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Application of Nanotechnology in the Agro-Food Sector

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Summary

Nanotechnology is an emerging field of research that has been widely applied in different scientific and engineering areas. The agro-food sector is not an exception, which considers its applicability in several areas of major interest for both consumers and producers. This review considers major concepts related to nanostructures and nano-based instruments used in the food sector, as well as their applications in agro-food products. Food safety through the use of nanosensors for pathogen detection, smart packaging, and valorisation of food products by nanoencapsulation/nanodelivery of food ingredients (*e.g.* flavours) are examples of important areas of nanotechnology. Consumers' apprehension regarding food stability and safety issues is also considered.

Key words: nanostructures, nanofiltration, food safety, food packaging, consumer safety

Introduction

Despite the scientific and technological progresses achieved in the new millennium, the aim to gain competitive advantage and market share in the agro-food sector requires a continuous update and the development of innovative strategies to improve production efficiency, food safety and food characteristics. In this context, a growing interest in the use of nanotechnology has been observed in different domains of food processing, food packaging, food safety and farm production systems (*1–8*), and so far it seems to have been a success due to the obtained value-added food and agricultural products. Despite the several nanotechnology applications that can be explored in the agro-food industry, their wide use is still very limited mainly due to the consumers' fears. In fact, several of the safety issues raised by consumers and regulators result both from the complexity and from many forms of nanotechnology that can be applied.

Although the food products are naturally composed of nanomaterials, based on the dimension of the particles and structures that constitute them, other nanostructures could be intentionally added or applied to manufactured food products. Over the past few years, nanotechnology has had an impact on various segments of the food and agricultural industry including the production, processing, storage, safety, authenticity and waste reduction. Several examples are reported in the literature concerning the development and application of different nanostructures ranging from intelligent packaging (*1,7*) to interactive food (*i.e.* in the sense that food products contain nanostructures, which can modulate the release of bioactive compounds, *e.g.* flavour or colour enhancers or nutritional elements, in response to nutritional deficiencies or allergies, detected through nanosensors, or personal taste preferences based on the concept of 'on-demand delivery') (*9*). Currently, major food researchers are using

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nanotechnology strategies (*10–14*) in order to: (*i*) improve the quality, safety and biosecurity of food products (*e.g.* nanosensors to detect pathogens or contaminants, and nanodevices to identify preservation and to track stocks); (*ii*) increase the range of food texture, colour and taste (*e.g.* encapsulation of flavour or odour enhancers) and decrease the use of fat, (*iii*) improve the efficiency of nutrient supplements and natural health care products (*e.g.* preparing carriers for protecting or delivering several antioxidant agents and nutraceuticals, such as quercetin, glycyrrhizinate and ellagic acid, resulting in higher stability and oral bioavailability); (*iv*) increase the efficiency of liquid filters; and (*v*) improve the shelf life. Nanotechnology applications are expected to provide more precise food manufacturing equipment, new packaging material, new methods for rapid identification of the authenticity of the products, as well as non-polluting, cheaper, and more efficient food processing techniques (*2*).

The present paper provides a comprehensive review of the latest achievements in nanotechnology applied to foods and food-related systems. Covered topics include nanostructures, nanofiltration, food safety, food packaging and instruments used in this area. An overview of food stability and consumer safety is also briefly discussed.

Concepts of Nanotechnology in Food Industry

Even though there is no precise definition of nanoscale material in the food sector, nanotechnology is commonly associated with the characterization, design, production and/or manipulation of structures, devices or material with submicron diameter (*15*). Nanostructures are defined as having one structural dimension with a diameter smaller than 100 nm (*16*), which are comparable to those of subcellular structures, including cell organelles or biological macromolecules like proteins (*17*). On this scale, the structures should display functional properties that are significantly different from the properties of macroscale material composed of the same substance (*7*). Consequently, interest and activities in this research area have greatly increased over the past years. Some of their characteristics comprise changing important organoleptic properties (*e.g.* colour), good physical stability, conductivity (heat and electrical), chemical reactivity, optical and magnetic properties, which when incorporated into products can enhance or improve their existing properties (*e.g.* enhance oral bioavailability) (*18,19*).

