Peer-reviewed | Manuscript received: December 19, 2011 | Revision accepted: February 10, 2013

Nanotechnology in the Food Industry

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Summary

Extreme surface-to-volume ratios of the particles are characteristic of nano-scaled materials. Compared with macro-scaled materials, this results in entirely different physical and chemical properties. These are the basis of the special applications and also the cause of the possible risks with nano-materials. The present overview deals above all with the four main areas of use in the food industry: packaging, process technology, microbiology and ingredients. On the one hand, we must distinguish between inorganic and organic nano-materials and, on the other hand, between the direct and indirect use of nano-materials in connection with foodstuffs. Besides research investigating new applications, toxicological investigations, for example for influencing bioavailability and the metabolism of nano-scaled substances, are required. Due to the special material properties, this also demands new analytical procedures.

Keywords: Nano-scaling, food technology, bioavailability, toxicology, food analysis

Introduction

The science of applied nano-technology is concerned with the characterisation, production and targeted modification of naturally-occurring or synthetically manufactured materials at the atomic, molecular or colloidal level [1]. While the original definition of nanotechnology referred to all structures having a characteristic size of less than 100 nanometres (10^{-7} m) in at least one dimension (see glossary), in recent years the definition of nano-structured materials has been limited to materials which show entirely new physical and chemical properties and which therefore differ considerably from macro-scaled materials with the same chemical structure [2].

Citation:
Weiss J, Gibis M (2013) Nanotechnology in the food industry. Ernaehrungs Umschau international 60(4): 44–51
This article is available online:
DOI: 10.4455/eu.2013.011

These divergent properties of nano-scaled materials are frequently the result of extreme surface-to-volume ratios of the particles and are the reason why boundary layer phenomena are decisive for the physical and chemical properties of these materials [3]. Thus, for example, many nano-scaled materials have a substantially higher chemical reactivity and entirely different interaction behaviour with electromagnetic waves than macroscopically structured materials. These different properties necessitate special toxicological considerations [2] (◆ Overview 1). At the same time, however, these represent the basis for special industrial applications and possible advantages for the consumer. Besides machine production, electrical engineering, textile finishing and pharmaceutics, this also applies to the food industry [1, 3–8]. The present contribution will focus primarily upon applications in the food industry.

Indirect applications of nanotechnology in foodstuffs

Four principal potential applications of nanotechnology in which intensive research is currently in progress can be identified in the food industry: packaging, process technology, microbiology and ingredients (◆ Figure 1) [5, 8]. Its use in foodstuffs can be classified as "direct" or "indirect".

Direct use refers to the integration of nano-structured substances and materials in foodstuffs and must also be declared as such.

Indirect use includes e.g. the use of nano-structured materials in packaging technology [4] or the use of efficiently nano-structured catalysers for the hydration of fats [9, 10]. It can therefore be expected that the majority of applications of nanotechnology are concerned with its indirect use.

It should be noted that in indirect use no nano-structured materials are directly incorporated in the foodstuff matrix. However, the materials come into contact with the foodstuff [2, 11]. The reason is that the materials must be in contact with the foodstuff in order to fulfil a certain function benefitting the foodstuff or

the foodstuff production, such as the catalysed hydration of fats with low trans-fatty acid contents. Special attention must therefore be given to the possible transfer of nano-structured materials to foodstuffs [2]. Such a transfer must be excluded in order to use these materials as contact materials or auxiliary process materials.

