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Innovations with protein nano-fibres

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Proteins in solution can form objects of various shapes. One fascinating possibility is the formation of fibres with a length up to micrometers, but with a thickness of a few nanometres, therefore referring to them as nano-fibres. Many proteins show this behaviour under the appropriate conditions. Gel properties of nano-fibre containing systems can be manipulated by adjusting the fibre properties. The systems exhibit particular behaviour under flow, which can be utilised in processing. Such nano-fibres have innovation potential for foods in terms of for example extremely low weight fraction gels, and their presence may give rise to unexpected novel, including sensory related, material properties.

Keywords: innovation; food proteins; nano-fibres; gels

Introduction

Defining an innovation as a (usually unexpected) step forward in technological development from which new products arise, one may challenge the innovation potential of food proteins, since exploration of the functionality of food proteins has received interest over many decades. Applications originating from these explorations find their way in emulsions, foams and gel materials, as, for example, in mayonnaises (McGee 2003), egg-white foams (McGee 1985) and in the control of the hardness of a cooked egg (Kimball 1999), respectively. Here we will focus on the latter area, *i.e.* gel materials based on food protein.

In this short paper a few insights will be given in one specific area of protein research, *i.e.* gelation of protein solutions. The paper is merely intended to give the reader an appreciation of the innovation potential of protein gelation research.

In order to define the area of interest we start with considering a solution of proteins. This solution can become a gel when the proteins aggregate into structures that (efficiently) fill up space. See *Figure 1*.

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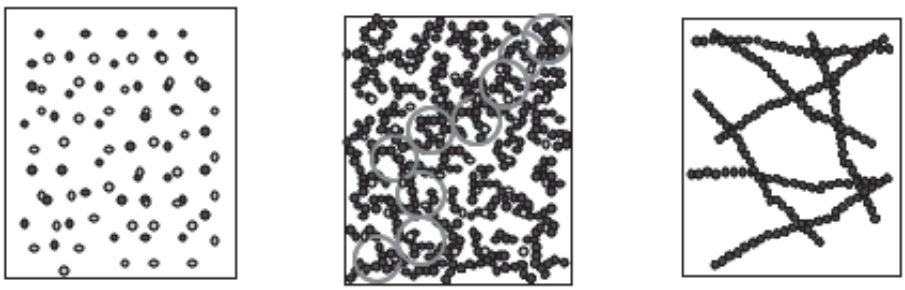

Figure 1 Proteins (left) that aggregate into clusters (branched or fibrillar), causing gelation.

In the middle part of *Figure 1* one has clusters of proteins that are relatively small (red circles). When the number of cluster-cluster contacts exceeds a certain value, the system starts to exhibit gel-like properties. In the right hand part of *Figure 1* one has very elongated protein aggregates, *i.e.* nano-fibres. Also in this case, when the number of fibre-fibre contacts exceeds a certain value, the fibre system starts to exhibit gel characteristics. In comparison, the protein fraction above which gel characteristics become apparent is much larger for a spherical cluster system than for a fibre system. In other words, a fibre system is a very weight efficient gel former.

It is known that the various types of aggregate structures that proteins can form in solution depend on temperature, pH, salt concentration, concentration of protein, and presence of co-solutes like alcohol. One thus should be able to determine the specific circumstances for a specific protein that can lead to the formation of fibrillar structures, *i.e.* which circumstances yield a weight efficient gel. Vice versa, the desire for controlling the preparation of weight efficient protein gels requires understanding of the aggregation mechanisms and according thermodynamic conditions in relation to the formation of elongated structures. It turns out that many different proteins actually are able to form elongated structures, up to micron size (see *e.g.* Aymard *et al.*, 1996, Aymard *et al.*, 1999, Veerman *et al.*, 2002, Veerman 2003a, Kavanagh *et al.*, 2000). The occurrence of elongated aggregates in general lies within a relatively large and practically accessible formulation window. This applies to many different food proteins like beta-lactoglobulin, soy-protein, BSA, ovalbumin, pea protein etc.

The fibres are different in terms of length of the fibres, stiffness, and in terms of the conditions necessary to make them (pH, salt concentration, temperature).

One interesting gel characteristic is the minimal (or critical) concentration of material to yield a gel. Once this concentration is known, one can predict for example the dependence of gel elasticity on the concentration protein (van der Linden and Sagis (2001)). The minimal gel concentration has been expressed in terms of the fibre characteristics like stiffness and as a function of salt concentration (Veerman 2004, Sagis *et al.*, 2004).

There are two important factors, which allow one to manipulate this minimal gel concentration to an extremely low value. The first factor is the following. It has been found (Veerman 2003a) that depending on the type of protein one may encounter assembly which is not reversible (after waiting for a few hours), or assembly that is reversible upon dilution. For betalactoglobulin (Veerman *et al.*, 2002) and ovalbumin (Veerman, Schiffaert *et al.*, 2003) it was found that the assembly was irreversible, while for BSA it was found to be reversible (Veerman, Heck *et al.*, 2003). For a short review on this matter see Veerman, Sagis *et al.*, 2003. This may be due to irreversible (or very unlikely to reverse within the experimental time frame) conformational changes of the protein once assembled, much like the so called skin formation of proteins that reside at for example an

air-water interface. Once assembly has taken place, and it would be irreversible upon dilution, one may use this (preparing at high concentration and subsequent dilution). In other words, the first important factor in making extremely low weight fraction gels is the nano-fibre being robust against dilution.