Regarding food applications, nanostructures can be produced by two different approaches, either 'bottom-up' or 'top-down' (*20–22*). The 'bottom-up' approach refers to the manipulation of single atoms and molecules which produce more complex molecular or supramolecular structures by a design based on self-organization of chemical or biological compounds. For example, in the biological application, the organization of casein micelles or starch and the folding of globular proteins and protein aggregates are self-assembling structures that produce stable entities (*21*). Alternatively, the 'top-down' approach is achieved by reducing the size and refinement of the smallest structure to the nanoscale dimension by physical or chemical techniques (*e.g.* mechanical milling, homogenization, ultrasound, lasers and evaporization followed by cooling) (*22,23*). For example, the antioxidant activity of green tea powder has been improved by using dry milling technology (*24*).

One important concept in this area is the so-called 'delivery system', which has essentially originated from research in the pharmaceutical field. However, similar concepts have been explored in foodstuffs. Delivery system is defined as a vehicle that is used to carry a bioactive compound (*e.g.* drug, functional food ingredient) to a desired site of action. In recent years, nanotechnology has been used in the agro-food sector to prepare carriers for delivering several functional food ingredients, such as quercetin, β-lactoglobulin, hesperetin, or tea catechins (*13,25–30*). In order to target a specific site, nanosystems have to be able to control the bioactive release (*26,29,30*). Additionally, nanocarriers can protect the bioactive compounds against degradation (*e.g.* chemical and/or biological) during processing, handling, storage, and utilization (*14,26,31,32*) maintaining the functional ingredient in its active state. Another requirement is its compatibility with other components, with physicochemical benefits and qualitative outcomes (*e.g.* texture) of the final product (*33*).

Nanostructures

A huge variety of nanostructures has been developed to be used in agriculture and food fields. The development of nanostructures to entrap or nanocarriers to deliver functional food ingredients (*e.g.* nutrients, supplements and additives) has been widely explored. As delivery systems, the aim is to develop biocompatible nanocarriers, in which the functional food ingredients (*e.g.* vitamins, probiotics, bioactive peptides, antioxidants and natural health products) are incorporated within the matrix, thus allowing a controlled bioactive release. This approach is very useful as it can protect the sensitive compounds against a range of environmental influences such as in the gastrointestinal tract (*e.g.* pH, enzymes, presence of other nutrients) and conditions encountered in food process (*e.g.* temperature, oxygen, light), but it is also capable of improving their stability and solubility. Consequently, the bioavailability and targeted bioactive delivery can be increased (*34–36*).

Within the agro-food chain, metal or metal oxide-based nanostructures (*e.g.* nano-Ag, nano-ZnO, nano-Cu, nano-TiO_2) have been investigated as pesticides (*37,38*). These particles are known to have different structures and shapes, which can be (homogeneous or heterogeneous) spherical, tubular, irregularly (non-spherical) shaped, or can exist in fused aggregated or agglomerated forms (*39*).

There are several types of nanostructures used to deliver functional food ingredients (*e.g.* nanoemulsions, micelles, liposomes, nanoparticles, cubosomes) (*25*), which can be based on lipids, proteins, polysaccharides, polymeric networks, and even combinations of these compounds (*40*). As far as food is concerned, delivery systems based on lipids, proteins, and/or polysaccharides are the most widely used and promising materials due to the biocompatibility concerns. Nanostructures based on polymers are prepared by the polymerization of more than one type of monomer, frequently combinations of hydrophobic and hydrophilic monomers are used, creating a carrier with opposite affinities for aqueous solvent (*41*), and therefore more versatile in terms of application. Different natural polymers such as albumin (protein), gelatin (protein), alginate (saccharide), collagen (protein), chitosan and pectin (saccharides), α-lactalbumin (milk protein) and zein (corn protein) have been applied (*14,21, 26,29,30,40,42,43*).

