Chapter 4

Nanostructured Colloids in Food Science

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Additional information is available at the end of the chapter

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Abstract

Nanostructured colloids are materials with at least one dimension in the nanometer range (<100 nm). Such materials find multiple and exciting applications in various areas of food science, and can lead to development of new and innovative food products and ingredients. Nanostructured colloids can be naturally present in food or they can be synthetically manufactured and added during different stages of food production and packaging. The building blocks of nanostructures in food consist of organic molecules (proteins, lipids, saccharides), inorganics (metal and metal oxides, carbon-based materials, clays) and combined organic and inorganic compounds. Some examples of nanostructured colloids naturally occurring in food include fat globules in homogenized milk, casein micelles, β-lactoglobulin fibers in milk. Synthetically manufactured colloids (artificial and engineered) include nanoemulsions, nanomicelles, nanocapsules, nanofoams, nanoliposomes, nanogels, nanofibers, metal and metal oxide nanoparticles. Synthetically manufactured nanostructures are normally added in food to enhance solubility, improve bioavailability, protect the biologically active compounds from degradation, increase the shelf life, color, flavor, and add nutritional value. Exciting fields of applications of nanostructured colloids in food science comprise: functional food ingredients, food additives, food supplements, food packaging and nanosensors.

Keywords: natural nanocolloids, artificial nanocolloids, engineered nanocolloids, applications, safety

1. Introduction

Nanotechnology provides innovative means of controlling and structuring food. Recently, nanotechnology has become an active research field in food science, especially related to the development of functional foods with improved functionality and value. Nanostructured colloids (nanocolloids) are nano-sized materials that can be inherently present in food, or they can be formed because of food processing technologies such as milling, homogenization, emulsification, electrospraying, spray-drying, supercritical CO_2-based techniques, gelation, foaming, etc. [1, 2].

According to the European Commission, "Nanomaterial" means a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range of 1–100 nm. In specific cases and where warranted by concerns for the environment, health, safety or competitiveness, the number size distribution threshold of 50% may be replaced by a threshold between 1 and 50% [3].

Additionally, engineered nanomaterial means any intentionally produced material that has one or more dimensions of the order of less than 100 nm, or that is composed of discrete functional parts, either internally or at the surface, many of which have one or more dimensions of the order of 100 nm or less, including structures, agglomerates or aggregates, which may have a size above the order of 100 nm but retain properties that are characteristic of the nanoscale. Properties characteristic of the nanoscale include: (i) those related to the large specific surface area of the materials considered; and/or (ii) specific physicochemical properties that are different from those of the non-nanoform of the same material [3].

Two approaches are commonly encountered in food science and industry to produce nano-sized particles: the so-called 'bottom-up' and 'top-down' approaches. The 'bottom-up' approach is related to the ability of molecules to self-assemble; it is a method based on atomic and molecular manipulation. Self-assembly is characteristic for the formation of naturally occurring nanostructures in foods. The 'top-down' approach leads to particle size reduction down to the nanometer range during physical processing of breaking-up bulk materials such as milling, homogenization, emulsification, and nanolithography [1, 4, 5].

Molecular self-assembly at the nanometer scale can be achieved by non-covalent interactions. The self-assembly of nanostructured food components is thermodynamically driven by non-covalent interactions such as van der Waals interactions, hydrogen bonds, electrostatic interactions, Coulomb interactions, coordinate bonding, or hydrophobic interactions. Proteins, peptides, lipids and polysaccharides have the ability to self-assemble into different types of nanostructures such as self-assembly of casein micelles, folding of globular proteins, formation of protein nanofibers, and formation of starch [6].

The 'top-down' approach most commonly includes dry milling, high-pressure homogenization, and ultrasound emulsification. In milling, mechanical energy is applied to break-up the particles and reduce their size to the nanometer range. Dry milling can be applied to reduce the size of wheat flour and increase thus its water-binding capacity. Also, applying dry milling to reduce the size of green tea particles resulted in increased oxygen-eliminating enzyme activity [5]. High-pressure homogenization or microfluidization converts high fluid pressure into shear forces, leading to uniform particle size reduction and formation of nanostructures. The technique is normally used in dairy sector for size reduction of fat globules which is useful to increase the stability of emulsions. Microfluidization results in size reduction and emulsion formation, leading to improved texture and aspect. It is applied for obtaining fillings and icings, salad dressings, yoghurts, syrups, flavored oil emulsions, creams, drinks [4, 5, 7]. Ultrasound emulsification is also used to prepare stable oil and water emulsions making use of high intensity ultrasound waves.

2. Types of nanostructured colloids in food science

2.1. Naturally occurring nanocolloids in food

In many cases, food products naturally contain ingredients in the nanometer range, which are different from the synthetically manufactured ones. There is a large variety of naturally occurring food structures that fall within the nanometer range at least in one dimension. Such nanostructures result from the self-assembly of the biological molecules through non-covalent interactions. The nanostructures can result from the arrangement of proteins, lipids, polysaccharides as-such, or in combinations, even combinations with small ligands. Also, most polysaccharides and lipids in food form linear polymeric chains less than 1 nm thick. Milk contains many naturally occurring nanostructures; during milk homogenization, lipid vesicles of around 100 nm are formed. Proteins in food are globular structures with size between 10 nm and hundreds of nm.

Some particular examples of commonly encountered protein nanostructures include: the casein molecule, β-lactoglobulin, bovine serum albumin, α-lactalbumin, lactoferrins (all present in milk), and lysozyme, ovalbumin, avidin (all present in egg white) [5, 8]. Most of the proteins of vegetal and animal origin are globular proteins, with few exceptions such as casein which forms micelles. Globular proteins such as whey proteins from milk have the ability to form particles with sizes of 40 nm.

Caseins belong to a family of phosphoprotein nanostructures present in milk that self-assemble into micellar granular structures which are suspended in the aqueous phase of milk. Ninety-five % of the caseins naturally self-assemble into micelles with 50–500 nm diameter [8, 9]. There are four varieties of caseins present in the mammalian milk: casein α_{s1}, α_{s2}, β, and κ [5, 10]. Casein accounts for 80% of the proteins in the cow's milk and 20–45% of the proteins in the human milk [11]. The casein micelle is stabilized by calcium-phosphate bonds. The high phosphate content of casein micelles allows it to associate with calcium and to form calcium phosphate salts. The high phosphate content of milk allows it to contain much more calcium than would be possible if all the calcium were dissolved in solution, thus casein proteins provide a good source of calcium for milk consumers. Thus, this type of colloidal micelles significantly increase the bioavailability of the calcium and phosphate ions [12]. Moreover, the casein micelles are important in milk digestion in the stomach and intestine, and constitute the basis for many of the milk products, being the major component of cheese (cheese is obtained by coagulation of casein). Casein is also used as food additive.

β-Lactoglobulin is the major whey protein in mammalian milk, except the human milk. The β-lactoglobulin monomer is a 3.6 nm long nanofiber which consists of a 162 amino acid sequence, and a molecular weight of 18.4 kDa. Depending on the pH, β-lactoglobulin can exist as slightly different structures such as dimmers, monomers, tetramers. The exact role of β-lactoglobulin has not yet been clearly established. It is believed to be involved in the transport of molecules due to its capacity to bind hydrophobic molecules and iron [5, 13].

