Part Two
Basic Applications

3
Nanotechnology in Food Production

Remko M. Boom

3.1
Introduction

The global supply of food is affected by a number of developments: growing world population, increased affluence of large groups, use of bioresources for fuel and chemicals production, intensification of agriculture, and monopolization of global food supply chains.

Nanotechnology may offer a way to produce very high-quality foods in a much more sustainable way, while offering better bioavailability of (micro)nutrients. An overview of current applications of nanotechnology in and around food products is given in this chapter. In addition, some examples are given on how the technology could contribute to the indicated problems: the strong improvement of bioavailability of lycopenes from nanocrystals or nanosized emulsions; the application of lipid-based delivery systems that may deliver components through the intestinal wall; the production of nanostructured plant-protein-based products; and the development of much better isolation and structuring methods.

The chapter concludes with a brief discussion of resistance to the use of the technology for food production. Even though rational arguments seem to favor the application of nanotechnology, human feelings are of prime importance in such an important subject as the supply of our food. They should not be ignored and may be sufficient reason for not applying the technology. However, this would leave the ethical issue of perpetuating our methods of food preparation at the expense of people who do not have sufficient supplies of food.

Nanotechnology in the Agri-Food Sector: Implications for the Future, First Edition. Edited by Lynn J. Frewer, Willem Norde, Arnout Fischer, Frans Kampers.

3.2 Food Production

3.2.1 Food and New Ways of Food Production

Food is among the most complex materials that we know. Biological tissues are generally structured down to the (macro)molecular level. When we convert living tissue into food, preservation ensures that our food will be safe, even if we do not consume it directly after harvesting, but can store it and prepare it later. The processing (preservation, storage, and preparation) induces changes that we often appreciate, and which may also improve the bioavailability of the nutrients inside the product.

Food is close to us. The expression "we are what we eat" is true in a literal sense, and in a metaphoric sense: our choice in the type and preparations of food, and the way we consume food, is closely connected to our personal and social identity. This implies that we do not like to consider large changes in the way we process foods. Our intuition tells us that we should use the same method as our grandparents used: what was good enough for them, must be good for us, as is claimed by Michael Pollan [1]. In this case, our intuition may not be reliable. Compared to 50–100 years ago, our food is now much safer, and no consumer would accept the risks of food consumption that were normal in the past. However, the statement by Pollan shows the power of our feelings for our food.

3.2.2 Why Do We Need New Processing and Preparation Methods?

If the introduction of new ways to prepare foods is so sensitive, why should we consider them? Why should we want to consider the use of new technology as nanotechnology for the processing of foods?

The main reason is the fact that food is becoming scarcer, expressed in higher market value. There are a variety of reasons for this. First, the world's population is still growing, and will reach approximately nine billion in 2050.

Second, at the same time, the population of large parts of the globe (mainly Asian countries, but also others) are quickly gaining affluence, which means that they are starting to consume more foods that have a high requirement for agricultural resources. For example, the production of meat is extremely costly in terms of usage of agricultural land or crops, use of water, and production of greenhouse gases by cattle. Growing feed for cattle consumes roughly 50% of all water in the USA, and 80% of the agricultural land. Cattle raised in the USA for food consume 90% of the soy, 80% of the corn, and 70% of the cereals.

With the increasing number of people in the world, and with increasing affluence in many regions, we will not be able to support meat production for all. If all the grain currently fed to livestock in the USA were consumed directly by people, the number of people who could be fed would be nearly 800 million [2].

Thus, it would make sense to try to produce high-quality, tasty protein foods directly from plants. Unfortunately, the structure of meat is so intricate that conventional structuring methods cannot nearly match nature; the quality of meat-replacing protein foods is considerably lower than the original.

A third factor is the increasing scarcity of fossil fuels. This makes the use of agricultural resources to produce biofuels (ethanol, biodiesel, and others) and biochemicals to replace chemicals from petrochemistry more attractive. However, this implies that the land used to produce these materials is not available for the production of food.[1)]

A fourth factor is that the increasingly intensive use of land leads to slow degradation of the agricultural land, via for example erosion or salination. This will slowly make the pressure on the remaining land even larger. And a fifth factor is the emergence of food as a political factor: the free, global market enables countries to swap their traditionally produced crops for high-value crops that yield more value.

3.2.3 More Efficient Fractionation of Crops

All these factors, and others, imply that it is important to consider processing technologies that can convert agricultural crops into as many useful (and edible) products as possible. The current technology is mostly aimed at the isolation and purification of a single product, or in some cases two products. Processing of sugar beet is optimized solely for the production of sugar, and the production process is only aimed at ensuring the quality of that product. The rest of the beet is thermally degraded, and is used as animal feed. In order to produce more than only sugar from such a crop, one needs to consider new ways of processing: for example, non-thermal methods, or methods that would enable very precise removal of components, while minimizing the change in the feedstock. Nanotechnologies may enable this, using for example molecular recognition techniques to isolate specific components.

3.2.4 More Efficient Product Structuring

A second issue requiring better and more sustainable processing technologies is the preparation or structuring of foods. Most people like meat as an important part of their diet – not only because of the nutritional value, but also because of the excellent taste of it. The fact that meat is a product that is fibrillar on a

1) This is at least true for the first-generation technology, which directly uses edible fractions such as starch and oil. The second-generation technology uses inedible fractions, such as cellulose and possibly lignin, which are now left on the land to fertilize and protect the soil. Using these for biofuels and biochemicals will reduce the nutrients left on the soil and may thus reduce productivity and promote land erosion in the long term.