The use of protein-based nanostructures to encapsulate compounds is particularly interesting because proteins present: (*i*) unique functional properties, especially those derived from food, (*ii*) high nutritional value, and (*iii*) a less dangerous application in food (*44*). Moreover, proteins offer additional advantages since they are relatively easy to produce and capable of forming complexes with polysaccharides, lipids or other biopolymers, allowing them to be used in a wide range of applications.

Polysaccharide-based nanostructures are also interesting for encapsulation of food ingredients due to both biocompatibility and biotolerability properties. Polysaccharides are polymers of monosaccharides (carbohydrates) that are linked together by glycosidic bonds. In the organism, these polymers are broken down to saccharides by the colonic microflora and are able to protect functional food ingredients from hostile conditions, such as those found in biological systems (*e.g.* stomach acidic pH). The glycosidic linkages are hydrolyzed on arrival in the colon, which triggers the release of the entrapped bioactive compounds. Two polymers frequently used are chitosan and pectin (*40*).

In the last years, several copolymers have been synthesized and used to prepare different nanostructures, such as micelles, nanospheres, polymersomes and nanocapsules (*45*). Polymeric micelles are described as nanoscopic core/shell structures formed by amphiphilic block copolymers. The inner core of these nanostrutures is composed of hydrophobic regions of the amphiphilic molecules in which hydrophobic compounds can be encapsulated (*e.g.* lipids, flavouring agents, antimicrobial agents, vitamins, antioxidants) (*44*). Polymeric nanospheres are solid colloidal particles in which compounds are dissolved, entrapped, encapsulated, chemically bound, or adsorbed to the polymer matrix (*41*). In some situations, it is not easy to differentiate between micelles and nanospheres if the inner core becomes more or less solid-like depending on the copolymer composition (*44*).

Nanocapsules and polymersomes are vesicular systems of colloidal size in which the compound is inside a cavity surrounded by a polymer, which is usually an amphiphilic synthetic block copolymer, membrane or coating. A nanocapsule is formed if the core is composed of an oily liquid and the surrounding polymer is a single layer, which is able to entrap hydrophobic compounds (*e.g.* lipids, hydrophobic vitamins, antioxidants). If the core of the vesicle is an aqueous phase and the surrounding coating is a bilayer polymer, the particle is considered a polymersome.

Nanostructures, such as surfactant micelles, vesicles, bilayers, reversed micelles, and liquid crystals, have been widely used to encapsulate and deliver polar, nonpolar and/or amphiphilic functional ingredients (*46*). They are thermodynamically favourable systems whose formation is normally driven by the hydrophobic effect. A different type of nanostructure depends on: (*i*) the nature of the resultant structures, (*ii*) the concentration and molecular characteristics of the surfactant, and (*iii*) the established environmental conditions. Among these colloidal systems, nanoemulsions are defined as non-equilibrium, heterogeneous systems, which are composed of oil droplets dispersed in an aqueous medium and stabilized by a surfactant. Nanoemulsions differ from conventional emulsions in nanometric droplet sizes (normally in the range of 10–100 nm), which makes them more stable for longer periods (*47*), being often produced by high-pressure valve homogenizers or microfluidizers. Functional food ingredients can be incorporated within the nanodroplets or in the continuous phase of these nanoemulsions (*48*). They are an example of lipid-based nanostructures. Particularly, the nanostructures based on lipids represent one of the most attractive encapsulation technologies due to their additional benefits since after oral administration they are expected to undergo similar metabolic pathways as the food-ingested lipids. Moreover, when compared to other nanostructured materials, lipid-based systems present additional advantages, such as: (*i*) they can be produced using natural ingredients, which are usually similar to the physiological lipids and have Generally Recognized as Safe (GRAS) status for oral and topical administration, therefore, a biocompatible and biodegradable nanocarrier is expected; (*ii*) they have high capacity of modulating the bioactive delivery; (*iii*) they can entrap efficiently compounds with different solubility, and (*iv*) they require low-cost production techniques, avoiding the use of organic solvents, and are easily scaled up to industrial production (*49–51*).