α-lactalbumin is a 123 amino acid residue milk protein, with 2.01 nm radius, 14.2 kDa molecular weight and an isoelectric point between 4.2 and 4.5. α-Lactalbumin is a component of lactose-synthase, the enzyme responsible for lactose synthesis in mammalian milk, so it has a key role in regulating lactose production in milk. It has been proved that some folding variants of α-lactalbumin have bactericidal activity and some of them cause apoptosis of tumor cell [12, 14].

Lactoferrin is a 689 amino acid iron-binding protein with 3.6 nm radius, 82.4 kDa molecular weight and 8.7 isoelectric point. Lactoferrin has antibacterial and antifungal activity, it plays a role in iron adsorption, and studies have shown that lactoferrin is involved in the immune system responses [5, 15]. Human colostrum has the highest lactoferrin content, providing protective and antibacterial activity to infants. Lactoferrin normally complexes with other milk components such as casein, β-lactoglobulin, DNA, polysaccharides, and some of its biological activity is due its complexated forms [15].

Lysozyme (C-type) is a 129 amino acids globular antibacterial protein found in egg white, with a diameter of about 4.2 nm. C-type lysozyme is related to α-lactalbumin regarding the sequence structure [16].

Ovalbumin is the main protein in egg white, accounting for 55% of its protein content. It is a 385 amino acids sequence with 42.7 kDa molecular weight and 6.1 nm diameter [5, 17].

Lipid based nanocolloids naturally occurring in food are represented by lipoproteins in egg yolk, oleosomes found in plant seeds, fat globules in milk. The lipid nanostructures in food are normally composed of a hydrophobic core (triacylglycerols, glycerols, esterified fatty acids), surrounded by other phospholipids or proteins. The lipoproteins present in egg yolk form spherical structures with 15–60 nm diameter. The oleosomes are specialized lipid-based organelles in plant seeds, 0.1–10 μm in diameter, used for energy storage and for preventing fats from oxidation during germination. They are good vitamin reservoirs, being composed of a vitamin and triacylglycerol-rich core, surrounded by a phospholipid layer and protein layer [18].

2.2. Artificial nanocolloids in food

The term artificial refers to synthetically manufactured organic structures to produce colloidal particles on the nanoscale. There is huge interest towards designing nano- and microstructures for the food industry sector, since bioactive molecules can be encapsulated in such nanostructures. In many situations, encapsulation offers significant advantages, such as enhancing the stability and solubility of bioactive compounds (e.g. carotenoids), improving bioavailability (e.g. carotenoids, vitamins, minerals), protecting nutrients and bioactive compounds from degradation during manufacturing and storage, facilitating controlled release, masking unpleasant taste during eating (e.g. fish oil, polyphenols) [19].

Artificial organic colloidal nanostructures are most often synthesized from proteins, polysaccharides, and lipid molecules. Artificial organic nanostructures can be build-up of one type of molecule only, or, alternatively they can be combinations of different molecules. Commonly encountered artificial nanocolloids include nanoemulsions, nanomicelles, nanocapsules, nanofoams, nanoliposomes, nanogels, and nanofibers. The development of such structures has impact on the stability, protection, delivery, and bioavailability of bioactive molecules.

2.2.1. Nanoemulsions

Nanoemulsions are dispersions of at least two immiscible liquids, usually oil-in-water dispersions with mean diameters of 10–100 nm. Basically, they are fine dispersions of droplets of one liquid in another one in which the first is not soluble (usually defined as the oil phase and aqueous phase). Compared to conventional emulsions, nanoemulsions offer significant advantages for certain applications. First, nanoemulsions are much more stable compared to traditional emulsions, due to the small particle size [4, 20, 21]. They have higher stability towards particle aggregation and gravitational separation. In addition, nanoemulsions are used to increase solubility and bioavailability of bioactive hydrophobic molecules, especially carotenoids (lutein, lycopene, β-carotene), polyphenols (resveratrol), vitamins (A, D, E_3), enzymes (coenzyme Q10), fatty acids (omega-3 fatty acids). Also, compared to traditional emulsions, the very small particle size of nanoemulsions, which can be smaller than the wavelength of light, only scatters light weakly. This does not alter the appearance of the food in which they are incorporated. So, they can be incorporated into optically transparent foods and beverages without affecting their clarity. Nanoemulsions can be prepared by high-pressure homogenization or ultrasound-assisted homogenization.

2.2.2. Nanoliposomes

Nanoliposomes are spherical bilayered vesicles made of amphiphilic molecules, such as phospholipids (e.g. lecithin, cholesterol). The molecules are arranged in two concentric circles, such that the hydrophilic end of the outer layer is exposed to the outer environment, while the inner hydrophilic end makes the hydrophilic core. The hydrophobic tails are in between the two hydrophilic layers. Their size varies between 20 and 400 nm usually. Liposomes can encapsulate both hydrophobic and hydrophilic molecules: hydrophobic molecules are encapsulated in the hydrophobic tail regions, while water soluble molecules are encapsulated in the hydrophilic core [4, 21].

2.2.3. Nanomicelles

Nanomicelles are spherical monolayered vesicles made of amphiphilic molecules. In a biological system, the molecules tend to arrange themselves in such a manner that the inner core is hydrophobic and the outer end is hydrophilic in nature. Thus, they can be used for encapsulation of hydrophobic molecules. Nanomicelles size varies in the range 5–100 nm.

2.2.4. Polymeric nanocapsules

Polymeric nanocapsules are synthetic colloidal nanostructures obtained from different natural or synthetic biocompatible polymers, with the final goal of encapsulating bioactive compounds. Most common polymers are poly lactic acid, poly-ε-caprolactone, poly-lactide-coglycolide, natural polysaccharides (chitosan, Xanthan gum, Arabic gum), whey protein, etc. [22]. Nanocapsules are synthetic vesicles in which the bioactive compounds of interest, solubilized in an aqueous or oil core are covered by a polymeric shell. Polymeric nanocapsules can be build-up of single or multilayered polymeric walls, such as for example the polyelectrolyte multilayer capsules made of alternating layers of positively and negatively charged polymers. The bioactive compounds are normally encapsulated within the nanocapsule's core. Examples of molecules that can be encapsulated in polymeric nanostructures are the carotenoids lutein, lycopene, β-carotene, bixin, but also quercetin, α-tocopherol, vitamin B12, turmeric oil, lemongrass oil, cinnamon oil, etc. [22].