- Up to now only little information is available on the subject of the specific toxicokinetics/toxicology of nano-scaled substances. The investigations of inorganic substances (metals, metal oxides, and titanium dioxide) far outnumber the investigations of organic nano-structures.
- The substantially different reactivity compared with macro-scaled substances creates analytical problems (detection, characterisation and measurement). Interactions with the surrounding matrix (e.g. foodstuffs) must therefore be taken into consideration.
- The tiny particle size and the high activity in small concentrations demand very time-consuming analytical procedures. Sufficient reference methods and reference substances for calibration are, however, not yet available.
- The tiny size and the substantially different reactivity can result in different absorption and distribution/accumulation behaviour in the organism (passage of the blood brain barrier, placenta mobility, membrane solubility, interaction with transport molecules, etc.).
- The knowledge of biotransformation and the elimination of nano-substances remain very limited.
- Nano-scaled substances can have catalytic properties or act as a crystallisation seed and influence the correct protein folding (tertiary or quaternary structure).
- The applicability and information value of existing toxicological test procedures for use with nano-materials must be investigated. Thus, the foodstuff additives titanium dioxide (E 171) and vegetable carbon (E 153) in nano-scaled form are known to cause damage to the DNA, which is not the case with macro-scaled forms.
- The activities of nano-scaled substances cannot be extrapolated from macro-scaled or dissolved reference substances.
- Due to the lacking declaration obligation until now, knowledge of the type and scope of nano-material applications is too limited, making the estimation of the exposure for consumers difficult.

Overview 1: **Toxicological aspects of nano-scaled materials** (after [2])

Use in packaging technology

For a number of years nanotechnological approaches have been used to improve the functional properties of packaging materials [4, 12–14]. The principal focus is upon the development of new packaging materials which prolong the shelf lives of foodstuffs as a result of improved protective functions (◆Figure 1). Thus, for example, the inclusion of impermeable nano-scaled bleaching earth mono-layers incorporated in a compound following chemical modification can reduce gas exchange (oxygen, nitrogen, carbon dioxide, etc.) with the environment [8].

Besides improving the barrier function of the packaging material, the incorporation of nano-scaled structures also substantially improves the mechanical properties of the packaging, such as the abrasion resistance [4].

Nano-scaled carrier systems for anti-oxidants and preservatives incorporated in packaging materials can transform "passive" packaging to "active" packaging and thus prolong the shelf life of the foodstuffs [15, 16]. The incorporation of nano-sensors and tracers which, with their increased sensitivity, can recognise and indicate even the smallest changes in the packaged goods (e.g. rotting or interruption of the refrigeration chain), make possible intelligent packaging able to indicate the presence of oxidation products or microbiologically produced secondary metabolites which can impair the quality or safety of the foodstuffs [6, 15, 16].
In addition, nanotechnology allows the modification of other functionalities, such as the processability of packaging machines, optical properties (transparency), biological degradability, wetting behaviour and the application of heat in microwave ovens.

Use in food processing technology

At the present time nanotechnology finds only limited use in food processing technology, although this situation could rapidly change. New research results which show dramatically different catalysation behaviour for nano-structured metal particles offer particular promise [9]. Accordingly, it would then be possible not only to accelerate reactions by a corresponding design, but also to control the reaction paths and consequently the concentrations and types of the products generated.

Specific applications include the reduced formation of trans-fatty acids during the hydration of fats and the accelerated production of protein-carbohydrate conjugates for the stabilisation of foodstuff emulsions.

Due to their very high surface-to-volume ratios and their well-defined structures, many nano-structures are particularly well suited for use as immobilisation systems for enzymes [1, 17]. With the process known as "electrospinning" the application of a high voltage to a polymer solution can, for example, produce nanofibres with more or less porous core-shell structures [18, 19]. With this process it is possible to define the size of the core material, which can consist of enzymes, thus allowing precise control over the material transport of the educts and the enzymes, as well as to remove the products without loss of the enzymes.

Besides their use in catalysis, nanotechnological approaches have also led to improved separation processes [20]. Thus, the modification of the surfaces of nano-structured filter materials results in better separating capacities and better specificities for the materials separated.

Use in food microbiology

In the area of food microbiology (cf. ◆ Box 1) there are two different fields of application (◆ Figure 1): (1) developing nano-sensors and (2) improving the effectiveness of preservatives, i.e. materials which inhibit the growth of or kill microorganisms [15, 19, 21–23]. However, as in this case nano-structures must be added directly to the foodstuffs the latter application must be allocated to the direct use of nanotechnology in foodstuffs.