Other interesting lipid-based nanostructures that have been reported as useful for food technologists are nanoliposomes, archaeosomes and nanocochleates (*52,53*). Nanoliposomes are structures of bilayer lipid vesicles (<30 or 30–100 nm). Due to their typical composition, nanoliposomes are versatile colloidal carriers capable of both hydrophilic and hydrophobic compound entrapment within a unique particle (*52*). Moreover, it has been reported that the lipid bilayer of nanoliposomes can fuse with physiological bilayers (*e.g.* cell membranes), mediating the release of functional ingredients into the cells (*e.g.* encountering specific cellular enzymes, due to pH or thermo sensitivity or after the binding of antigen with the tagged antibody) (*54*). In addition, these lipid-based nanostructures have demonstrated good biocompatibility, low toxicity, lack of immune system activation and, therefore, high versatility in use as colloidal carrier systems (*55*). Nanoliposomes have been used in the food industry to encapsulate and control ingredient delivery in functional food (*e.g.* flavours and nutrients), enhancing their biovailability, stability and shelf life (*e.g.* milk proteins) and, more recently, have been investigated for their ability to incorporate antimicrobials that could aid in the protection of food products against microbial contamination (*53*).

Archaeosomes represent another variant of liposome vesicles, which are prepared from archaeobacterial membrane lipids (*56*). The archaeobacterial lipids exhibit unusual lipid structures, which contribute significantly to the vesicle stability (*e.g.* thermostable and stress resistant) and are well tolerated *in vivo* (murine models) (*57*).

Archaeosomes have proved to be an ideal carrier for protection of food supplements, such as antioxidants, during food processing (*e.g.* against chemical influences) (*56*).

Nanocochleates are obtained by the addition of calcium ions to small phosphatidylserine vesicles and they comprise a multilayered structure consisting of a continuous, solid lipid layer sheet rolled up in a spiral fashion with little or no internal aqueous space producing a nanocrystalline structure (*i.e.* microstructural features on a nanometer scale) (*52*), which can also encapsulate and deliver both hydrophilic and hydrophobic compounds. It is well documented that the structures of crystalline and intercrystalline regions vary with decrease in crystallite size. These lipid-based nanostructures deliver their inside content to target cells through fusion mechanisms. Their solid layer protects the 'encochleated' material from harsh environmental conditions (*e.g.* pH) or enzymes, being able to resist the degradation in the gastrointestinal tract, which makes them attractive carriers for oral delivery (*58*). They protect micronutrients and antioxidants from degradation during manufacture and storage (*2*).

Furthermore, nanostructured lipid carrier (NLC) and nanosized self-assembled structured liquid (NSSL) are also demonstrating successful achievements in food technology. NLC is composed of a blend of solid lipid (melting point above 40 °C) within very tiny nanocompartments of liquid lipids (oil), which is solid at both body and room temperatures. NLC usually provides a high payload and prevents bioactive expulsion during storage (*51,59*). Hentschel *et al.* (*60*) reported a sucessful encapsulation of β-carotene in NLC to be dispersed in beverages. NSSL involves the development of minute compressed micelles called 'nanodrops'. These nanodrops serve as liquid carriers, allowing penetration of health components, such as vitamins, minerals and phytochemicals, which are insoluble in water or fats. This technology has been used in the canola active oil by Shemen Industries and they report that the phytosterols added to the micelles can pass effectively the digestive system without breaking down (*61*). Additionally, these colloidal systems compete with cholesterol from the micelles produced by bile acids, inhibiting the move of cholesterol from the digestive system into the blood stream (*62*).