2.3. Engineered food nanocolloids

Engineered food nanocolloids mainly refer to synthetic metal and metal oxide nanoparticles. According to the European Commission, these nanoparticles are attracting great interest because they are increasingly used through sunscreen creams or other cosmetics, paints, plastics, dyes, food, medicines. Commonly used engineered colloidal systems in food industry include silver nanoparticles (AgNPs), titanium dioxide nanoparticles (TiO_2-NPs), zinc oxide nanoparticles (ZnO-NPs), silicon dioxide nanoparticles (SiO_2-NPs), nickel oxide nanoparticles (NiO-NPs), copper oxide nanoparticles (CuO-NPs), tin oxide nanoparticles (SnO_2-NPs), chromium oxide nanoparticles (Cr_2O_3-NPs), or composites between such nanoparticles [23]. A more complete list containing nanomaterials currently used in the European Union is presented in **Table 1**, based on an inventory of the European Food Safety Authority (EFSA) [3].

Naturally occurring nanomaterials	Nisin	Proteins	Casein
	Cellulose	Green tea	Lysozyme
	Starch	Enzymes	Zeolites
	Nanodroplets	Nanolipids	Nanosalts
Artificial nanomaterials	Nanocapsules (liposomes, micelles, nanocapsules, nanoemulsions)		
Engineered nanomaterials	Silver	Copper	Nickel
	Titanium dioxide	Fullerenes	Cerium oxide
	Nanocomposites	Selenium	Aluminum
	Zinc oxide	Calcium	Aluminum oxide
	Clays	Calcium carbonate	Carbon black
	Synthetic amorphous Silica	Calcium silicate	Organic Pigments
	Carbon nanotubes	Calcium phosphate	Platinum
	Silicon dioxide	Copper oxide	Sulfur
	Gold	Chromium	Amorphous Na-Al silicate
	Iron	Lead	Titanium nitride

Table 1. Nanomaterials in the agricultural, food and feed sector, as reported by an EFSA inventory [3].

Even if there is great potential for applications of such colloidal nanostructures in food industry, this class of structures is also related to potential food safety implications, health and environmental hazards. There is currently a continuous debate in this regard. The health hazards related to human exposure to such engineered nanostructures is due to the fact that their accumulation in tissues and cells is not entirely understood, and there is a fear of long term systemic toxicity. Environmental concerns are closely related to the fact that hundreds of tons of nanoparticles end up annually into the environment and further into the food chain. Plants play a critical role nanoparticles fate in the environment by assimilation, being exposed to nanoparticles due to agricultural soils fertilization with sewage sludge, as well as due to the use of nanoparticles in phytosanitary products.

3. Applications of nanostructured colloids in food science

The unique physical, chemical, and biological properties of colloidal nanostructures are considerably different from their bulk counterparts and this opens them unique and new possibilities for applications. Several research papers and reviews have identified potential applications of nanostructures for the food sector to improve food quality and safety. Increasing the shelf life of the food (preservation), pathogen detection, sensing, coloring, flavoring, and increasing nutritional value, are some of the important applications of nanostructures in the food and agri-food sectors.

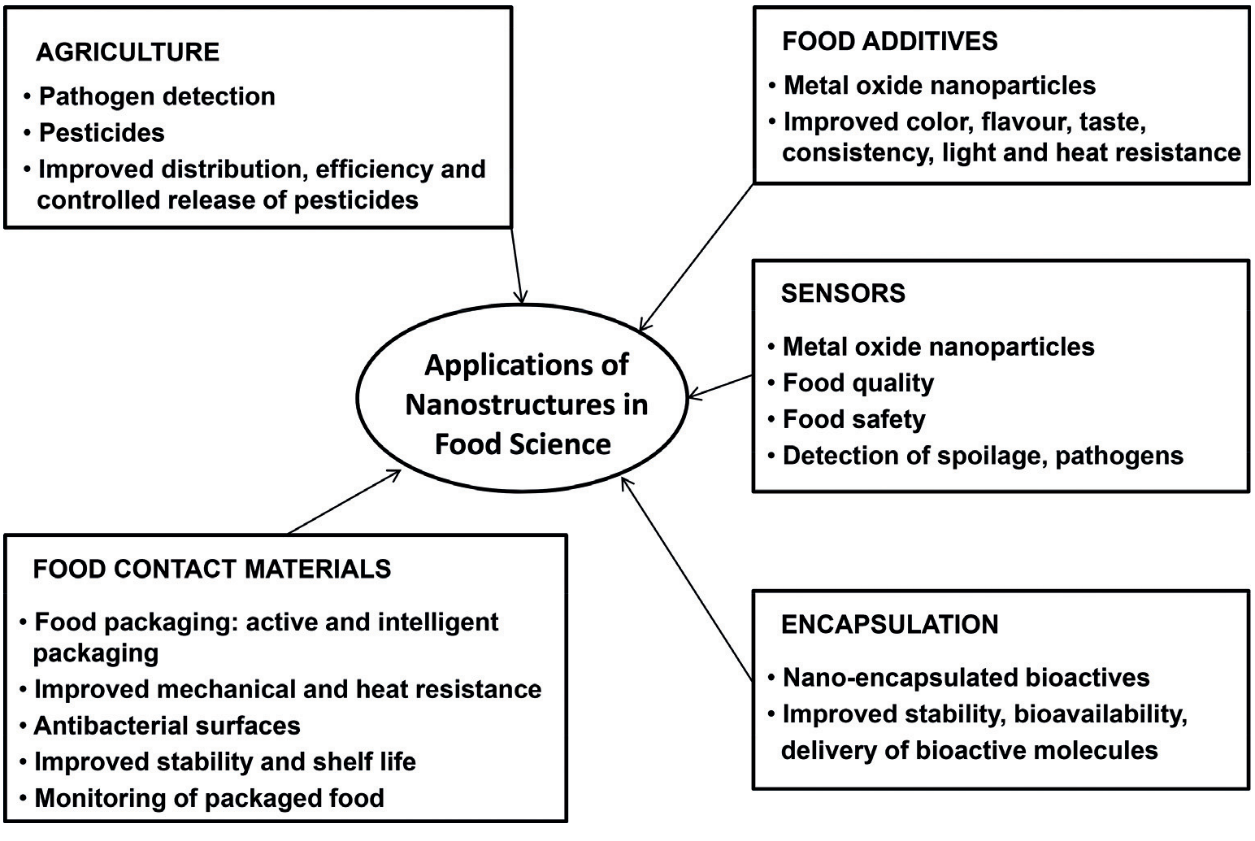

Figure 1. Applications of colloidal nanostructures in the food and agri-food sectors.

According to a report of EFSA [3], AgNPs and TiO_2-NPs are most used nanomaterials, with food additives and food contact materials being the most encountered applications. Some of the common potential fields of application of nanostructures and nanotechnology in food science and agriculture sectors are highlighted in **Figure 1**.

3.1. Sensing

Lately, there is an increasing number of sensing devices with applications in the food analysis sector, especially food quality control. Such devices are mainly based on colloidal metal oxide nanoparticles. They can mainly detect unwanted gases and volatile organic compounds that are produced in food products due to their spoilage. The working principle of such sensors is based on the interaction between the gas molecules and the colloidal metal oxide particles, which generates the change of a physical parameter in the transduction mechanism, making it possible to identify and in some situations to quantify the unwanted gas molecules. The colloidal metal oxide nanoparticle sensors can be independent devices used for food quality monitoring or they can be incorporated in intelligent packaging systems (see Section 3.3).