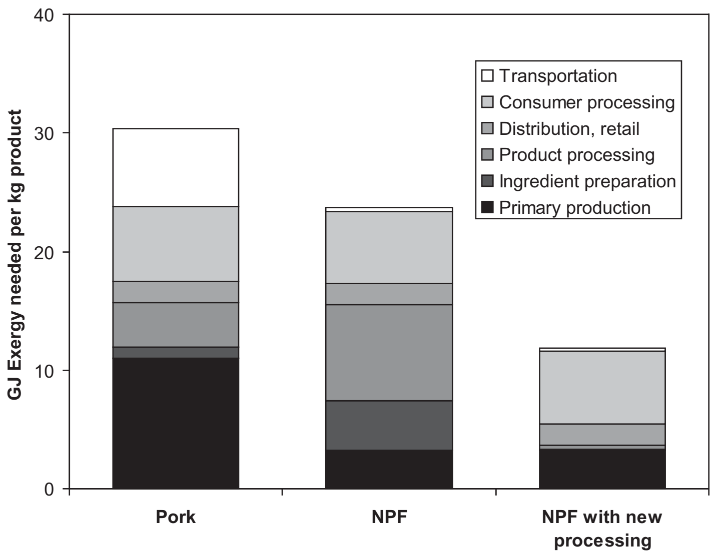


Figure 3.1 Exergy demand of producing 1 kg of pork meat and 1 kg of pea-protein-based meat replacer ("novel protein food", NPF) using conventional processing technology, and possible exergy demand when new and much better processing could be used (exergy demand for new processing estimated at 10% of the original processing). Partly based on [3].

nanometer scale is mainly responsible for this: the flavor components are only gradually released upon chewing, giving a good taste experience during the complete mastication.

One could envisage the preparation of a similarly nanostructured product, with the help of nanotechnology, but now based on plant proteins. The preparation of such a product would require considerably less agricultural resources, since the plant proteins are used directly for food preparation, instead of first being converted into animal protein (saving a factor 4–10). In addition, this direct plant-based product preparation would reduce animal suffering due to poor conditions during their life, and before slaughtering. This development would be welcomed for an additional reason. Especially in the Western world, there is resistance to the meat industry on animal welfare grounds. Nanotechnologies could offer techniques both to fractionate the biofeedstock efficiently into high-value fractions (such as plant protein) and also to nanostructure products that may replace meat in some of our meals.

The case below may serve to illustrate this. Apaiah *et al.* [3] calculated the total "exergy", that is, the potential to perform work (resources) needed to produce one kilogram of pork meat, and the same for a product based on pea protein, which was first purified from peas, and then converted into a meat-replacing product using extrusion (Figure 3.1).

The primary production of the pea-protein-based product is much more sustainable (i.e., requires less exergy), but the current technologies for purification and structuring of the protein product are not efficient. In addition, the product made with extrusion is clearly inferior in quality compared to the original and will therefore not be chosen by consumers. Overall, the new product is only marginally better in terms of sustainability and inferior in quality. It is therefore clear that new and better production technologies are required. If one could reduce the exergy needed both to fractionate the peas into valuable fractions and to structure the protein fraction into a good product, the figure shows that big steps could be taken, especially when the new technologies could lead to a product that is comparable in quality to meat, and would therefore be chosen by consumers, not only on idealistic grounds, but also because of the taste.

3.2.5 Optimizing Nutritional Value

A further driver would be to optimize products in terms of nutritional value. This is currently an important trend in the developed markets, usually referred to as functional foods. Nanotechnologies could enhance the nutritional value by increasing the rate of uptake of specific nutrients in the gastrointestinal tract. This can be done by using specific form of encapsulation, or by shaping the nutrient into nano-sized droplets or crystals.

3.2.6 Nanotechnology for Food Production?

Considering the above factors, it may be clear that, especially now, nanotechnology may play an important role in establishing a more sustainable supply of high-quality food products for the global population. However, one should not forget that food and food preparation represent more than just the rational supply of adequate food.

Perhaps surprisingly to many people, new methods for fractionation and structuring seem a logical match with biological (or sustainable) farming. A more sustainable primary production (e.g., in terms of soil use, and fertilizer and pesticide usage) combined with a more sustainable processing would yield much better sustainable food production. However, this would require a merger of the biological farming world, which currently is on a somewhat technophobic track, and the nanotechnology world, which is very much the reverse.

A last look at Figure 3.1 on the exergy demand for the preparation of protein products shows that, as soon as the fractionation and structuring steps have become much more sustainable, the main factor remaining is the preparation of the food at home. Using newly available technologies to make this process more sustainable would once more contribute to a more sustainable world. Whether this would be accepted by consumers is, however, unclear.

3.3 Nanotechnology and Food

3.3.1 What Is Nanotechnology?

Before continuing toward the developments in nanotechnology relevant for food production, it is important first to define what we mean by the term "nanotechnology", because there is a bewildering range of definitions available. While many say it is the technology that concerns itself with arranging molecules or clusters of molecules smaller than 100 nm, some argue that this could also be a description of colloid science for sizes between 1 and 100 nm or organic/inorganic chemistry for sizes between 0.1 and 1 nm.