Direct use of nanotechnology in foodstuffs

The direct application of nano-structured materials in foodstuffs [5, 7, 11, 24–29] is of particular interest. Possibilities exist here for the incorporation of functional foodstuff ingredients, such as fragrances, colouring agents, anti-oxidants, preservatives (see above) and biologically active components (vitamins, omega-3 fatty acids, polyphenols, etc.) in nano-structured particles or fibrous structures. In this respect, the possible advantages of improved bioavailability must be weighed against the risks of a possible overdosis (see below). This requires further research. Thus, the modification of the pharmacokinetics of biologically active materials for use in these carrier systems could require rethinking of the permissible concentrations, as the risk of over-dosage during consumption then increases [7]. On the other hand, it is possible that the use of nano-scaled substances reduces unwanted substances, such as salt, fat or sugar, in foodstuffs.

The requirement for the use of new material structures as functional foodstuff ingredients is justified by their frequently inadequate physical and chemical stability in foodstuff matrices [26, 27]. Due to their chemical structure these ingredients can be optimally incorporated in the existing biological structures (cells and cell compartments such as vacuoles or membranes) of the starting material, e.g. fresh fruit or vegetables and thus stabilise these. On the other hand, in processed foods these materials are subject to rapid oxidation or polymerisation reactions following their incorporation, detrimentally affecting the quality of the respective foodstuff. The incorporation of components in nano-structured carrier systems can greatly reduce the extent of such destabilising reactions and prevent the physical separation of the components from the foodstuff matrix [29].

Contrary to inorganic nano-materials, which are predominantly used in chemistry and the material sciences, for direct use in foodstuffs only organic substances approved for use in foodstuffs which are either broken down in the human body analo-

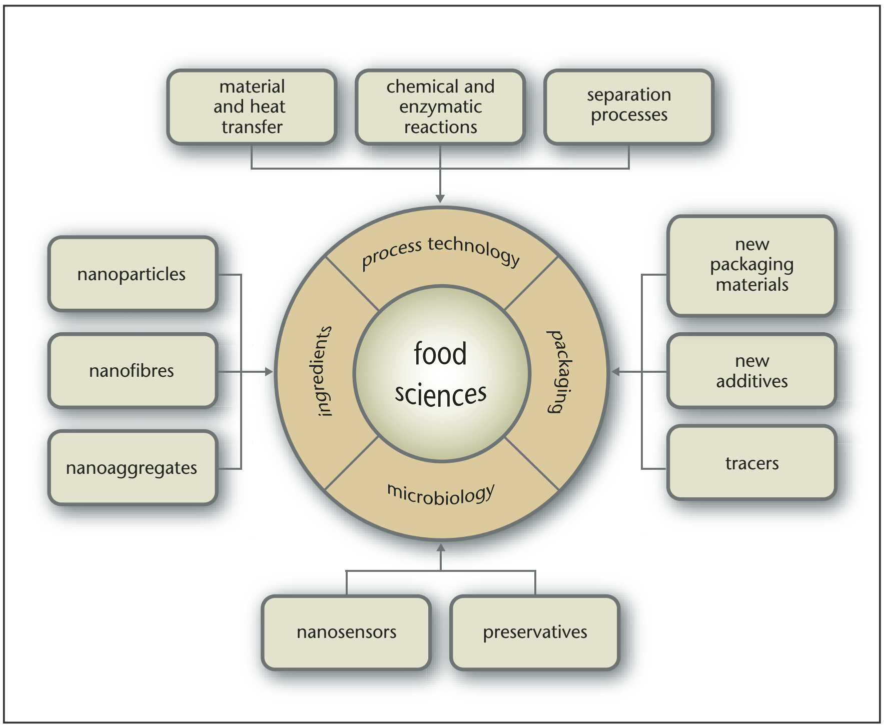

Figure 1: **Schematic overview of the potential areas of application for nanotechnology in the food industry**

gously to conventional ingredients or directly eliminated come into question.
The justified toxicological reservations in respect of inorganic nanoparticles, such as silver or titanium dioxide, mentioned overproportionately in the media preclude their direct use in foodstuffs [2].