The second important factor is the robustness of the fibres against other treatments. In general of course the robustness of the fibres will aid to their applicability. In the current case of interest, for example beta-lactoglobulin exhibits robustness against deterioration by pH change (Veerman, Sagis *et al.*, 2003, Veerman, Baptist *et al.*, 2003). The fibres being prepared at low pH (pH=2) do not show any dissociation or dissolution upon a pH change towards pH=7.

One may now use the above two factors to one's advantage for producing an extremely low weight fraction gel as follows. The first step is to make a dilute solution of fibres at low pH. The second step is to adjust the pH to 7. The system is still a solution at this pH. The third step is to add Ca ions, inducing attractive interaction between the fibres, land this leads to gelation. Using this procedure, gels with 0.07 % (w/w) of protein have been reported (Veerman, Sagis *et al.*, 2003). This is a significant advantage over the conventional method of heating, cooling and subsequent addition of salt, all at pH 7, which yields a minimum gel concentration of 0.5%. This is one example of the innovation potential of protein nano-fibres.

Another example of using protein nano-fibres for innovation purposes may be based on the following considerations. One of the issues for making gels (based on fibres) is how the systems that will become a gel will behave under flow while being prepared. The picture which arises from the experiments (Veerman *et al.*, 2005), can be given in simple terms. The gel point is determined by how much the fibres interfere with one another. Without flow, the fibres orient themselves isotropically, *i.e.* without any preferred symmetry. Once flow is exerted on the fibre containing system, there will exist a preferred orientation of the fibres along the direction of the flow. This preferred orientation implies that one needs more fibres per unit of volume to have them interfere to the same extent as in the case for the gel point at rest. Thus, the minimal gel concentration will increase with flow. This implies that one might prepare, under flow conditions, a fibre solution which does not show gelation (under that flow), but which does exhibit gelation as soon as the systems stops flowing, when for example it has been put in a bottle.

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References

AYMARD, P., DURAND, D. and NICOLAI, T. (1996) The effect of temperature and ionic strength on the demerisation of β-lactoglobulin. *International Journal Biological Macromolecules* **19**: 213-221.

AYMARD, P., NICOLAI, T. and DURAND, D. (1999) Static and Dynamic Scattering of β-Lactoglobulin Aggregates Formed after Heat-Induced Denaturation at pH 2. *Macromolecules* **32**: 2542-2552.

McGEE, H. (2003) On food and cooking, the science and lore of the kitchen, *Scribner*, New York, ISBN 0-684-84328-5.

KAVANAGH, G.M., CLARK, A.H. and ROSS-MURPHY, S. (2000) Heat-induced gelation of globular proteins: part 3. Molecular studies on low pH β-lactoglobulin gels. *International Journal Biological Macromolecules* **28**: 41-50.

KIMBALL, C. *et al.* (the editors of "Cook's illustrated Magazine) (1999) The best recipe, Boston Common Press, Massachusetts, USA.

LINDEN, E. VAN DER and SAGIS, L.M.C. (2001) Isotropic-force percolation in protein gels. *Langmuir* **17**: 5821-5824.

SAGIS, L.M.C., VEERMAN, C. and LINDEN, E. VAN DER (2004) Mesoscopic properties of semi-flexible amyloid fibrils. *Langmuir* **20**: 924-927.

VEERMAN, C., RUIS, H., SAGIS, L.M.C. and LINDEN, E. VAN DER (2002) Effect of Electrostatic Interactions on the Percolation Concentration of Fibrillar β-Lactoglobulin Gels. *Biomacromolecules* **3**: 869-873.

VEERMAN, C., SAGIS, L.M.C. and LINDEN, E. VAN DER (2003) Gels at Extremely Low Weight Fractions Formed by Irreversible Self-Assembly. *Macromolecules Bioscience* **3**: 243-247.

VEERMAN, C., HECK, J., SAGIS, L.M.C. and LINDEN, E. VAN DER (2003) Mesostructure of fibrillar bovine serum albumin gels. *International Journal Biological Macromolecules* **31**:139-146.

VEERMAN, C., SCHIFFAERT, G. DE, SAGIS, L.M.C. and LINDEN, E. VAN DER (2003) Irreversible self-assembly of ovalbumin into fibrils and the resulting network rheology. *International Journal Biological Macromolecules* **33**: 121-127.

VEERMAN, C., BAPTIST, H., SAGIS, L.M.C. and LINDEN, E. VAN DER (2003) A New Multistep Ca^{2+}-Induced Cold Gelation Process for β-Lactoglobulin. *Journal Food Chemistry* **51**: 3880-3885.

VEERMAN, C. (2004) Properties of fibrillar protein assemblies and their percolating networks. Thesis Wageningen University, The Netherlands.

VEERMAN, C., SAGIS, L.M.C., VENEMA, P. and LINDEN, E. VAN DER (2005) The effect of shear flow on the percolation concentration of fibrillar protein assemblies. *Journal of Rheology* **49**: 355-368.