Another interesting nanostructure with potential application in food systems and industrial food processing equipment is the nanotube membrane. Carbon nanotubes represent a newer generation of nanostructures. Carbon nanotubes are tube-shaped structures which are constructed of a hexagonal array of carbon atoms. These structures have interesting features that make them useful in food packaging to improve its mechanical properties. Moreover, Kang *et al.* (*63*) have reported that carbon nanotubes demonstrated powerful antimicrobial activity and that *Escherichia coli* died on immediate contact with long, thin nanotubes.

When combined with molecular biological tools, the developed nanostructures may offer new biotechnological applications including bioanalysis (*64*). This type of nanostructures has already been applied for different purposes such as bioimaging, biosensing or bioactive delivery, with an enormous potential still to be explored (*65*), increasing the prospects of nanotechnology transfer to other scientific and technological areas.

Applications in the Food Industry

Applications of nanotechnology in the food industry are emerging quickly. Considering food, there are two areas of interest, the first which involves food packaging (*i.e.* nanotechnology application outside the food product), and the other concerning the product *per se* (*i.e.* nanotechnology application inside the food product). Therefore, according to Moraru *et al.* (*66*), the four main topics in food industry where nanotechnology could have a significant impact are: (*i*) encapsulation of nutraceuticals and other functional food ingredients to develop new functional material, (*ii*) micro- and nanoscale processing, (*iii*) product development, and (*iv*) design of methods (*e.g.* nanofiltration) and instrumentation (*e.g.* nanosensors) for food safety and biosecurity.

In the last years, some successful examples of food-related nanotechnology applications have included the development of smart delivery systems of nutrients or other food ingredients, rapid sampling of biological and chemical contaminants (*i.e.* food safety), and nanoencapsulation of nutraceuticals for controlled release, water purification and cell-wall rupture (*10,11,44,67,68*). Several applications of nanotechnology in food have recently been reported, but only some examples will be explored.

Application in product per se – food manufacture and encapsulation of food additives

Functional ingredients are essential components of a wide range of industrial products, including pharmaceuticals, natural health-care products (*e.g.* quercetin, glycyrrhizinate, tea cathechins, ellagic acid), cosmetics, agrochemicals, and foodstuff. Examples of functional ingredients used in food products include vitamins, antimicrobials, antioxidants, probiotics, prebiotics, peptides and proteins, carotenoids, omega fatty acids, flavourings, colourants and preservatives, and they can present a variety of different molecular and physical forms. In general, they are not administrated directly in their pure form, but they are often incorporated into the delivery system (*e.g.* nanostructures). Functional ingredients are incorporated into food matrices to develop innovative functional food, known as nutraceuticals. Even though the involvement of these products in physiological functions is not yet fully understood, it is widely accepted that their addition to food systems may have physiological benefits or disease preventing properties (*69*). An example of the benefits is the antihypertensive effect of dietary peptides derived from milk proteins, mediated by angiotensin-converting enzyme inhibition (*70*).

Concerning encapsulation and delivery nanotechnology application, functional food ingredients can be incorporated, absorbed, or dispersed in nanostructures. New food products containing nanostructures have been introduced or are being currently developed for different purposes (*2,25,40,71*): (*i*) to protect nutraceuticals against degradation during manufacturing, distribution and storage, improving their stability, (*ii*) to enhance the bioavailability of poorly soluble functional food ingredients (*e.g.*

hydrophobic vitamins), improving their nutritional value, (*iii*) to increase food shelf life, (*iv*) to produce low fat, carbohydrate or calorie products (*e.g.* mayonnaise, spreads and ice creams), (*v*) to optimize and modify the sensory characteristics of food products creating new consumer sensations (*e.g.* texture, consistency, development of new taste or taste masking, flavour enhancement, colour alteration), (*vi*) to control functional food ingredient delivery (*e.g.* flavour, nutrient). Additionally, nutraceutical digestion or absorption may increase or decrease depending on the structural, chemical, and physical state of the developed nanostructures and the entrappement of the functional food ingredient. Therefore, reducing the size of the encapsulates can be related to prolonged gastrointestinal retention time caused by bioadhesive improvements in the mucus that covers the intestinal epithelium (*72*).

