic structure formation was observed using well-defined shear flow (in rheometers), including the key phenomena for the structure formation

<table>
<thead>
<tr>
<th>Model system</th>
<th>Structures</th>
<th>References</th>
<th>General remarks about phenomena</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Wormlike micelle solutions</strong></td>
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</table>
| Cetylpyridinium chloride and sodium salicylate (CPyCl–NaSal) | Shear bands | Mendez-Sanchez, Lopez-Gonzalez, Rolon-Garrido, Perez-Gonzalez, & de Vargas, 2003<sup>a</sup>; Lopez-Gonzalez, Holmes, & Callaghan, 2006<sup>d</sup>; Herle, Fischer, & Windhab, 2005<sup>b</sup>,<sup>e</sup>; Fischer, 2000, Fischer et al., 2002<sup>a</sup>,<sup>b</sup>,<sup>c</sup>; Lopez-Gonzalez, Holmes, & Callaghan, 2006<sup>d</sup>,<sup>e</sup>; Herle, Fischer, & Windhab, 2005<sup>b</sup>,<sup>c</sup> | - Semi-dilute concentrations  
- Non-monotonic stress–shear rate curves  
- Flow instabilities  
- Usually 2–3 bands, perpendicular to \( \nabla \) or \( \omega \)  
- Oscillations in stress and normal stress  
- Shear-induced concentration fluctuations  
- Phase separation (accompanied by turbidity)  
- Shear thinning, shear thickening |
| | | | |
| Cetyltrimethylammonium bromide (CTAB) and NaSal or KBr | Shear bands | Azzouzi, Decruppe, Lerouge, & Greffier, 2005<sup>a</sup>,<sup>c</sup>,<sup>e</sup>,<sup>f</sup>; Lerouge, Decruppe, & Olmsted, 2004<sup>a</sup>,<sup>e</sup> | |
| **Polymer solutions** | | | |
| Polystyrene/diethyl malonate solution | Elongated structures in flow direction (\( \perp \nabla \)) | Endoh, Takenaka, & Hashimoto, 2006<sup>b</sup>,<sup>c</sup>; Saito, Hashimoto, Morin, Lindner, & Boue, 2002<sup>a</sup>,<sup>b</sup>,<sup>g</sup> | - Semi-dilute concentrations  
- Spinodal decomposition and demixing  
- Shear-induced concentration fluctuations  
- Butterfly and streak patterns in SALSSANS  
- Shear thinning  
- Structures disappear after cessation flow |
| High \( M_w \) deuterated polystyrene in dioctyl-phthalate | Structures (\( \perp \nabla \)) | Steiger et al., 2004; Steiger & Richtering, 2003<sup>a</sup>,<sup>b</sup>,<sup>g</sup> | |
| Aqueous solutions of linear poly(\( N \)-isopropylacrylamide) and PNiPAM microgel particles | Lamellar structures (closely packed), demixing | | |
| Ultrahigh \( M_w \) polyethylene in paraffin wax as solvent | Optically anisotropic fibrils in flow direction | Murase, Kume, Hashimoto, & Ohta, 2005<sup>a</sup>,<sup>b</sup>,<sup>c</sup>,<sup>e</sup> | |
| **Colloidal suspensions** | | | |
| Polystyrene latex dispersions, charge stabilized by sulfate surface groups (92, 120, 143 nm) | Layers (hexagonal) | Dux et al., 1998<sup>g</sup> | - Dense colloidal dispersions  
- Structures depend on particle concentration  
- Structures disappear after cessation flow  
- Long-range interactions favor bundle formation  
- Shear thinning (alignment in flow direction)  
- Shear-induced concentration fluctuations  
- Interactions (particle, depletion) important |
| Two aqueous poly(butylacrylate-styrene) lattices (average diameter 127, 254 nm) | Bundle-like structures in flow direction | Vermant et al., 1999<sup>a</sup>,<sup>b</sup>,<sup>e</sup> | |
| Poly(butylacrylate-styrene) latex particles (83 nm) and two telechelic polymers based on polyacrylamide and polyacrylic acid | Aligned particles in flow direction and in \( \omega \) direction | Belzung et al., 2000<sup>a</sup>,<sup>b</sup> | |
| **Macroscopic particle suspensions** | | | |
| Polyacrylamide solution in water–glycerol; poly(methyl-methaacrylate) (PMMA) spheres (126–150 and 53–63 \( \mu \)m) | String formation in flow direction | Lyon et al., 2001<sup>a</sup>,<sup>c</sup> | - Coarser strings when particle concentration \( \uparrow \)  
- Shear thinning essential  
- \( W \)l not only determining for particle string formation (no criterion possible)  
- Bulk phenomenon |
| Glycerin, ethylene glycol, polyacrylamide; xanthan-sucrose solutions; PMMA spheres (300–355 \( \mu \)m) | String formation in flow direction | Won & Kim, 2004<sup>a</sup>,<sup>e</sup> | |
| Polysisobutylene (PIB) in polybutene; PIB in decalin; hydroxypropylcellulose and carboxymethylcellulose sodium salt; polystyrene spheres (2.7 \( \mu \)m) | String formation in flow direction | Scirocco et al., 2004<sup>a</sup>,<sup>b</sup>,<sup>g</sup> | |
dispersions independent of the internal particle structure (Stieger, Lindner, & Richtering, 2004).