Nanotechnology in general is characterized by a high degree of multidisciplinarity: chemical concepts such as self-assembly and molecular recognition are used, in conjunction with physical methods, such as use of atomic force microscopy, but also principles from biology, such as bilayer formation. One of the challenges for nanotechnology is to translate the level of control that, for example, organic chemistry has over the composition and conformation of molecules, to larger size scales. Next to the molecular scale is supramolecular chemistry, which concerns the assembly of a moderate number of molecules in well-defined clusters. Perhaps the ultimate challenge is to translate the concept into all levels of size, from the supramolcular to the colloidal, the mesoscopic, and all the way up to the level that humans can directly perceive, by touch, taste, or smell.

This is especially important for foods: they are characterized to a high degree by their internal hierarchical structure, that is, highly defined and well structured from the (macro)molecular (nanometers) level all the way up to the macrolevel (centimeters). While we will discuss this in more detail later on, we would like here to define nanotechnology for this chapter as the directed assembly of molecules or clusters of molecules into well-defined structures from the level of the clusters up to the larger scales relevant to direct human perception. This means that we will not strictly adhere to the size limit of 100 nm, but rather explore the hierarchical construction of product structure on several scales of size: while the fundamental building blocks are in the range of 1–100 nm, the structures built with them should be much larger in at least one dimension. The control over the structure should be there over all dimensions, from 100 nm to centimeter scales.

3.3.2 Nanotechnology in Food Production

Nanotechnology often makes use of the natural tendency of molecules to self-assemble into specifically shaped aggregates. Typical examples are the self-assembly of amphiphilic molecules (surfactants, some proteins) into micelles at lower concentrations, and into lamellar mesophases at higher concentrations. By somewhat changing the properties of the molecules, one can change the shape and morphology of the aggregates that are formed.

Lipids, such as phospholipids, self-assemble into bilayers, consisting of two layers of the molecules, with the hydrophobic parts of the molecules in between the layers, and the hydrophilic groups to the outside world. Phospholipids have the tendency to form vesicles (in fact, they form the membranes of living cells as well in this way) that are more or less spherical, and that have water inside and outside. Addition of ceramides, which are a different type of lipid, induces the bilayer to become less curved, and this induces the bilayer to form not spherical vesicles, but tubular ones. There are numerous shapes and morphologies of vesicles, but the shape is mostly governed by the molecular properties, plus the precise conditions in the suspending fluid, which determine the intermolecular forces acting between the molecules.

Vesicles and other structured aggregates can be used to encapsulate bioactive food ingredients, such as flavors, enzymes, prebiotics or even probiotics. They typically provide a barrier against the hostile environment in the stomach, and, due to their membrane-like structure, can deliver their contents to the cells of the intestinal wall. An example here is the use of cochleates, phospholipid–cation crystalline structures that form spiral lipid sheets with little or no internal aqueous space. They can encapsulate relatively hydrophobic components by taking them up in their bilayers. Cochleates that were loaded with a vaccine have been shown to give immune response after they were orally administered, which illustrates that they could deliver the active components into the cells; the vaccine in itself would have passed the intestinal wall [4].

There are many more examples of encapsulates for delivering ingredients to specific locations in the gastrointestinal walls. Since the techniques in the field of nanotechnology offer the possibility to precisely assemble a structure from individual molecules, it is clear that they can be applied for preparing these encapsulates. These encapsulates themselves are usually in the size range of 1–10 μm: smaller encapsulates would have an enormous interfacial area, complicating effective encapsulation, while for example probiotics consist of bacterial cells, which would make smaller encapsulates impossible. Encapsulates larger than 10 μm would make them perceptible to the human organoleptic system.

Vesicles can serve as encapsulates for specific ingredients to be dispersed into a food, but they are not a food matrix itself. A food matrix is characterized by the presence of structure on different scales. An example is the structure of meat, which is made of individual protein filaments only a nanometer thick, bundled into fibrils, which in turn are bundled into fibers, which in turn are bundled into fasciculi. The structure of meat cannot at this moment be imitated with plant proteins by existing technologies. Even though the flavors and the color of the meat can be easily matched, it is the complex structure that makes meat still unique.

Nanotechnology can contribute to this by providing ways to precisely position molecules into fibrils, bundling them together into fibers, and bundling them together into fasciculi-like structures. From this, it is also clear that the new technology should not stop at only arranging individual molecules into molecular strings; instead, it should enable us to arrange the clusters on several scales of magnitude at the same time. This challenge is present wherever we consider the preparation of food matrices, instead of food ingredients (e.g., encapsulates).

The use of nanotechnologies for food ingredients will be discussed, with the example of fibrils from protein that can serve as rheology enhancer even at very low concentrations, but which can also serve as building block of encapsulates. We will then continue with the discussion of the preparation of food matrices. This will be done with an example in which anisotropic structuring over a range of scales of magnitude was achieved.

There are many more examples possible in both categories; however, it is not the purpose to give a complete overview in this chapter; it rather attempts to sketch the challenges that future technology could and should aim at.