The significant improvement in the detection of pathogenic bacilli or toxins produced by pathogenic bacilli which pose a danger to the safety of the foodstuff is one of the most important indirect applications of nanotechnology in microbiology [6]. The superior qualitative and quantitative detection of microorganisms and their metabolic products is once again based upon the development of new materials, of which quantum dots, nano-composites, conductive polymers, Langmuir-Blodgett films and carbon nanotubes deserve particular mention.

Here, above all the fact that nano-scaled detection systems have extremely high sensitivities and very fast detection speeds is exploited. Thus, nano-sensors allow the *in-situ* determination of concentration with a detection sensitivity several orders of magnitude superior to that of conventional determinations and which, in the most favourable cases, yields a result within only a few seconds. In the case of micro-biological organisms, for example, cell concentrations of 10^{-2} CFU/ml can be determined. For oligosaccharides the minimum concentrations which can be measured are of the order of less than 10^{-14} M, corresponding to around six molecules/µl.

Box 1: **Nanotechnology for the detection of pathogenic bacilli and toxins**

Top-down and bottom-up food production

One of the basic principles of nanotechnology is the manufacture of structures by the "bottom-up principle" (◆ Figure 2). Contrary to the top-down principle, in which raw materials are introduced to the food product via conventional, above all, mechanical processes, such as mixing, grinding, separation and agglomeration, with the bottom-up principle structures comprised of individual molecules are systematically transformed to nano-structures and micro-structures. These then serve as the basic systems for the structure of the final food product.

A key principle of bottom-up is the so-called self-assembly. This describes the spontaneous formation of ordered thermodynamically stable structures without the application of classical mechanical methods. An example of this is the formation of emulsifier micelles [21]. These colloidal particles are formed as soon as active boundary-layer amphiphilic emulsifier molecules are introduced in sufficiently high concentrations to a polar solvent, such as water. The redistribution of the hydrophobic emulsifier monomer groups into the inside of a spherical or cylindrical aggregate minimises the thermodynamically unfavourable interactions between the polar solvent and the hydrophobic groups and maximises the thermodynamically favourable interactions between the hydrophilic emulsifier head groups and the polar solvent. The system is therefore able to achieve a state in which the free energy is less than in the original disordered state.

Exactly which structures can be formed by self-assembly depends upon the chemical structure of the individual molecules and the ambient conditions (pH, temperature, ionic strength and pressure). Controlling the self-assembly requires a vast knowledge of the molecular and colloidal interactions (such as van der Waals, Coulomb, steric, depletion, hydration and hydrophobic interactions) [8].

Micelles are also capable of assimilating covalent components, for example anti-microbial ethereal oils having low solubility (also referred to as solubilisation) into their interior structure. Assimilating these substances enlarges the micelles, which are then referred to as loaded or swollen micelles or as microemulsions [30].

In a further step it is necessary to develop an understanding for such processes as phase separations, network formations and phase transitions which transform the self-assembly structures produced, most of which have sizes of the order of a few nanometres, to larger structures. Only then can these be further processed by classical methods to food products. It can therefore be expected that in future a combination of bottom-up and top-down foodstuff production will be increasingly employed in food technology.

Functional nano-structures in foodstuffs

Both the bottom-up principle described above and the top-down principle can be utilised for the production of the functional nanostructures used in food products. Fundamentally, one can distinguish between simple particulate nanostructures, so-called nanoparticles, nanofibres and complex nanoaggregates (◆ Figure 3) [5, 8, 24].

The nanoparticles used are predominantly in the form of nano-emulsions, i.e. oil in water or water in oil dispersions, in which the mean droplet sizes are well below 100 nm. Because of their tiny droplet size nano-emulsions do not tend towards