Shear flow affects phase transitions in near-critical solutions, which can lead to changes in structure (Onuki, 1995; Wang, 1991). Phase separation induced by shear flow, for example through spinodal decomposition, is closely related to concentration fluctuations (Fischer, Wheeler, & Fuller, 2002; Grand, Arrault, & Cates, 1997; Le Meins & Tassin, 2001). Therefore, susceptibility to shear-induced phase separation depends on the thermodynamic state of the solutions. Attractive interactions in microgels of dense polymer particles lead to demixing as a result of shear-induced concentration fluctuations whereas repulsive interactions yield closely packed lamellar structures (Stieger et al., 2004).

The formation of bundle-like structures in dense latex suspensions oriented in the flow direction depended on the presence of long-range interactions and on the particle concentration (Vermant, Raynaud, Mewis, Ernst, & Fuller, 1999). Weakly aggregating colloidal particles formed banded structures in the vorticity direction rather than in the flow direction (Belzung et al., 2000; De Groot, Macosko, Kume, & Hashimoto, 1994). Ordering of colloidal aggregates in the vorticity direction increased with increasing shear rate and particle concentration (De Groot et al., 1994). The structures formed were also related to shear-induced concentration fluctuations.

On a macroscopic scale, alignment of solid particles and emulsion droplets was obtained in viscoelastic solutions. The so-called string formation of solid particles was accompanied by the presence of normal stresses in the viscoelastic suspensions, expressed as the Weissenberg number ($Wi$), and...
shear thinning (Lyon, Mead, Elliott, & Leal, 2001; Michele, Patzold, & Donis, 1977; Won & Kim, 2004). However, the effect of viscoelasticity (through Wi) on aligning particles could not be proven, which led to the conclusion that string formation is mainly a hydrodynamically driven phenomenon (Scirocco, Vermant, & Mewis, 2004).

Bi-continuous string formation oriented in the flow direction has been observed in various two-phase systems (Table 2). A polymer blend showed string formation under strong shear flow, which was related to spinodal decomposition of the system (Hashimoto, Matsuzaka, Moses, & Onuki, 1995). A biopolymer mixture of gelatin and dextran also exhibited similar anisotropic structures, which were stable under steady shear flow (Wolf & Frith, 2003). The string formation depended on the viscosity ratio, shear rate and phase volumes used. Well-defined deformation and simultaneous solidification through cooling of a dilute polysaccharide mixture of gellan and κ-carrageenan also yielded elongated structures (Wolf, Frith, Singleton, Tassieri, & Norton, 2001). Confinement accounted for a transition of droplets into strings for concentrated polymer blends (Migler, 2001).

Emulsion droplets in polymer blends were reported to form structures in the vorticity direction at high shear rates (>450 s⁻¹), which was explained by the viscoelasticity of the phases involved (Hobbie & Migler, 1999). A shear thinning emulsion with attractive interactions formed cylindrical aggregates in the vorticity direction, which was accompanied by negative normal stresses (Montesi, Pena, & Pasquali, 2004).