Some examples include sensors for the detection of trimethylamine, dimethylamine and ammonia, which are gas molecules naturally formed during biodegradation of plants, fish and animal tissues. Their presence is directly related to the degree of freshness of the fish and seafood products. Different metal oxide nanoparticle sensors have been reported for the detection of trimethylamine such as TiO_2-NPs, Au-WO_3-NPs, ZnO-Cr_2O_3-NPs, Cr_2O_3-SnO_2-NPs [23, 24]. For dimethylamine detection different formulations of ZnO-NPs have been reported. Besides being used for detection of fish meat freshness, detection of ammonia is also useful to give information about spoilage of other meat products.

3.2. Encapsulation and functional foods

Incorporation of several bioactive molecules in various food systems is limited by their poor water solubility and instability in presence of light, heat, and oxygen. As mentioned above, bioactive food compounds can be encapsulated by inclusion in different nano-sized structures, such as nanoemulsions, nanomicelles, nanoliposomes, nanocapsules. Vitamins, probiotics, fatty acids, lipids, antioxidants, preservatives, proteins, enzymes, peptides have been incorporated in several nanocapsule-based systems.

To summarize, the main objectives of encapsulation are:

- to enhance the solubility (e.g. coloring agents, antioxidants);
- to improve the bioavailability of the compounds, meaning the amount of bioactive compound which is absorbed by the human body (antioxidants, vitamins, minerals, enzymes);
- to improve stability and shelf life;
- to protect bioactive molecules and micronutrients during manufacture, storage, retail;
- to allow controlled release of bioactive molecules and drugs (only at desired target organs, only in particular parts of the gastrointestinal tract; e.g. pH-driven release, light-driven release).

As particular example, enzymes are a very important class of bioactive molecules for which colloidal nanoencapsulation can bring advances regarding improvement of their stability, activity, avoiding of denaturation, improving absorption in the gastro-intestinal tract. Enzymes would benefit delivery through functional foods and supplements, but since they are susceptible to denaturation, encapsulation is one was to overcome the problem. Food-grade colloidal enzyme delivery nanocapsules are normally composed of lipids, proteins, polysaccharides (starch, carrageenan, etc.), and encapsulating structures include colloidal nanoemulsions, solid-lipid nanoparticles, liposomes, gels [25].

Micelles and liposomes are used as encapsulating and carrier structures for different hydrophobic molecules such as essential oils, flavors, antioxidants (polyphenols, carotenoids, coenzyme Q10), vitamins, minerals, proteins, nutrients, nutraceuticals [4, 21].

Nanoemulsions find applications in the production of table spreads and yoghurts, flavored oils, personalized beverages, sweeteners, fortification of milk with vitamins, minerals, antioxidants [4, 5].

Nanoliposomes in food were reported as vesicles for encapsulation of antioxidants, fatty acids, oils. Stable nanoliposomes made of ω-3 and ω-6 fatty acids, tocopherol and vitamin C encapsulated in soy phosphatidylcholine were studied as functional ingredients in acidic foods, such as the orange juice [26].

Nanocapsules can be incorporated in interactive foods and drinks which can release flavors, aromas, colors by breaking up at certain pH values or at certain infrared frequencies. Breaking up causes delivery and release of the content. One such example of colloidal polymeric nanocapsules is the light-responsive polyelectrolyte capsules containing gold nanoparticles as light responsive nanomaterial [27]. These colloidal nanocapsules are formed by alternating layers of positively and negatively charged, biocompatible polymers, hold together by electrostatic interactions. The bioactive molecules are encapsulated into the nanocapsules core, while the optically addressable colloidal AuNPs are embedded within the nanocapsule walls. The AuNPs within the capsule walls absorb light in the near infrared region, where most tissues show only weak absorption; the laser energy is then efficiently transformed into heat, being possible to open the capsules and achieve laser-induced release of the encapsulated molecules, with minimal damage of the surrounding tissues and of the bioactive molecules. There is currently a lot of effort being put into developing this type of light-responsive systems. One potential application could be the encapsulation of antioxidants such as carotenoids and their efficient delivery to retina cells—to be used as food supplements and to prevent and treat age-dependent macular degeneration.

3.3. Food packaging and antimicrobials

Lately, several innovative food packaging materials based on colloidal nanoparticles have been developed. Nano-packaging is a new generation of food packaging technology which represents a radical alternative to the conventional food packaging. Basically, nanomaterials are incorporated in the polymer matrix to offer mechanical strength or to function as a barrier against gases, volatile components (e.g. flavors) or moisture. Widely used colloidal nanostructures in food packaging include colloidal AgNPs, ZnO-NPs, TiO_2-NPs, mostly used for antimicrobial activity and ultraviolet protection, also nanoclays used to develop materials with enhanced gas-barrier properties, colloidal Silica nanoparticles used for surface coating of packaging materials. Titanium nitride in the nanoparticle form is authorized in the EU for use as additive or polymer production aid in plastic food contact materials (EU Regulation 1183/2012) [28].

The concept of active packaging, e.g. antimicrobial packaging involving incorporation of nanoparticles with antibacterial and antioxidant properties (AgNPs, ZnO-NPs, MgO-NPs, TiO_2-NPs) into the food package has attracted increasing interest due to its potential huge impact in the food safety sector [29]. An active packaging system involves interaction between the packaging material and the food to provide desirable effects, such as microbial safety, extended shelf life of foods. For example, colloidal AgNPs have been for long known to be effective antibacterials. They are reported by several authors [30, 31] to be incorporated in antibacterial coatings, efficient against different fungi, gram positive and gram-negative pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Aspergillus niger*, *Penicillium funiculosum*, *Chaetomium globosum*, *Aspergillus terreus*, and *Aureobasidium pullulans*.

Other nanocolloidal systems (e.g. TiO_2-NPs, SiO_2-NPs, nanoclays) can ensure good food preservation by blocking the UV radiation, improving mechanical and heat-resistance properties, reducing the permeability of foils, deodorizing, antimicrobials [32]. Colloidal TiO_2-NPs are UV blocking agents, used as filler particles in foils, food packaging, plastic containers. Nanoclays are among the first polymer nanostructures to emerge on the market as improved materials for food packaging [33]. Clays are one of the oldest and most important types of available colloidal materials. Nanoclay based food packaging increases the shelf life of oxygen sensitive foods by increasing the water and gas barrier of the polymer material. Uses have been reported for manufacturing of bottles for beers and carbonated drinks [33]. Nanoclays have been also reported to improve mechanical properties, thermal stability and fire resistance of polyethylene, polypropylene, nylon, poly(e-caprolactone), polyethylene terephthalate polymers.

The possibility to improve the performances of polymers for food packaging by adding nanoparticles has led to the development of a variety of polymer nanomaterials. Polymers with nanofillers, e.g. polymer nanocomposites, are created by dispersing an inert, nanofiller into a polymeric matrix. Filler materials can include nanoclays as mentioned above, SiO_2-NPs, carbon nanotubes, starch nanocrystals, cellulose-based nanofibers, chitin and chitosan nanoparticles [29, 32]. Compared to conventional polymers, polymer nanocomposites show improved packaging properties by having better mechanical resistance, flame resistance, and better thermal properties (e.g. melting points, degradation and glass transition temperatures). Polymer nanocomposites can improve quality of meat and meat products by reducing moisture loss, reducing lipid peroxidation, improving thus the appearance of the products by maintaining the flavors, color, and texture.