3.4 Applications of Nanotechnology in Foods

3.4.1 Sensing

Nanotechnology is associated with a range of applications in foods. One of the earliest was the development of sensors for detecting a specific molecule that is associated with the condition of a food product. One can think here of the detection of food spoilage by sensing metabolic products of spoilage bacteria, or direct detection of spoilage bacteria. While this is very challenging indeed, given the low concentrations of bacteria and their metabolic products, an even more challenging target is the detection of pathogenic bacteria, as their concentrations are even much lower. Not only would a sensor need to be sensitive to a single molecule, but even that might not be sufficient. One might need to concentrate a large amount of the product (liters), and then detect a single bacterium in the concentrate. The levels of sensitivity indicate clearly why one looks at nanotechnology to deliver these sensitivities. The sensors should probably be made in a very inexpensive way, to incorporate them into packaging of food products (see Chapter 5). This poses a further challenge to the technology, as expensive materials cannot be used, and the mode of production should be suitable for mass production, yet remain absolutely reliable, as the health of consumers may depend on it.

3.4.2 Packaging

A second application is the development of active packaging. A package with a build-in sensor showing the state of freshness of the product can be regarded as an active package, but there are other types. There has been a development in the incorporation of nano-sized particles into the packaging material itself. Incorporation of crystalline nanoparticles, such as nanoclays, can make the packaging material more impermeable to oxygen or modified-atmosphere gases such as nitrogen or carbon dioxide while improving its strength.

Another application is the incorporation of nano-sized silver particles, which give the material antibacterial properties. While the application of silver for this purpose is not new, the use of silver nanoparticles is; and it is claimed that the silver is more antimicrobially active in this form [5].

Another example is a packaging material composed of potato starch and calcium carbonate. This foam has good thermal insulation properties, is lightweight and biodegradable, and has been developed to replace the polystyrene "clam-shell" used for fast food [6].

3.4.3 Encapsulation

A third application is the nano-engineering of food ingredients and encapsulation. This is a wide field, which was initiated for medical purposes (delivery of active ingredients into the targeted area in the human body without spreading into other areas), but which may soon be a major application in the area of foods, especially for fortified or functional foods. Probiotic bacteria are, for example, at least partially inactivated by the adverse conditions in the stomach and other parts of the gastrointestinal tract. These bacteria may be protected until they have reached the large intestine, which is where they are supposed to be active. A similar argument holds for prebiotic ingredients (ingredients that cannot be directly digested by humans, but are nutrients to the probiotic micro-organisms in our gut): some of them will be digested partially even before reaching the large intestine.

An interesting development as a crossover between food and medicine is the development of oral vaccines. A major impediment in vaccination is the necessity for injection. In the developed world some groups do not want vaccination for religious reasons; in the developing world the costs involved and the assurance of hygiene with the injection is an important issue. Oral vaccination could alleviate some of these problems, but vaccines usually do not survive the conditions in the stomach, and when they do survive, they will not pass the intestinal wall, as this barrier evolved exactly to protect against the passage of pathogens. Encapsulation of the vaccine, such that it would be resistant to the stomach's conditions, and also would be delivered into the cells of the body, would make oral vaccination possible. Encapsulates that are (nano-)engineered to this end would therefore be a good development; there are developments in this area that are encouraging [4].

Apart from encapsulation, another development is the nano-engineering of food ingredients such that they become more bioavailable. This is achieved, for example, by preparing nano-sized crystals (in fact, the crystal size is not in the nanometer but in the submillimeter range) or emulsions that contain a supersaturated solution of a nutrient. The effectiveness of these routes in actual products and in the gastrointestinal tract is still under discussion, but it indicates the potential for nano-engineering ingredients to influence their destination in the human body.

Apart from nano-engineering food components, a new area of application could be the nano-engineering of food matrices. Food products in general are characterized by a very high degree of structure on a range of size scales (see also Section 3.3.1). Until now, nano-engineering has only been associated with engineering on the nanoscale. The successive arrangement of these structures on larger scales has not yet received much attention. Further, many of the nano-engineering procedures have been developed for use in a diluted environment, not in a concentrated, semi-solid matrix. However, the development of nano-engineering instruments to do that would have great value in the realm of food production.

The subjects of sensing and packaging are dealt with in different chapters and will therefore not be discussed further here. Therefore we will focus on some examples in the area of nano-engineering food ingredients. In addition, we will discuss an example of precisely structuring food matrices by combining directed assembly with well-defined process conditions, which results in the formation of a hierarchically well-defined structure on many size levels.

3.4.4 Nano-Engineering Food Ingredients to Improve Bioavailability

3.4.4.1 Nanocrystalline Food Ingredients

Many micronutrients and pharmaceutical components are poorly soluble in water, for example, lipids such as omega-3 fatty acids, flavors, antimicrobial components, antioxidants such as tocopherols, carotenoids such as β-carotene and lycopene, and also components like phytosterols. However, most foods have an aqueous continuous phase, as have the intestinal contents. The kinetics of uptake can therefore be slow, and in many cases the fraction taken up by the body is quite small. One way to improve this is by preparing so-called nanosuspensions or nanocrystals [7]. An example is the production of β-carotene nanocrystals [8]. Typical crystal aggregates with a size of 120 nm can be obtained, stabilized by gelatin for example, that contain crystallites around 30 nm (Figure 3.2). These particles can be created by forcefully mixing a solution of the carotene (in, e.g., an alcohol) into water usually containing a polymeric stabilizer. Tan and Nakajima [9] and Chu *et al.* [10] developed a method based on emulsification, where a β-carotene solution in hexane was emulsified in water containing sodium caseinate as stabilizer. By subsequent evaporation of the hexane, nanoparticles of 17 nm were created.