Factors relevant for anisotropic structure formation

In all studies, the thermodynamic and rheological properties of the systems were important for obtaining anisotropic shear-induced structures. The investigated systems were either close to the point of phase separation or intrinsically phase separated.

In case of wormlike micelles, the time scale of the formation and breakage of entanglements, therewith influencing the micellar length, is important for structure formation under shear (Forster, Konrad, & Lindner, 2005; Ganapathy & Sood, 2006). For colloidal suspensions, long-range electrostatic interactions and short-range van der Waals interactions (Vermant et al., 1999), or depletion interactions in the presence of a polymer (Belzung et al., 2000) can affect structure formation. Therefore, interactions between the structural elements in the model systems cause structure formation. These interactions are affected by the concentration used, the presence of ions, or other components that can induce interactions, and temperature.

The role of viscoelasticity during structure formation is not clear yet. Many shear-induced structural changes are accompanied by the presence of normal stresses (Table 2). However, it may as well be a result of ordering of structural elements, suggesting that cause and result are not clear in the various systems.

Structure formation of protein-rich systems using well-defined flow

In the light of the studies concerning polymer model systems, it seems logical to start with the fundamental mechanisms of structure formation identified in the previous sections, and from this, define conditions that lead to the same type of structure formation in concentrated protein foods. This first necessitates the discussion of the influence of well-defined flow on structuring in protein-rich foods.

Fibrous protein-rich structures formed using well-defined flow

Fig. 2 illustrates the type of structures that were obtained after shearing solely dense calcium caseinate dispersions (30% w/w), and a two-phase system comprising the same

![Fig. 2](image-url)
protein and palm fat (13% w/w) in a shear cell device, which was developed based on a rheometer concept (Manski, Van der Goot, & Boom, 2007; Peighambardoust, Van der Goot, Hamer, & Boom, 2004). Shear flow was essential to align the dense calcium caseinate dispersions into macroscopic layers. Simultaneous shearing and crosslinking of calcium caseinate using the enzyme transglutaminase were required to obtain fibers (~100–200 nm) in both the one- and two-phase system (Manski et al., 2007; Manski, Van der Zalm, Van der Goot, & Boom, in press). The degree of fibrousness in the products was high, based on the ratio of the yield stresses measured parallel and perpendicular to the fiber direction, which varied between 8 and 14 depending on process conditions and composition. The layered calcium caseinate product resulted in a yield stress ratio of 2.

Shear rate was found to be an important process parameter to control both the formation of fibrous structures and the degree of anisotropy. Based on the process parameters involved, we concluded that the crosslinking rate and shear rate should be balanced to successfully obtain fibrous products (Manski et al., in press).

Structure formation is a mutual effort of protein properties and processing

Recent research showed that the formation of the fibrous protein-rich structures using well-defined flow and enzymatic crosslinking is dependent on the precise properties of the system and does not occur in any system. After processing dense sodium caseinate dispersions (30% w/w) at similar process conditions as calcium caseinate, isotropic materials without any observable orientation at macro- or microscale were obtained (Manski, Van Riemsdijk, Van der Goot, & Boom, submitted for publication).

The different behavior of calcium caseinate and sodium caseinate under shear flow could be related to the difference in structural elements present. The dimensionless numbers Péclet \((\text{Pe} = (6\pi a^2 \eta \gamma)/kT)\), where \(a\) is the particle radius, \(\gamma\) the shear rate, \(\eta\) the viscosity, \(k\) the Boltzmann’s constant, \(T\) the temperature, and Deborah \((\tau\gamma)\), where \(\tau\) is the relaxation time, indicate that the presence of structural elements with a certain size or relaxation time is essential for shear-induced ordering (Macosko, 1994). Calcium caseinate dispersions comprised both larger protein aggregates in the order of 100–300 nm (De Kruif, 1998; Dickinson et al., 2001) and stronger attractive interactions due to the presence of calcium ions compared to sodium caseinate, which were in the order of 20–50 nm (Lucey, Srinivasan, Singh, & Munro, 2000), therewith favoring shear-induced ordering.