Very recent work in the field of nanocomposites and antimicrobial packaging reports on the use of polyvinyl alcohol/nanocellulose/Ag nanocomposite films with antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* [34]. Other advances report the development of nanocomposite gelatin based antifungal films efficient against *Aspergillus niger* [35, 36]. For the gelatin-based nanocomposite preparation either chitin nanofibers and ZnO-NPs, or chitin nanofibers and corn oil were used.

Intelligent packaging involves incorporation of nanosensors into food packaging to improve detection and tracking of the physicochemical changes in food during storage and transport. It gives information based on its ability to detect changes in the product's environment [29].

Despite tremendous advances in applying nanostructures in food packaging, there is continuous debate regarding the potential diffusion in time of nano-sized materials from food packaging into the packaged food and this is a topic that requires further and careful investigation [37].

3.4. Food additives

3.4.1. Titanium dioxide (TiO_2)

TiO_2 is a food coloring agent approved by EFSA, known as E171. It is used as whitener and colorant. It is also used as a food additive and flavor enhancer in a variety of non-white foods, including dried vegetables, nuts, seeds, soups, and mustard, as well as beer and wine. E171 contains TiO_2 particles, partially in the nanometer size. The average particle size is 200–300 nm, part of this bulk material may contain a fraction of particles with sizes <100 nm. A recent study showed that 5–36% of the TiO_2 in food products is in the nano-size range [38]. Colloidal TiO_2 can be found in products such as candies, sweets, chewing gum, toothpaste, nutritional supplements, sunscreen protection products [39].

Other metals in the form of colloidal nano-sized particles are available as food or health supplements. These include selenium nanoparticles [40], calcium nanoparticles, iron nanoparticles, and colloidal suspensions of metal particles, e.g. cobalt nanoparticles, gold nanoparticles, platinum nanoparticles, silver nanoparticles, molybdenum nanoparticles, paladium nanoparticles, titanium nanoparticles, and zinc nanoparticles.

Extensive use of TiO_2 has been, in some studies linked with adverse health effects. The European Chemicals Agency has concluded that titanium dioxide may cause cancer if inhaled [41]. In their study on mice, Rizk et al. [42] found that some biochemical parameters and the liver structure were influenced by colloidal TiO_2-NPs in a dose-dependent manner. There is continuous debate and further studies are needed to elucidate all the safety aspects of TiO_2 in its nano- and non-nanoforms on pharmaceutical and food applications. In September 2016, EFSA published an opinion on the re-evaluation of E171, based on a detailed literature review on TiO_2 nanoparticles. It was concluded that current exposure of consumers to E171 related to its use in foodstuffs is not likely to constitute a health risk, but that it was not possible to establish an acceptable daily intake [43].

3.4.2. Zinc oxide (ZnO)

Zinc oxide nanoparticles (ZnO-NPs) are used in some food contact materials such as polypropylene and polyethylene. ZnO is used as a transparent ultraviolet light absorber in unplasticized polymers at up to 2% by weight [44]. It is added as nanopowder in the formulations and thus colloidal nanoparticles are present in the final polymer. It absorbs UV light without re-emitting it as heat, improving thus the stability of the polymers. It is also used in nutritional supplements, for example vitamins [39].

There are several *in vitro* studies demonstrating some degree of toxicity of ZnO-NPs. A study on RKO human colon carcinoma cells found that the toxicity is not related to the colloidal ZnO-NPs concentration, but to the direct particle-cell contact [45]. *In vivo* studies have shown that ZnO in the nanometer form tends to accumulate in liver, spleen, kidney tissues in higher amounts compared to the micrometer ZnO particles. EFSA has performed a safety assessment study of ZnO-NPs used in food contact materials [44]. Considering previous knowledge on the nanoparticles diffusion in polymers and the solubility characteristics of the ZnO-NPs, it was concluded that ZnO-NPs do not migrate and the focus should be on the migration of soluble ionic Zn, which complies with the current specific migration limit.

3.4.3. Silicon dioxide (SiO_2)

Silicon dioxide (SiO_2) is a licensed food additive (E551), used as anticaking agent. It is one of the most important caking agents, present in many powdered food items, chewing gums, cheese, seasonings, cooking salt [3]. It is also used for clearing of beverages. E551contains primary particles, aggregates and agglomerates, and partially it contains SiO_2 in the nanometer range, with sizes <100 nm.

3.5. Agriculture and animal feed

Nanostructured colloidal materials have been reported to have contributions in the delivery of agro-chemicals such as pesticides and herbicides through, for example controlled delivery systems based on encapsulation. Such systems can decrease the amounts of sprayed chemicals by more efficient delivery of bioactive compounds. Different nanostructured delivery systems could be applied to store, protect, deliver, release of pesticides, nutrients, fertilizers [5]. Some formulations of nanocapsules with pesticides and herbicides have been reported. Some companies have produced nanosuspensions and nanoemulsions containing water or oil soluble pesticides and herbicides. Nanocapsules can also be used for the delivery of DNA and chemicals to plant tissues, with the final goal of ensuring protection against several pests and diseases [5].

Additionally, uses of colloidal nanostructures in agriculture have been reported for wastewater treatment, disinfectants (AgNPs mainly), sensors for detection of pesticides, fertilizers, herbicides, pathogens, aflatoxins, soil pH and moisture, etc. The efficiency of colloidal AgNPs and colloidal gold nanoparticles (AuNPs) as larvicide against *Aedes aegypti* was reported [46]. AgNPs were also found to be effective against powdery mildew in cucumber and pumpkin, under different cultivation conditions *in vitro* and *in vivo* [47]. Powdery mildew is one of the most devastating diseases in cucurbits, which can lead to serious crop yield decrease. Colloidal AgNPs have been studied also as an alternative for the antibiotics used in the poultry production [48].

Colloidal nano-sized minerals, vitamin or other additives could equally be used for animal feed, to ensure improved delivery of veterinary drugs and to improve availability and quality of nutrients in feed.

3.6. Some examples of commercial nanofood products

Many food companies are investigating or already employing nanocolloids to change the structure of food and drinks. Examples include the interactive foods and drinks containing colloidal nanocapsules that can change color and flavor, spreads and ice creams with nanoparticle emulsions that improve food texture [49]. In the following, some examples of commercial nanofood products existing on the market will be provided.

Nanotea [50] is a colloidal formulation containing nano-selenium in concentration of 3–5 ppm, prepared using nanotechnology. The nanotea can release effectively all the excellent essences of the tea, being a good selenium supplement (10-fold increase).

Nanoceuticals™ Slim Shake Chocolate [51] is a slimming product containing Cocoa nanoclusters reported to enhance the taste benefits of the product.