Co-precipitation with a biopolymer such as poly(lactic acid) can result in very stable nanoparticles, obviously at the cost of having a lower concentration of carotene, due to the presence of the biopolymer [11]. A strongly enhanced solubilization of the active components was noted, for example, by Trotta *et al.* [12] for nanoparticles of poorly soluble active components.

There is some evidence that particles in the range of 10 nm show a different structure than larger particles: their properties become different from the bulk properties. This, and the fact that their very large surface area allows their contents to be much more bioavailable, shows the potential of designing and producing

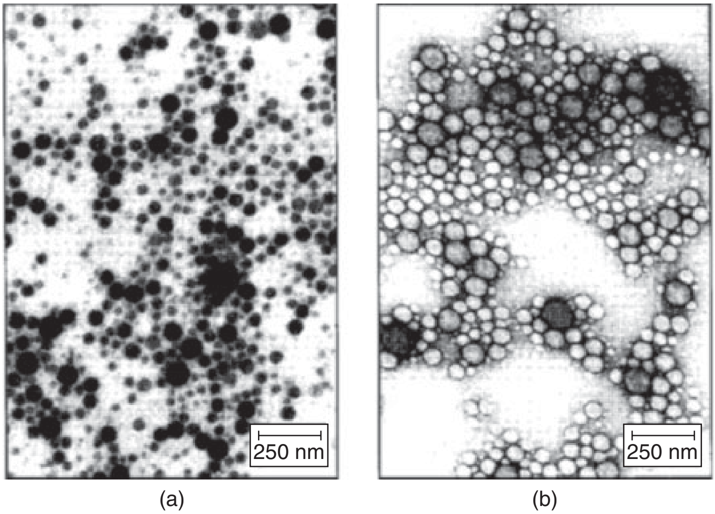


Figure 3.2 Transmission electron microscope (TEM) pictures of β-carotene nanoparticles: (a) stained with OsO_4, showing the β-carotene particles; (b) stained with uranyl acetate, showing the stabilizing gelatin coating. From [8].

particles of very small dimensions. Nanotechnology can help in the preparation of these particles, and in stabilizing them (e.g., by using microchannel emulsification methods, as has been developed by [13]), such that they have good shelf-life, and that they can be incorporated into complex food products.

3.4.4.2 Nano-Emulsions

Ribeiro *et al.* [14] have developed an interesting route for making carotene more bioavailable. Most lycopenes are completely insoluble in water and only slightly in oil. Therefore, only a small fraction of the lycopenes in our food is digested; most of it is excreted unused. The lycopene is typically at 180 °C in oil at a concentration of 15–30 wt%, which they then quickly emulsified into water using high-pressure emulsification. The resulting emulsion droplets are around 100–150 nm, which is so small that they do not contain sufficient material to form a critical nucleus; thus, the lycopene stays in solution and will be more available for digestion. It is obvious that the smaller the emulsion droplets are, the higher the lycopene concentration can be. Thus, engineering nano-emulsions would give added value. A similar system has been patented (Figure 3.3).

3.4.4.3 Nano-Engineered Protein Fibrils as Ingredient Building Blocks

Protein-Based Nanofibrils

Many proteins have the tendency to form aggregates when subjected to conditions under which they are less soluble. This may stem from a change in either solvent quality or the protein molecule itself (in fact, the two are not independent of each other: a change in solvent quality induces a change in protein conformation).

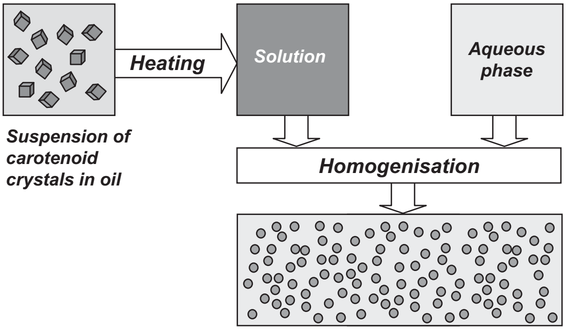


Figure 3.3 Procedure for making supersaturated emulsions of β-carotene. According to Schweikert and Kolter [15].

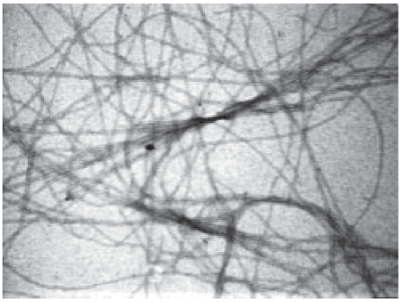
Owing to the conformational change, hydrophobic parts of the proteins become more available for interaction with other proteins. These interactions then lead to the formation of aggregates.