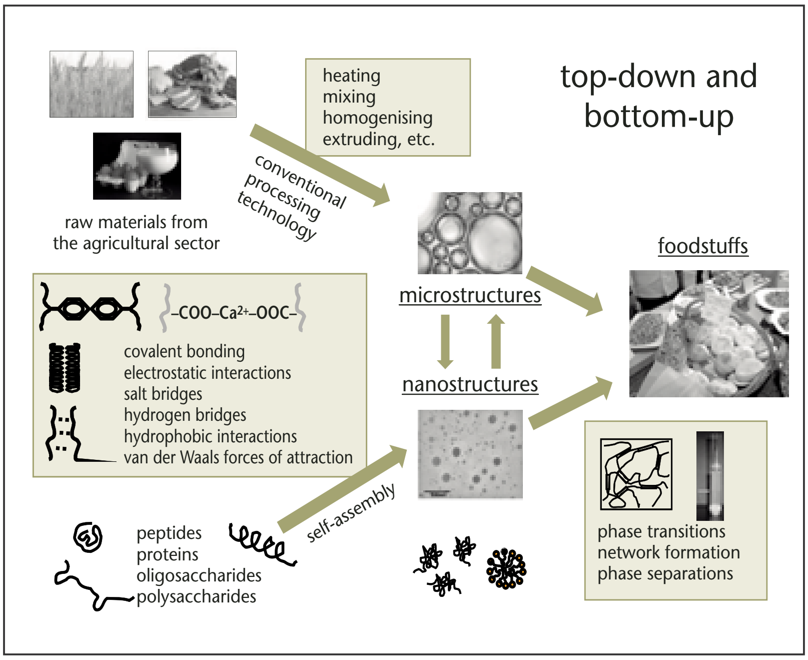

Figure 2: **Top-down and bottom-up approaches for the production of foodstuffs**

creaming, are transparent and – when charged emulsifiers are used – frequently have an unusually high viscosity. Due to their transparency concentrated fragrance nano-emulsions in which ethereal fragrance components are encapsulated in an oil in water emulsion and which also have an anti-microbial effect in addition to the fragrance, for example, are well suited as additives for colourless beverage systems [31, 32].

The production of liquid nano-emulsions from the melting of fats which crystallise following cooling results in so-called solid lipid nanoparticles. These are especially suitable as carrier systems for bio-active substances [29, 33, 34]. Thus, for example, beta carotene or vitamin D_2 can be encapsulated in solid lipid nanoparticles in order to protect them from light and oxidation. Here also, their transparency makes them suitable for use as nutritional supplements in colourless beverage systems [34, 35]. Due to their surface-initiated crystallisation with triazyl glycerides, solid lipid nanoparticles possess unusual fat crystal structures, which are particularly well suited for protecting chemically unstable bio-active substances against oxidation processes.

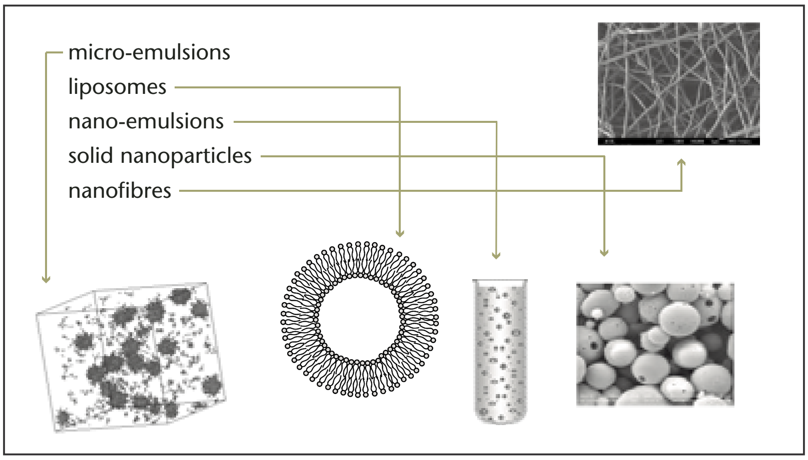

Figure 3: **Functional nano-structures which come into question for use in foodstuffs**

In principle, the bioavailability of many biologically active substances in foodstuffs is low, for example as a result of their bonding and incorporation in cell compartments and structures [33]. Both nano-emulsions and solid lipid nanoparticles are capable of improving the bioavailability of many biologically active substances, as nanoparticles possess large surfaces in relation to their volumes, enhancing their absorption in the intestinal wall and allowing effective micellation with the bile salts. Furthermore, nanoencapsulation also suppresses a number of unwanted interactions with the foodstuff matrix which reduce absorption in the intestinal tract [26].