Canola Active Oil [52] produced by Shemen Industries, Israel, is a product obtained by the nano-sized self-assembled structured liquids (NSSL) technique, giving compressed micelles, called nanodrops. These micelles act as liquid carriers, allowing encapsulation of different bioactive components (vitamins, minerals, phytosterols). The micelles are added to the food products for improving bioavailability of the bioactives, being able to pass through the digestive system effectively, without breaking up, and thus effectively reaching the absorption site.

Hydracel [53] is a food supplement containing nanocolloidal minerals, made of water, silicon, magnesium sulfate, potassium carbonate, potassium hydroxide, sunflower oil, reported to improve the cell life cycle.

Nano calcium [54] is a food supplement containing calcium in the nanoparticle colloidal form, reported to enhance and fasten calcium absorption in the body.

Production of carotenoid preparations in the form of coldwater-dispersible nano powders with up to 200 nm size, and the use of the novel carotenoid preparations are reported by US patent 5968251A [55].

LycoVit [56] is a BASF product containing lycopene in the nanosize range. Its intended use is for dietary supplement, beverage and food applications such as turbid beverages, carbonated drinks, cake and biscuit fillings, dairy desserts, candies, pastas, meats and soups. It is reported to be a safe, highly effective substitute for artificial colors, which may have an adverse effect on activity and attention in children.

NovaSOL curcumin [57] is a nanomicelle-based compound, stable through the digestive process, able to deliver curcumin to the intestinal wall with maximum therapeutic effect. It is reported that NovaSOL curcumin has 185× increased bioavailability than native powdered turmeric/curcumin.

NutraLease Ltd. Company [58], through the nano-sized self-assembled structured liquids (NSSL) technology develops nanocarriers to be incorporated in food systems and cosmetics. The technology allows enhanced solubility and bioavailability of compounds in water-based or oil-based media. The encapsulated compounds include lycopene, beta-carotene, lutein, phytosterols, coenzyme Q10, lipoic acid, and DHA/EPA. The company focuses on fortifying foods and beverages.

4. Regulatory aspects of nanotechnology in the agri-food sector in the EU countries

As mentioned, nanotechnology and the use of colloidal nanoparticles in the food science and agricultural sectors open up the possibility to produce new ingredients and foods with improved and beneficial properties. However, as the research in the field is intensifying, in some cases several health and environmental concerns appear. Therefore, a careful assessment of the potential risks is essential before approval of any nanostructured ingredients. Efforts are being made worldwide to ensure the production and safe use and handling of nanomaterials in the agricultural and food sector.

Nanomaterials are present in many commercial preparations. Some of the nanoparticles are ingested by humans through food consumption. EFSA observed that organic artificial nanomaterials (vesicles, nanoemulsions) present a low risk for health as they are most likely completely metabolized. In contrast, the agency considered that the research effort has to be concentrated on inorganic, synthetic nanomaterials, whose faith and accumulation in the human body is not exactly certain, nor are their effects upon long term exposure.

There is no single legislation fully dedicated to nanomaterials, but there are several regulations addressing different aspects of nanomaterials use in the agri-food sector. **Table 2** summarizes relevant European regulations connected to the use of nanomaterials in food. The "General Principles and Requirements of Food Law" are established by the EC Regulation 178/2002, containing several articles on novel foods, novel foods ingredients, food and feed additives,

Legislation number	Legislation topic
(EC) 178/2002	General principles and requirements of food law
(EU) 2283/2015	Novel foods, novel food ingredients, food and feed additives, food supplements, vitamins, minerals, food contact materials
(EC) 1332/2008	Food enzymes
(EC) 1333/2008	Food additives
(EC) 1334/2008	Food flavorings
(EC) 46/2002	Food supplements
(EC) 10/2011	Plastic materials and food contact materials
(EC) 450/2009	Active and intelligent materials and food contact materials
(EU) 528/2012	Biocides
(EC) 1107/2009	Plant protection products

Table 2. Overview of the European legislation on the agri-food sector, in relation to nanomaterials.

food supplements, vitamins, minerals and food contact materials. Regulation (EU) 2283/2015 concerning novel foods and novel food ingredients covers aspects of the use of nanotechnology in food production and repeals the older Regulation (EC) 258/97. Food additives are covered by several regulations: Regulation (EC) 1333/2008 on food additives, Regulation (EC) 1332/2008 on food enzymes, Regulation (EC) 1334/2008 on flavorings and certain food ingredients with flavoring properties for use in food. Regulation 1331/2008 establishes a common authorization procedure for food additives, enzymes and flavorings prior to their entrance on the market. Vitamins and minerals are regulated by Directive (EC) 46/2002 on food supplements. Regulation (EC) 10/2011 establishes specific rules for plastic materials and articles intended to come in contact with food, to be applied for their safe use. Regulation (EC) 450/2009 deals with active and intelligent materials and articles intended to come into contact with food. The Biocidal Products Regulation (EU) 528/2012 lays down the provisions for the use of non-agricultural pesticides by both professionals and consumers and Regulation (EC) 1107/2009 concerns placing of plant protection products on the market [3, 59, 60].

5. Conclusions

Applications of colloidal nanostructures in food science are receiving increased attention due to the possibility of developing structures and materials with improved properties compared to the conventional ones. In the future, enabling a better control over the colloidal nanostructure formation will enhance the control over food structure formation, leading to the design of new food products with improved characteristics and enhanced impact on consumers'. It is the unique physical, chemical, optical and biological properties of the nanocolloidal systems, considerably different from those of the corresponding bulk materials that are responsible for their unique properties. Colloidal nanostructures are also raising a series of health and environmental concerns, since not so much information is known on the long-term exposure of organisms to such materials, even if at the present time most of them are considered safe. In the future, more studies and regulations regarding the health and environmental impact of colloidal nanostructures will be needed.

Despite the fate and potential toxicity of such colloidal nanostructures being not fully understood, it is obvious that this research field will bring significant advances in the food sector, which will most likely impact the food safety and nutrition sectors, the production of new functional foods and ingredients, the developing of innovative packaging with great potential to transform the future of food packaging, will as well help assisting in the detection of pesticides, pathogens, toxins, etc.

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Conflict of interest

The authors declare there is no conflict of interest.