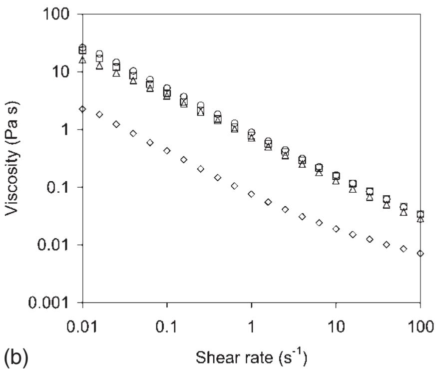
Aggregation due to protein unfolding (denaturation) is well known, and is one of the fundamental mechanisms underlying the preparation of food: boiling or frying an egg results in solidification of the egg white, due to the aggregation of the protein into a fine, random network. Meat, when cooked, becomes firmer, due to the (partial) denaturation of the proteins.

By selecting the conditions, one can tune the properties of the resulting solution. For example, when making a sauce or ice cream, one often heats the (egg protein) solution to a specific temperature (around 70°C): at this temperature, the egg proteins do not denature completely, the interactions between the molecules remain mild, and the consistency of the solution becomes more viscous, without leading to large-scale aggregation and hence flocculation (curdling).

By partially denaturing a protein solution at conditions of low ionic strength and pH, one can make the aggregation process highly specific. At low pH the protein molecules have a high charge, while the low ionic strength ensures that the molecules will repel each other, although the partial denaturation enables hydrophobic interaction. Once at very specific spots the molecules will have the chance to interact and form a bond. This gives rise to string formation in the form of fibrils. The precise method of formation of such fibrils is not yet completely understood, but it is generally agreed that β-sheet formation plays an important role. Recent findings indicate that partial hydrolysis is necessary for some proteins to form fibrils [16]. After some time, the bonds between the individual molecules, that were initially reversible, become irreversible.

A system known for its tendency to form fibrils is β-lactoglobulin, a protein from whey. One typically heats a diluted solution of it (1–5 wt%) for 6–24 h at 80 °C and pH 2 and low ionic strength. This results in fibrils 1–8 μm long with a diameter of 4 nm, representing a thickness of one or two individual protein molecules (or significant fractions of them). The resulting fibrillar solution shows strongly increased viscosity (up to 10 000 times and more; see Figure 3.4) and shear thin-





(a) (b)

Figure 3.4 (a) TEM picture of fibrils from β-lactoglobulin, made by 2 h heating at 90 °C while shearing at 200 s^{-1}. (b) Flow curves for solutions of 5.2 wt% whey protein prepared at different shear rates: ◇ 0 s^{-1}, □ 168 s^{-1}, ○ 337 s^{-1}, △ 673 s^{-1}. The viscosity of the solvent without protein is 0.001 Pa s. From Akkermans *et al.* [16].

ning behavior, assumed to be caused by percolation of the fibrils, forming a network, which is successively destroyed when applying shear.

A recent finding was that the self-assembly kinetics of this process can be influenced by applying mild shear during the assembly process [16]. Figure 3.4 shows that application of shear increases the viscosity by an order of magnitude. This is caused by a much higher yield. A higher shear rate does result in more fibrils, but this is not evident in the viscosities: at higher concentrations, the fibrils cannot assume a random orientation any more due to steric hindrance and they form nematic, liquid-crystalline domains, in which they align and hence have less influence on the viscosity. This was supported by the observation of birefringence in the solutions, indicating fibril alignment in the solution.

It is not clear how flow positively influences the self-assembly process. It may speed up the diffusion of proteins (or their fragments) toward the active tips of the fibrils. The Peclet number calculated with a shear rate of 168 s^{-1} is, however, only 3×10^{-5} (using a diffusion coefficient for β-lactoglobulin of $9.7 \times 10^{-11}\,m^2\,s^{-1}$ and a molecular size of 4 nm), which indicates that direct influence of the flow on the mass transfer should not be expected. However, when the fibrils start to form more densely packed, liquid-crystalline domains, diffusion from the surrounding solutions into these domains may become limiting. It is clear indication, however, that even a macroscopic parameter such as shear flow may influence a molecular process such as self-assembly of a protein. The fact that the fibrils start to line up into liquid-crystalline domains also indicates that larger-scale structure may be formed. Aligning these domains by applying shear flow (evident in the shear thinning behavior) may further yield options for forming the fibrils into a matrix, when one could fix the fibrils while aligned.

Using Nanofibrils for Microstructure Assembly Apart from their use as an ingredient to modify the rheological properties of a product, fibrils may have further use.

At low pH, the fibrils themselves are highly positively charged. This enables assembly of the fibrils into larger structures using electrostatic interactions. Recently, Sagis *et al.* [17] used this for structural reinforcement of encapsulates (Figure 3.5). They used emulsion droplets stabilized by β-lactoglobulin at low pH, and exposed them alternately to high-methoxyl pectin and fibrillized whey protein isolate (consisting mainly of β-lactoglobulin). The pectin was negatively charged and therefore formed a nanometer-thin layer on top of the positively charged droplet surface. The positively charged whey protein isolate fibrils then formed a layer on this negative surface, and so forth. Shells with only a few layers do not have much mechanical strength; however, the application of six layers gave the encapsulates considerable strength.

While the fibrils were created by using the combination of specific hydrophobic interaction with general electrostatic repulsion (to reduce random aggregation), the larger aggregates could be assembled by using electrostatic attraction.

There are many examples of using self-assembled or directionally assembled aggregates for encapsulation.