Nanostructures (*e.g.* nanoemulsions, liposomes) containing functional food ingredients (*e.g.* polyphenols, minerals, and micronutrients) can be used to protect them from oxygen and water (*73*), and from harsh conditions of the GI tract (*74*), improving their shelf life. Nanostructures of certain inorganic materials have also been studied as food additives in form of coating to provide moisture or oxygen barrier (*e.g.* silicon dioxide (E551), magnesium oxide (E530), titanium dioxide (E171)) (*75*), and antibacterial 'active' coating, especially silver (*76*). However, very little is confirmed about the specific effect of silver-based nanoparticles on human health which limits their addition directly to foods. The administration of ω-3 polyunsaturated fatty acids can be of major importance due to their effectiveness in preventing diseases (*77,78*). However, due to their high sensitivity to oxidation, they require both stabilization procedures and protection against deterioration factors. A recent study by Zimet *et al.* (*68*) reported about two systems based on casein (casein nanostructures and reformed casein micelles) that showed a remarkable protective effect against docosahexaenoic acid oxidation. These systems proved to have a good colloidal stability and to preserve the effect of their functional ingredient up to 37 days at 4 °C. Recently, natural dipeptide antioxidants (*e.g.* L-carnosine) are receiving increasing attention due to their potential application as biopreservatives in food technology. However, their direct application in food can result in a decrease of their activity due to stability issues (*e.g.* proteolytic degradation and a potential interaction of peptide with food components). In this context, Maherani *et al.* (*79*) have successfully encapsulated these natural antioxidant peptides by nanoliposomes to overcome the limitations related to the direct application in food. BioDeliverySciences International has developed Bioral^{TM} nanocochleate nutrient delivery system, which is a phosphatidylserine carrier (~50 nm), derived from soya bean (GRAS status). This system has been used for protecting micronutrients and antioxidants from degradation during manufacture and storage (*76*).

Although several benefits can be claimed in the development of nano-sized food ingredients, the main aim appears to be the improved uptake, absorption and bioavailability of these ingredients. In this context, nanotechnology renders hydrophilic substances fat soluble and liphophylic ones water soluble, allowing nanostructures of some functional food ingredients (*e.g.* carotenoids, phytosterols, and antioxidants) to be dispersed in water or fruit drinks (*10*). Nanostructures of the synthetic form of tomato carotenoid, lycopene (particle size of 100 nm, from BASF's US patent US5968251), have been developed and accepted as GRAS substance by the Food and Drug Administration (FDA). The water-dispersible lycopene nanostructures can be added to soft drinks to provide colour and health benefits (*80*). For example, the mentioned authors demonstrated that synthetic lycopene in association with vitamin E can inhibit the growth of prostate cancer in nude mice. Lycopene has been included in other products such as baking mixtures and blancmanges (*76*). Another example of nanostructure application in nutraceuticals is related to coenzyme Q10 (CoQ10), which is an antioxidant agent with well-established pharmacological activities. However, CoQ10 is only marketed as a nutritional supplement without any claim of its therapeutic activity probably due to its poor physicochemical and biopharmaceutical properties leading to its low oral bioavailability, which does not allow it to reach its therapeutic concentration easily. Ankola *et al.* (*81*) demonstrated the potential of nanotechnology in improving the therapeutic value of molecules like CoQ10, facilitating its usage as first-line therapeutic agent for prophylaxis and therapy by overcoming the problems associated with its delivery in physiological conditions. These authors developed biodegradable polymeric nanostructures based on poly(lactide-co-glycolide) (PLGA), using quaternary ammonium salt didodecyldimethylammonium bromide as a stabilizer. Essential oils present poor water solubility, which limits their application. Recently, Wu *et al.* (*43*) have demonstrated that the entrapment of essential oils within zein nanostructure allows their dispersion in water, enhancing their potential for use as antioxidant and antimicrobial in food preservation and control of human pathogenic bacterium, *Escherichia coli*. Another example includes catechins (natural health care product), which are present in tea and can act as an antioxidant agent. However, the oral bioavailability of tea catechins is known to be very low probably due to digestive instability and poor intestinal uptake/transport (*82,83*). Recently, Tang *et al.* (*30*) developed self-assembled nanostructures composed of chitosan and an edible polypeptide, poly(γ-glutamic acid), for oral delivery of tea catechins, which can be used as food additives for drinks, foods and dietary supplements. Li *et al.* (*13*) reported that solid lipid nanoparticles are valuable as an oral delivery carrier to enhance the gastrointestinal absorption and, thus, the bioavailability of quercetin, a natural health care product. There are many other nutraceuticals that are poorly absorbed, such as vitamin K2, vitamin E and many phytonutrients, especially the polyphenols and terpenes (*e.g.* carotenoids, chromanols, limonoids and saponins), where nanotechnology has or can have a great potential to enhance the bioavailability and to generate new nutraceutical products.