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References

[1] Huang Q, editor. Nanotechnology in the Food, Beverage and Nutraceutical Industries. Cambridge: Woodhead Publishing in Food Science Technology and Nutrition; 2012. DOI: 10.1533/9780857095657

[2] Dickinson E, van Vliet T, editors. Food Colloids, Biopolymers and Materials. Cambridge: The Royal Society of Chemistry; 2003. DOI: 10.1016/s0927-7765(03)00133-4

[3] Inventory of Nanotechnology applications in the agricultural, feed and food sector [Internet]. 2014. Available from: https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/sp.efsa.2014.EN-621 [Accessed: 2018-05-08]

[4] Pathakoti K, Manubolu M, Hwang HM. Nanostructures: Current uses and future applications in food science. Journal of Food and Drug Analysis. 2017;**25**(2):245-253. DOI: 10.1016/j.jfda.2017.02.004

[5] Axelos MAV, Van de Voorde M, editors. Nanotechnology in Agriculture and Food Science. Weinheim: Wiley-VCH; 2017. DOI: 10.1002/9783527697724

[6] Whitesides GM, Boncheva M. Beyond molecules: Self-assembly of mesoscopic and macroscopic components. Proceedings of the National Academy of Sciences of the United States of America. 2002;**99**(8):4769-4774. DOI: 10.1073/pnas.082065899

[7] Augustin MA, Sanguansri P. Nanostructured materials in the food industry. Advances in Food and Nutrition Research. 2009;**58**:183-213. DOI: 10.1016/S1043-4526(09)58005-9

[8] Rogers MA. Naturally occurring nanoparticles in food. Current Opinion in Food Science. 2016;**7**:14-19. DOI: 10.1016/j.cofs.2015.08.005

[9] Holt C, Horne DS. The hairy casein micelle: Evolution of the concept and its implications for dairy technology. Netherlands Milk and Dairy Journal. 1996;**50**(2):85-111

[10] Dalgleish DG. Casein micelles as colloids: Surface structures and stabilities. Journal of Dairy Science. 1998;**81**(11):3013-3018. DOI: 10.3168/jds.S0022-0302(98)75865-5

[11] Kunz C, Lonnerdal B. Human-milk proteins—Analysis of casein subunits by anion-exchange chromatography. Gel-Electrophoresis, and Specific Stainign Methods. American Journal of Clinical Nutrition. 1990;**51**(1):37-46

[12] Milk Composition [Internet]. Available from: http://www.milkfacts.info/Milk%20Composition/Protein.htm#MilkProtPhysProp [Accessed: 2018-03-15]

[13] Brownlow S, Cabral JHM, Cooper R, Flower DR, Yewdall SJ, Polikarpov I, et al. Bovine beta-lactoglobulin at 1.8 angstrom resolution—Still an enigmatic lipocalin. Structure. 1997;**5**(4):481-495. DOI: 10.1016/s0969-2126(97)00205-0

[14] Permyakov EA, Berliner LJ. Alpha-lactalbumin: Structure and function. FEBS Letters. 2000;**473**(3):269-274. DOI: 10.1016/s0014-5793(00)01546-5

[15] Sanchez-Valdes S, Ortega-Ortiz H, Valle L, Medellin-Rodriguez FJ, Guedea-Miranda R. Mechanical and antimicrobial properties of multilayer films with a polyethylene/silver nanocomposite layer. Journal of Applied Polymer Science. 2009;**111**(2):953-962. DOI: 10.1002/app.29051

[16] Peters CWB, Kruse U, Pollwein R, Grzeschik KH, Sippel AE. The human lysozyme gene-sequence organization and chromosomal localization. European Journal of Biochemistry. 1989;**182**(3):507-516. DOI: 10.1111/j.1432-1033.1989.tb14857.x

[17] Nisbet AD, Saundry RH, Moir AJG, Fothergill LA, Fothergill JE. The complete amino acid sequence of hen ovalbumin. European Journal of Biochemistry. 1981;**115**(2):335-345. DOI: 10.1111/j.1432-1033.1981.tb05243.x

[18] Stumpf PK, Mudd JB, Nes WD. The Metabolism, Structure, and Function of Plant Lipids. New York: Springer; 1987. DOI: 10.1007/978-1-4684-5263-1

[19] Nedovic V, Kalusevic A, Manojlovic V, Levic S, Bugarski B. An overview of encapsulation technologies for food applications. In: Saravacos G, Taoukis P, Krokida M, Karathanos V, Lazarides H, Stoforos N, et al., editors. 11th International Congress on Engineering and Food. Amsterdam: Procedia Food Science; 2011. pp. 1806-1815. DOI: 10.1016/j.profoo.2011.09.266

[20] Choi AJ, Kim CJ, Cho YJ, Hwang JK, Kim CT. Characterization of capsaicin-loaded nanoemulsions stabilized with alginate and chitosan by self-assembly. Food and Bioprocess Technology. 2011;**4**(6):1119-1126. DOI: 10.1007/s11947-011-0568-9

[21] Greßler S, Gazsó A, Simkó M, Nentwich M, Fiedeler U. Nanoparticles and nanostructured materials in the food industry. NanoTrust Dossiers. 2010;**4**:1-7. DOI: 10.1016/S1043-4526(09)58005-9

[22] dos Santos PP, Flores SH, Rios AD, Chiste RC. Biodegradable polymers as wall materials to the synthesis of bioactive compound nanocapsules. Trends in Food Science & Technology. 2016;**53**:23-33. DOI: 10.1016/j.tifs.2016.05.005

[23] Galstyan V, Bhandari MP, Sberveglieri V, Sberveglieri G, Comini E. Metal oxide nanostructures in food applications: Quality control and packaging. Chemosensors. 2018;**6**(2):1-21. DOI: 10.3390/chemosensors6020016

[24] Perillo PM, Rodriguez DF. Low temperature trimethylamine flexible gas sensor based on TiO_2 membrane nanotubes. Journal of Alloys and Compounds. 2016;**657**:765-769. DOI: 10.1016/j.jallcom.2015.10.167

[25] McClements DJ. Encapsulation, protection, and delivery of bioactive proteins and peptides using nanoparticle and microparticle systems: A review. Advances in Colloid and Interface Science. 2018;**253**:1-22. DOI: 10.1016/j.cis.2018.02.002

[26] Marsanasco M, Piotrkowski B, Calabro V, Alonso SD, Chiaramoni NS. Bioactive constituents in liposomes incorporated in orange juice as new functional food: Thermal stability, rheological and organoleptic properties. Journal of Food Science and Technology-Mysore. 2015;**52**(12):7828-7838. DOI: 10.1007/s13197-015-1924-y

[27] Sukhorukov GB, Volodkin DV, Gunther AM, Petrov AI, Shenoy DB, Mohwald H. Porous calcium carbonate microparticles as templates for encapsulation of bioactive compounds. Journal of Materials Chemistry. 2004;**14**(14):2073-2081. DOI: 10.1039/b402617a

[28] Commission Regulation (EU) No 1183/2012 [Internet]. 2012. Available from: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32012R1183 [Accessed: 2018-04-28]

[29] Huang JY, Li X, Zhou WB. Safety assessment of nanocomposite for food packaging application. Trends in Food Science & Technology. 2015;**45**(2):187-199. DOI: 10.1016/j.tifs.2015.07.002

[30] Gunell M, Haapanen J, Brobbey KJ, Saarinen JJ, Toivakka M, Makela JM, et al. Antimicrobial characterization of silver nanoparticle-coated surfaces by "touch test" method. Nanotechnology Science and Applications. 2017;**10**:137-145. DOI: 10.2147/nsa.S139505

[31] Chen KH, Ye WJ, Cai SL, Huang L, Zhong TS, Chen LK, et al. Green antimicrobial coating based on quaternised chitosan/organic montmorillonite/Ag NPs nanocomposites. Journal of Experimental Nanoscience. 2016;**11**(17):1360-1371. DOI: 10.1080/17458080.2016.1227095