3.4.5
Preparation of Food Matrices

In Section 3.2.4 we discussed the relevance of being able to create food matrices with a well-defined hierarchical structure. Especially matrices with a fibrillar structure would be of relevance (e.g., to act as meat-replacing protein foods).

In principle, one could use the fibrillization procedures as discussed above for such a purpose. Recently, Akkermans *et al.* [16] showed that fibrils could be formed not only from proteins of animal origin, but also from proteins of plant origin: both soy glycinin and soy protein (a mixture of mostly glycinin and conglycinin)

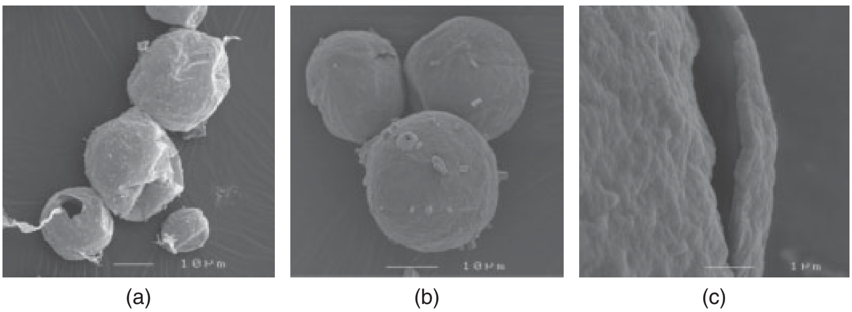


Figure 3.5 Shells made with alternating layers of high-methoxyl pectin and whey protein fibrils. Owing to the electrostatic interaction, the fibers do not stand out but are attached to the surface over their whole length. The fibres strengthen the encapsulates considerably. (a) These shells have only two layers (i.e., one pectin, one fibril layer). (b) Here the shells have six (three pectin, three fibril) layers. (c) A close-up of the wall of one of the six-layered shells, indicating a typical shell thickness of around 50 nm. From [17].

were shown to form fibrils. However, the fibrillization procedure is only effective with dilute systems: one cannot use concentrations higher than a few percent of protein: the system will form a dense gel already before fibrillization, while shear flow during fibrillization results in much shorter fibrils.

Formation of the fibrils at low concentrations, and subsequent concentration into a highly concentrated product, would not be sustainable: very large volumes would be necessary, and an excessive amount of (acidified) water would be needed. The same is true for many other methods to form fibrils; for example, wet spinning and electrospraying only yield low volumes of fibrils; thus one would need very large equipment to produce industrial volumes of protein product.

It is therefore important to start with systems having the same range of concentrations as would be required for the ultimate product. For a high-protein food such as meat, this would be in the range of 20–30 wt% of protein (raw meat).

Conventional technology in this range is the use of extrusion technology. The product ingredients are brought together, heated and compressed, and forced through a small die. Many protein-based systems will form a fibrillar structure under the influence of the extensional flow related with the focusing flow just before the die. The product is however not finely fibrillar, while the process consumes ample amounts of energy due to the intense process conditions. Other methods like wet or dry spinning produce limited numbers of fibers at the same time, and thus have limited scope for upscaling. An interesting process is the one used for the production of Valess, a product based on casein. By mixing the casein with a carbohydrate, and subsequently solidifying the carbohydrate phase, one can produce a product matrix that is fibrillar, down to a level of tens of micrometers. Even though this is a successful product, it cannot yet compete directly with meat, as its structure is still several orders of magnitude coarser.

A new process was proposed by Manski *et al.* [18, 19]. A 30 wt% calcium caseinate dispersion in water was subjected to plain shear flow in a special device. A cross-linking enzyme was added, solidifying the dispersion while it was being sheared. Under the right conditions, the caseinate was found to align into long fibrils with diameter of around 100–150 nm. The shear stresses applied were relatively low, so the process can be considered mild. The solid fibrillar product closely resembled meat in terms of structure (Figure 3.6).

The fibrillization was ascribed to what one might call directed self-assembly. Calcium caseinate is present in the dispersion as micelles of size around 125 nm, which is big enough to be aligned by the shear force. It is well known that particles in (non-Newtonian) suspensions have the tendency to align under plain shear flow (e.g., [20–22]). The calcium caseinate micelles have the tendency to cluster or "stick together", due to the divalency of the calcium ions, which can serve as a bridge between them. This is probably important in the alignment process. By concurrent cross-linking of the aligned micelles with the help of an enzyme, one can fix the strings of micelles, effectively creating a (soft) solid fibrillar matrix, because of the high concentration in the system.

The proposed mechanism was supported by the rheology of the dispersions. Calcium caseinate dispersions showed evidence of structure formation under

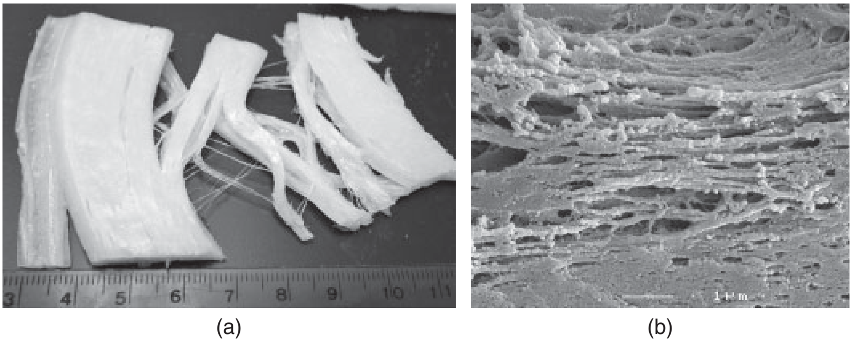


Figure 3.6 Hierarchically fibrillar protein structures made by shearing a 30 wt% dispersion of calcium caseinate that is slowly solidified by crosslinking with transglutaminase [18, 19]. The structure was shown to consist of fibrils of ~100 nm diameter, packed into bundles of ~100 μm thick (b), which themselves are arranged into larger-scale bundles evident in (a).