In this area, different commercial products are currently available. Nanotea, from Shenzhen Become Industry & Trade Co., Ltd., Shenzen, China, is a nano-selenium-enriched tea, which is claimed to enhance selenium uptake and bioavailability in certain regions of China that lack this mineral, providing health benefits (*2*). Novasol®

from Aquanova® AG (Darmstadt, Germany) is a nano-micelle based carrier system for use in food and beverage. Such system can encapsulate a variety of functional food ingredients (*e.g.* benzoic acid, citric acid, vitamins A and E, soya bean isoflavones, β-carotene, lutein, ω-3 fatty acids, CoQ10). An increase in bioactive bioavailability with Novasol® has also been claimed. Nutralease®, developed by the scientists of the Hebrew University of Jerusalem, Israel, is a technology used to improve functional food ingredient solubility and bioavailability (*84*). It is a nanosized self-assembled liquid structure that can carry different nutraceuticals such as coenzyme Q10, lutein, lycopene, vitamins, or phytosterols. Applying this technology, Shemen Industries Ltd. (Haifa, Israel) developed a canola active oil fortified with supplements (*e.g.* phytosterols) (*85*). In this area, another example of a commercial product is Nutri-Nano™ CoQ-10 Solgar (Leonia, NJ, USA), a commercial product which enhances the absorption of fat-soluble nutrients through their conversion into water-soluble ones.

Cushen *et al.* (*22*) reported that the use of nanoemulsions in food products can be a good alternative to reduce significantly the quantity of fat needed. The products produced using these nanoemulsions are as 'creamy' as conventional food products, without compromising the mouthfeel and flavour, being an alternative to full-fat food products. Cushen *et al.* (*22*) suggested that as the size of the droplets in an emulsion is reduced, it is less likely that the emulsion will break down and separate. Therefore, nanoemulsions may reduce the need for certain stabilizers. Such products include low-fat nanostructured mayonnaise, spreads and ice creams (*2,86*).

Additionaly, the undesirable tastes of functional ingredients can be minimised in the finished food product if they are carried in nanostructured delivery systems. Some examples of food additives entrapped into nanostructures are the ones that have been used to mask the taste and odour of tuna fish oil added to bread for health benefits and the addition of live probiotic microbes to promote gut functions (*2*). Wen *et al.* (*87*) described food industry applications of liposomes for oral delivery of functional food ingredients such as proteins, enzymes, flavours, and antimicrobial compounds. An example of this application is the entrapment of proteolytic enzymes in liposomes for cheese production (*52,88*), thus reducing the production time to half without losing flavour and texture properties. The use of zein nanostructures, a major protein found in corn, has also been explored as a vehicle of flavour compounds and essential oils (*e.g.* thymol and carvacrol), because they have the potential to form a tubular network resistant to microorganisms (*21, 43*). Another nanostructure proposed to encapsulate nutraceuticals (*e.g.* vitamins) or to mask disagreeable flavour/odour compounds is the α-lactalbumin nanotube, which can be obtained from milk protein (*42,89*). Based on the origin of these nanostructures, milk protein in the case of α-lactalbumin nanotubes, or corn protein in the case of zein nanostructures, can both be regarded as food grade, which makes their common application in the entrapment of functional food ingredients easier (*22*).