Structure formation induced by shear flow was proposed to be a generic property of near-critical and phase separated systems (Hobbie, Lin-Gibson, Wang, Pathak, & Kim, 2004; Onuki, 1995). We rationalized that the closely packed calcium caseinate system with strong attractive interactions was thermodynamically close to instability, resulting in shear-induced micro-phase separation in protein-rich and protein-poor phases, which led to layer formation. In addition, the rheological behavior of dense calcium caseinate was non-monotonic and shear-thinning, in contrast to sodium caseinate, and resembled the rheological behavior of polymer model systems that exhibited shear-induced concentration fluctuations. Presumably, in the presence of the crosslinking enzyme, fixation of the shear-induced structures can occur, resulting in a fibrous structure.

Decreasing the calcium caseinate concentration from 30 to 20% did not result in the formation of an anisotropic structure after shearing and crosslinking (Manski et al., submitted for publication). Obviously, a lower protein concentration results in a less closely packed dispersion, therewith affecting the interactions and the thermodynamics of the system, and in turn the susceptibility to shear-induced structure formation. This illustrates the subtlety in finding the correct combination of formulation and process conditions (Fig. 3).

**Effect of well-defined flow on protein molecules**

The physical properties of proteins determine to a large extent the structure obtained using well-defined flow. In high shear processes, such as extrusion and mixing, proteins can be altered as a result of breakage or crosslinking (Areas, 1992; Ledward & Tester, 1994). This raises the question to what extent shear flow can affect proteins. Systematic studies concerning the effect of shear rate and shear stress on concentrated protein systems are scarce. A few studies have been conducted to study molecular changes of proteins in dilute and viscous media using various shear devices. Small globular proteins (<40 kDa) in dilute water solutions were not affected by shear rates up to \(10^5\) s\(^{-1}\).
(Jaspe & Hagen, 2006; Maa & Hsu, 1996). In contrast, the shear stresses applied during shearing of the enzyme α-amylase (48 kDa) in viscous starch dispersions were thought to reduce the thermo-stability of the enzyme. Inactivation of α-amylase was reported to occur only at very high shear stresses exceeding 50 kPa (Van der Veen, Van Iersel, Van der Goot, & Boom, 2004).

Glutenin macropeptide aggregates, which are an important fraction of the protein gluten, were shown to stay intact during shearing of wheat dough, while the aggregates do break up during conventional mixing. At similar levels of specific mechanical energy (SME), which is typically used in mixing processes to determine the energy input per kilogram material, shearing (<50 s^{-1}) appeared a much milder process compared to mixing of various types of wheat dough (Peighambardoust, Van der Goot, Hamer, & Boom, 2005; Peighambardoust, Van der Goot, Van Vliet, Hamer, & Boom, 2006). In addition, the maximum shear stress (time-independent) occurring during shearing of low-moisture starch appeared to determine the breakage of starch molecules rather than the energy input (time-dependent) during shearing (Van den Einde, Akkermans, Van der Goot, & Boom, 2004; Van den Einde, Van der Linden, Van der Goot, & Boom, 2004).

Concluding, the physical properties of proteins are not expected to alter in well-defined flow due to their small molecular size. Therefore, the use of well-defined flow at moderate temperatures can preserve protein functionality, which is in contrast with the effect of a thermo-mechanical treatment such as extrusion.

**Challenges for research on structure formation in protein-rich systems**

Creation of novel protein structures requires in-depth knowledge of protein interactions (thermodynamic state) and rheological properties in concentrated systems. This imposes challenges to current analytical methods to study these properties and structure formation capabilities of concentrated protein systems, as these methods are often only suited to analyze properties of dilute systems.

Studies on the interactions in dense protein systems are scarce. A few researchers used osmotic measurements and rheology to study concentrated sodium caseinate and bovine serum albumin dispersions, which were regarded as glassy materials (Farrer & Lips, 1999; Ikeda & Nishinari, 2000). In general, dense protein systems pose limitations to the geometries and shear rates that are accessible with rheometers due to their high viscoelasticity (Macosko, 1994). Improved design of geometries is needed to prevent expulsion of viscoelastic materials at high shear rates.