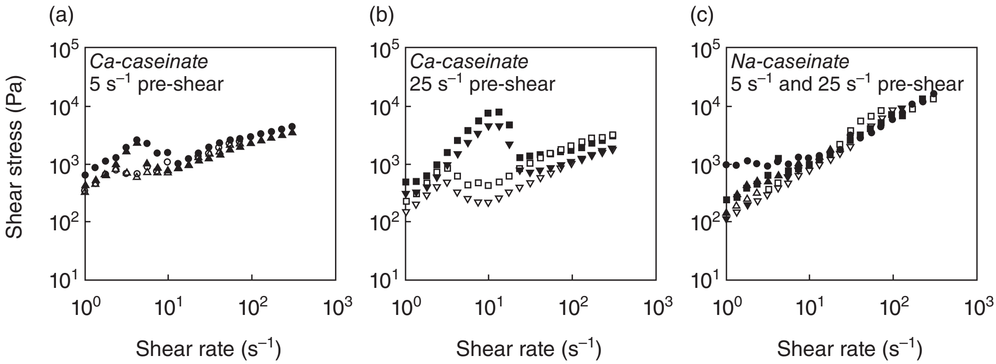


Figure 3.7 Rheology of calcium and sodium caseinate dispersions: closed symbols, up-sweep; open symbols, down-sweep; circles and squares, using pre-shearing directly before measuring; triangles, using pre-shearing, a rest period of 7 minutes, and then measuring. From Manski *et al*. [23].

shear flow, but sodium caseinate dispersions did not (Figure 3.7). Sodium caseinate micelles are smaller, therefore less easily aligned, and show no tendency to cluster or aggregate.

This example shows that a combination of self-assembly (well-defined protein clusters such as micelles) and positional assembly (alignment under shear flow) can yield better-structured food matrices. It seems probable that the range of examples can be extended when well-defined process conditions are used.

3.5 Concerns about Using Nanotechnology in Food Production

3.5.1 Risks of Nanotechnology

Nanotechnology in foods is usually associated in the media with the use of nanoparticles in foods, especially nanoparticles from non-biological origin, such as buckyballs (C_{60}) or carbon nanotubes. It is not yet clear how human physiology responds to these types of component, and one should therefore be very careful with application of this category of components.

Nanostructured components of biological nature such as fibrillized proteins (through β-sheet formation) may be safer, as these structures occur in nature, and thus our body has probably evolved in the presence of these components. The fact that some diseases seem to be correlated with the occurrence of amyloids may however warn us to be cautious. One should point out that there is no evidence that indicates that the consumption of, for example, fibrillized soy protein would in any way stimulate the formation of amyloids in the human body.

Another development is the use of nanoparticles of natural components that are smaller than those that occur in nature. It is unlikely that the use of nano-sized crystals or lycopenes would in any way be dangerous: these crystals have the tendency to disappear by dissolution quicker than the natural crystals do. Similarly, nano-sized emulsions will be broken down or absorbed very quickly, as they are less stable than larger emulsions. Emulsions with droplet sizes much smaller than 1 μm have been used extensively without any indication of any risk related to the droplet size.

Lipid-based encapsulates such as cochleates seem to be able to deliver deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) through the intestinal wall, and therefore may be a bigger risk factor: any contamination by, for example, a virus or other harmful components might also be transported through the intestinal wall. These components may therefore have some risk involved.

The fibrillized food matrices as described will almost certainly be safe, as the structure is natural in size scales, and all the components are completely food-grade. The process merely influences the spatial arrangement of the elements that were present together.

So, the emergence of nanostructured food components shows no reason for caution any more than the development of any new component would merit, but of course every caution needed should be taken.

3.5.2 Rational Argumentation Versus Human Feelings

Many of the more popular discussions on the use of nanotechnology evoke statements such as the one of Pollan [1] (see above): we should use the same method for food preparation as our grandparents used – what was good enough for them, must be good for us. This type of statement is not part of a rational discussion, but has an intuitive and emotional background.

Even though, on a rational basis, there seem to be ample reasons to consider the use of nanotechnology for food production (surrounded by reliable precautions), the emotional side to it is just as relevant. Emotional repulsion from application of new technologies for our food is sufficient reason not to apply them, when there is no issue in obtaining sufficient food of high quality. The global situation however is different. Not considering the use of better technology may ultimately imply the deprivation of adequate food supply for many humans on our planet.

The application of the new technologies will therefore depend on the severity of the problems surrounding food and bioproduction. It seems important that engineers and scientists at least work on sustainable methods to produce high-quality food, at least to enable the societal choice between the different alternatives that will be apparent in the future.

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