Alternatively to fat-based nanoparticles, it is also possible to produce nano-structured hydrogel particles, for example by complex coacervation or controlled biopolymer phase separation in combination with high-pressure homogenisation processing. Nanogel particles are suited above all as carrier systems for hydrophilic substances, as a component of multiple emulsions (e. g. water in oil in water) and for the formation of complex aggregates which can produce new textural properties in foodstuffs. In this way, retinol has been protected in silicone particles with multiple emulsions (W/O/W) and by means of the sol-gel method against light, heat and oxygen in order to allow its use in pharmaceutical products and nutritional supplements [36].

Finally, the self-assembly systems referred to above, which – besides the micelles – also include the phospholipid double-layer liposomes also belong to the nanoparticles [23, 28, 37].

In addition to particulate systems, current research is increasingly focussing upon the production of nanofibre structures (◆ Figure 3) [18, 19]. These can be produced by either controlled directionally oriented growth or by electrospinning (see above) from polymer solutions.

At the present time, the most intensive research is concerned with the formation of mixed aggregate structures consisting of individual simple nano-structures [26, 37, 38]. These include, for example, the colloidosomes, particulate core-shell structures in which the shell is comprised of individual or fused nanoparticles surrounding a liquid or a solid core. Such systems serve for the protection of bio-active substances or the targeted release of bio-active substances, for example omega-3 fatty acids [39].

Due to their superior stability in respect of storage and freezing, emulsions with multiple emulsifier layers built up utilising the layer-by-layer principle by the sequential adsorption of differently charged emulsifiers already find use in the food industry (e.g. deep-frozen ready-made meals, dried soups and sauces in powdered form) [27].

Starch-based spherulites are being investigated as a new form of carrier systems for biologically active substances. Liposomes, on the surface of which nanogel particles produced by complex coacervation are anchored by electrostatic forces of attraction, enable vastly better control over the release of encapsulated substances [34].

Glossary:

milli, micro, nano: These units are, from left to right, one thousandth of the preceding unit: 1 m = 10^3 mm = 10^6 m = 10^9 nm

The traditional **classification of nano-structures** distinguishes between "punctiform" (less than 100 nm in all three dimensions, e.g. nanocrystals or emulsions), linear (nano-scaled in two dimensions, e.g. nanofibres, nanotubes and nanogrooves), and layer structures (nano-scaled in only one dimension). Pores can be described as "inverse" nano-structures.*

The exceptional **reactivity of nanoparticles** results from the extreme surface-to-volume ratio: For a particle with a diameter of 10 nm around 20 % of all atoms or molecules are located on the surface. For a particle with a diameter of 1 nm this can even be more than 90 %.* The enhanced reactivity is observed up to an order of magnitude of 300 nm.

The liposomes familiar to the general public for the most part from the cosmetics and pharmaceutical sectors have sizes between 50 and 1,000 nm and, according to this definition, therefore are partly above the size for nano-structures.

bleaching earth mono-layers, nano-scaled: also known as betonite mono-layers; betonites have a highly adsorbent and catalytic character.

Immobilisation = here: fixation of enzyme molecules by bonding to a carrier or by incorporation in a (nano-scaled) matrix structure in order to prevent transfer to the foodstuff.

CFU: colony forming unit, a measure of the number of bacilli in a sample

amphiphilic: substance which is both hydrophilic and lipophilic, used e. g. as an emulsifier

coacervation: formation of extremely tiny droplet-shaped structures in mixtures of different macro-molecular substances (e. g. proteins)

spherulites: polycrystalline polymer structures with a radially symmetric form and anisotropic character, i. e. with different physical and chemical properties along the different spatial axes

quantum dots: nano-structures which incorporate semiconductor material fractions

nanocomposites: solid multi-phase systems in which nano-materials are integrated in polymer structures

conductive polymers: electrically conductive plastics

Langmuir-Blodgett films: Layers comprised of one or more single layers of organic material (mostly amphiphilic substances) transferred by immersion in a liquid from the boundary layer to a solid material

carbon nanotubes: tubular nano-structures. Because of their good conductivity and their affinity for biological molecules these are increasingly employed in the design of biosensors [1]. Carbon nanotubes coated with antibodies, capable of conducting weak electric currents virtually loss-free enable the detection of the bonding of individual molecules, whereby the surface can be quickly regenerated with relatively little effort following measurement.

self-assembly: Thermodynamic processes in which a structural or pattern formation occurs without external influences as a result of the existing molecular and colloidal interactions.