Small-angle light, neutron or X-ray scattering (SALS, SANS or SAXS) has frequently been applied to study polymer model systems under flow (Table 2). These techniques probe structures at various length scales, being 0.2–100 μm using light, 1–100 nm using X-rays and 1–20 nm using neutrons (Larson, 1999). In dense food systems, length scales of 0.2–100 μm are interesting for probing, which is usually performed using optical techniques. However, light cannot be applied to opaque samples. Therefore, developments are needed with respect to techniques that can probe relevant length scales in food, preferably online, as the phenomena tend to be time-dependent.

**New processing concepts for food structuring**

To make optimal use of structure formation capabilities of highly viscous or viscoelastic materials such as protein-rich dispersions, the development of process equipment dedicated to food structuring purposes needs to be stimulated. With respect to the implementation of well-defined flow, it seems that new processes should be designed in such a way that shear rate is a controllable process parameter. Van den Einde, Akkermans, et al. (2004) and Van den Einde, Van der Linden, et al. (2004) introduced a prototype of an equipment in which the determination of a more quantitative relation between process parameters and changes in product properties was obtained. The combination of this knowledge with information on structure formation in polymer systems will increase our understanding of food structuring.

In addition, the use of well-defined flow offers opportunities for the creation of new structures in novel food products. Therefore, pilot-scale equipment should be developed to test the industrial applicability of processes based on well-defined flow. An alternative to a cone and plate configuration (as in the shear cell device) is a Couette configuration that can be applied to approach a constant shear profile. Peighambardoust, Van Brenk, Van der Goot, Hamer, and Boom (2007) showed that a device based on this configuration was also suitable to create novel structures in a concentrated biopolymer system. The design of an industrial process for well-defined flow could initially be based on this configuration, as it resembles current industrial vessels when modifying the conventional stirrers present. Since novel structures in dense caseinate systems and in dough systems were created at a moderate (<60°C) and constant temperature, scaling-up of these structuring processes will not be hindered by severe temperature requirements.

**Conclusions and future outlook**

Structuring is considered an important activity in food engineering (Bimbenet, Schubert, & Trystram, 2007). However, traditional processes to produce fibrous protein-rich products limit innovations in anisotropic structures due to the design of the current equipment. When considering deformation and solidification as key processes in structure formation of soft solid foods, it is clear that we need to start with good fundamental insight into phenomena and characteristics that lead to the formation of the desired structure. Instead of adapting existing, proven equipment, it is important to take the step to design a new equipment solely based on optimally achieving the specific structuring mechanism. In this light, equipment based on well-defined flow,
therewith enabling the use of shear rate as design parameter, appears promising. In addition, novel technologies based on mild processing can increase the functionality of products because the potential of the ingredients may be preserved in a better way.

Research and innovation in food structuring will benefit tremendously by matching the use of well-defined flow and specific initial ingredient properties. The rheological properties and the interactions present in the starting ingredients appear to be successful tools to estimate the performance of these ingredients during processing. Therefore, research should be devoted to understand the effect of interactions in concentrated protein systems on structure formation capabilities. Parallels between protein systems and polymer model systems are useful to interpret these relations, and therefore, the existence of these parallels should be explored further. Besides experimental insight, the acquired knowledge can be applied in developing numerical models to describe the structure formation of dense protein dispersions as function of interactions, concentration and deformation.

Both the increasing interest to implement high concentrations of proteins in novel foods and the relevance of concentrated protein dispersions for shear-induced structure formation demand a new approach towards investigating protein systems. It is important that in future the interactions and rheological properties of the starting mixtures, here a concentrated protein system, are characterized and quantified to predict its behavior under flow. In addition, the structures (i.e., aggregates, micelles) present in the concentrated dispersions should be unraveled. This requires developments in analytical techniques. Finally, introducing the concept of well-defined flow in processing can result in highly relevant and valuable scientific insight into structure formation of complex materials like foods, which will stimulate innovations in that area.

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