[32] Duncan TV. Applications of nanotechnology in food packaging and food safety: Barrier materials, antimicrobials and sensors. Journal of Colloid and Interface Science. 2011; **363**(1):1-24. DOI: 10.1016/j.jcis.2011.07.017

[33] Silvestre C, Duraccio D, Cimmino S. Food packaging based on polymer nanomaterials. Progress in Polymer Science. 2011;**36**(12):1766-1782. DOI: 10.1016/j.progpolymsci.2011.02.003

[34] Sarwar MS, Niazi MBK, Jahan Z, Ahmad T, Hussain A. Preparation and characterization of PVA/nanocellulose/Ag nanocomposite films for antimicrobial food packaging. Carbohydrate Polymers. 2018;**184**:453-464. DOI: 10.1016/j.carbpol.2017.12.068

[35] Sahraee S, Ghanbarzadeh B, Milani JM, Hamishehkar H. Development of gelatin bionanocomposite films containing chitin and ZnO nanoparticles. Food and Bioprocess Technology. 2017;**10**(8):1441-1453. DOI: 10.1007/s11947-017-1907-2

[36] Sahraee S, Milani JM, Ghanbarzadeh B, Hamishehkar H. Effect of corn oil on physical, thermal, and antifungal properties of gelatin-based nanocomposite films containing nano chitin. LWT—Food Science and Technology. 2017;**76**:33-39. DOI: 10.1016/j.lwt.2016.10.028

[37] Mackevica A, Olsson ME, Hansen SF. Silver nanoparticle release from commercially available plastic food containers into food simulants. Journal of Nanoparticle Research. 2016;**18**(1):1-11. DOI: 10.1007/s11051-015-3313-x

[38] Weir A, Westerhoff P, Fabricius L, Hristovski K, von Goetz N. Titanium dioxide nanoparticles in food and personal care products. Environmental Science & Technology. 2012;**46**(4):2242-2250. DOI: 10.1021/es204168d

[39] Martirosyan A, Schneider YJ. Engineered nanomaterials in food: Implications for food safety and consumer health. International Journal of Environmental Research and Public Health. 2014;**11**(6):5720-5750. DOI: 10.3390/ijerph110605720

[40] Xu J, Yang FM, An XX, Hu QH. Anticarcinogenic activity of selenium-enriched green tea extracts in vivo. Journal of Agricultural and Food Chemistry. 2007;**55**(13):5349-5353. DOI: 10.1021/jf070568s

[41] European chemicals body links titanium dioxide to cancer [Internet]. 2017. Available from: https://www.chemistryworld.com/news/european-chemicals-body-links-titanium-dioxide-to-cancer/3007557.article [Accessed: 2018-05-23]

[42] Rizk MZ, Ali SA, Hamed MA, El-Rigal NS, Aly HF, Salah HH. Toxicity of titanium dioxide nanoparticles: Effect of dose and time on biochemical disturbance, oxidative stress and genotoxicity in mice. Biomedicine & Pharmacotherapy. 2017;**90**:466-472. DOI: 10.1016/j.biopha.2017.03.089

[43] Aguilar F, Crebelli R, Di Domenico A, Dusemund B, Frutos MJ, Galtier P, et al. Re-evaluation of titanium dioxide (E 171) as a food additive. EFSA Journal. 2016;**14**(9):4545. DOI: 10.2903/j.efsa.2016.4545

[44] Bolognesi C, Castle L, Cravedi JP, Engel KH, Franz R, Fowler P, et al. Safety assessment of the substance zinc oxide, nanoparticles, for use in food contact materials. EFSA Journal. 2016;**14**(3):4408. DOI: 10.2903/j.efsa.2016.4408

[45] Moos PJ, Chung K, Woessner D, Honeggar M, Cutler NS, Veranth JM. ZnO particulate matter requires cell contact for toxicity in human colon cancer cells. Chemical Research in Toxicology. 2010;**23**(4):733-739. DOI: 10.1021/tx900203v

[46] Soni N, Prakash S. Efficacy of fungus mediated silver and gold nanoparticles against *Aedes aegypti* larvae. Parasitology Research. 2012;**110**(1):175-184. DOI: 10.1007/s00436-011-2467-4

[47] Lamsal K, Kim SW, Jung JH, Kim YS, Kim KS, Lee YS. Inhibition effects of silver nanoparticles against powdery mildews on cucumber and pumpkin. Mycobiology. 2011;**39**(1): 26-32. DOI: 10.4489/MYCO.2011.39.1.026

[48] Pineda L, Chwalibog A, Sawosz E, Lauridsen C, Engberg R, Elnif J, et al. Effect of silver nanoparticles on growth performance, metabolism and microbial profile of broiler chickens. Archives of Animal Nutrition. 2012;**66**(5):416-429. DOI: 10.1080/1745039x.2012.710081

[49] Ngô C, Van, De Voorde M. Nanotechnology in a Nutshell: From Simple to Complex Systems. Amsterdam: Atlantis Press; 2014. 1-495 p. DOI: 10.2991/978-94-6239-012-6-1

[50] Nanotea [Internet]. 2007. Available from: http://www.nanotechproject.org/cpi/products/nanotea/ [Accessed: 2018-05-30]

[51] Nanoceuticals™ Slim Shake Chocolate [Internet]. 2007. Available from: http://www.nanotechproject.org/cpi/products/nanoceuticalstm-slim-shake-chocolate/ [Accessed: 2018-05-30]

[52] Canola Active Oil [Internet]. 2007. Available from: http://www.nanotechproject.org/cpi/products/canola-active-oil/ [Accessed: 2018-05-30]

[53] HydraCel [Internet]. Available from: https://behealthyeveryday.eu/en/hydracel/ [Accessed: 2018-30-05]

[54] Nano Calcium [Internet]. 2010. Available from: http://natu-health.com/healthy-bones--and-joint/nano-calcium.html [Accessed: 2018-05-30]

[55] US patent—Production of carotenoid preparations in the form of coldwater-dispersible powders, and the use of the novel carotenoid preparations [Internet]. 1996. Available from: https://patents.google.com/patent/US5968251 [Accessed: 2018-05-30]

[56] LycoVit® [Internet]. Available from: https://nutrition.basf.com/en/Human-nutrition/LycoVit.html [Accessed: 2018-05-30]

[57] NovaSOL Curcumin [Internet]. 2014. Available from: http://novasolcurcumin.com/ [Accessed: 2018-05-30]

[58] Company Overview of NutraLease Ltd. [Internet]. 2018. Available from: www.bloomberg.com/research/stocks/private/snapshot.asp?privcapId=7222521 [Accessed: 2018-05-30]

[59] EUR-lex. Access to European Union Law [Internet]. 2018. Available from: https://eur-lex.europa.eu/homepage.html [Accessed: 2018-05-10]

[60] Amenta V, Aschberger K, Arena M, Bouwmeester H, Moniz FB, Brandhoff P, et al. Regulatory aspects of nanotechnology in the agri/feed/food sector in EU and non-EU countries. Regulatory Toxicology and Pharmacology. 2015;**73**(1):463-476. DOI: 10.1016/j.yrtph.2015.06.016