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*Definition according to: Büro für Technikfolgenabschätzung (2006) TA-Projekt Nanotechnologie. Endbericht. URL: www.tab-beim-bundestag.de/de/pdf/publikationen/berichte/TAB-Arbeitsbericht-ab092.pdf Zugriff 08.01.13

Summary and outlook

Due to their extreme surface-to-volume ratios, many nano-structures show very substantial alterations in their physical and chemical properties. These can alter the functionality and/or the bioavailability of many ingredients. For the consumer it is of interest that nanotechnology enables improved foodstuff safety (for example by reducing the annual number of food poisoning cases) and can also enhance the uptake of health-related biologically active substances.

It is important to ensure that all structures produced are degraded in or eliminated by the body without impairment of health. For nano-structures formed from food ingredients and for which enzyme systems exist which allow their degradation, according to assessments to date there are no serious toxicological reservations. Nevertheless, the altered bioavailability of encapsulated substances, such as vitamin D_3 or A, must be more closely investigated in respect of their safe use, as an overdose of these could be injurious to health.

At the same time nanomaterials can also be present in food in natural form [40]. Thus, naturally occurring nanosystems, such as the casein micelle, are known which are well suited for ensuring the required uptake of vitamin D_3 in the human body [41].

The application of inorganic nanostructures must be viewed very differently. Currently, these are the subject of intensive nanotoxicological research.

Thus, for example silver and also silver salts are approved within the scope of additive approval legislation as E 174 for certain applications and can, for example, be used as a colouring agent for the "quantum satis" (in the amount required to achieve the desired effect) coating of confectioneries or for the preservation of drinking water.

However, in accordance with the relevant EC regulation (1333/2008) the use of silver (salts) in the form of silver nanoparticles is not approved, as an additive produced by nanotechnology is regarded as a new additive and is therefore subject to the regulation concerning novel foods and novel food ingredients (EC 258/97), which regulates the obligation to obtain approval for foodstuffs with nanomaterials in the EU.

Silver nanoparticles are nevertheless already in use as anti-microbial substances in surface coatings, for example in refrigerators or in packaging materials (nanoclay).

The German Federal Office for Risk Assessment (BfR) recommends that producers do not employ nano-scaled silver or nano-scaled silver compounds in foodstuffs and products for daily use until the available data allow a final assessment of health risks and the use of these products is known to be safe.

Grounds: Studies have shown that the bactericidal effect of nano-scaled silver depends upon the concentration, the form (spherical, rod-shaped, cubic or filamentous, irregular, or nanofilm) and can also greatly vary according to the surface reactivity [42]. Natural barriers, such as the intestinal mucosa, can also be more easily passed, so that silver could enter the bloodstream and be distributed over different organs. Furthermore, bacterial resistance can be expected with the use of silver nanoparticles [43].

Currently partly nano-scaled systems are mostly employed as excipients, for example as a secondary product of larger silicon dioxide aggregates used to ensure the free flow and trickling of ingredients in powder form. Organic nanoparticles are also employed in the form of micelles for the solubilisation of ethereal oils (spice extracts), enabling their use as anti-oxidative substances incorporated in sausage products or ready-made meals. These prevent oxidative alterations, such as rancidness.

In the food sector more intensive investigations of the digestion of nano-structured foodstuff components are required. In relation to new legislation currently being formulated in Europe and the USA, this would be entirely welcome. The role of food technologists during product development should be to consider toxicological testing early on in order to achieve a better balance between benefits, costs and risks for the new materials and structures.

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Conflict of interest:
Professor Weiss is a member of the board of directors of the association FoodDACH e. V. for the networking of research and industry in Germany, Austria and Switzerland.

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DOI: 10.4455/eu.2013.011