The enhancement of food flavour through the increase on the surface area that hits the taste buds has also been explored. NanoCluster™, from RBC Life Sciences® Inc. (Irving, TX, USA), is another delivery system for food products. Different products have been developed based on Nanoclusters™ system, such as Slim Shake Chocolate, which incorporates silica nanoparticles that are coated with cocoa to enhance the chocolate flavour.

Furthermore, as far as sensorial properties are concerned, the colour of the food products can be altered by nanoemulsion technology. Astete *et al.* (*90*) reported the use of β-carotene, an oil-soluble pigment compound, to colour water-based foods.

Nanofiltration

Nanofiltration includes several aspects of food technology and it may be divided into several fields of application: (*i*) water treatment for reuse, (*ii*) product quality improvement, and (*iii*) molecule isolation.

The application of nanofiltration in water treatment is linked directly to the worldwide increase of human population, which leads to a rising demand for food supply. The growth of food needs has been pressuring the agriculture sector in order to intensify production through the increasing usage of pesticides, herbicides and fertilizeres. The huge amount of residues produced by these practices is emerging as water contaminants. The same is true when considering animal farms, which have problems related to animal detritus and also to the antibiotic and hormone usage. Nanofiltration has been successfully applied in drinking water treatment plants (*91–93*). However, not all pesticides presented in the guidelines for drinking water by World Health Organization (*94*) have been considered in the referred study.

Nanofiltration has been reported to be directly used in combination with powdered activated carbon to remove effluent organic matter from a municipal wastewater (*95*). The combined effect revealed to be a promising treatment for high quality water reuse applications, especially for direct injection. Many membrane technologies have been applied to the dairy industry to treat water for further reuse in its processes (*96*).

Olive oil production at an industrial level produces large amounts of wastewaters that are considered highly polluting for the enviroment, since the contaminants are highly concentrated (*97*). A combined process using microfiltration followed by nanofiltration allowed to recover almost all available polyphenols, which are considered as pollutants, and to use these subproducts for preparing food, cosmetic and pharmaceutical formulations (*98*).

Because nanofiltration can separate low-molecular mass solutes from mineral salt solutions, the technique is very attractive for industrial applications in the food and pharmaceutical industries. In dairy industry nanofiltration is also used to improve the quality of the products (*96*), and to separate mineral salts from lactose, after removing the proteins by ultrafiltration. Both proteins and lactose can be used as raw materials to prepare a variety of products. Whey is the main by-product obtained from cheese production. Worldwide an increasing amount of whey is industrially processed to produce whey powders and other high-quality, protein-rich products meant for human or animal consumption. However, whey contains high salt levels which need to be removed. The only way to achieve this goal is through its concentration and demineralization, which is only possible using nanofiltration. Nanofiltration membranes have a high permeability for (monovalent) salts (*e.g.* NaCl or KCl) and a very low permeability for organic compounds (*e.g.* lactose, proteins, and urea). The separation mechanisms are still not clear, and are determined by complex steric and electrical effects or electroneutrality principle such as in Donnan effect (*99*). The nanofiltration membrane behaviour is influenced by the feed solution characteristics (*100*) and by the membrane material properties (*101,102*).

Similar applications may be found in other by-products. Processing xylan-containing raw materials in aqueous media under suitable operational conditions generates xylooligosaccharides, which have food, medical, and pharmaceutical applications. It was possible to fractionate and purify xylooligosaccharides from monosaccharides and other low-molecular mass materials, such as salts, from rice husk xylan using nanofiltration; the concentrate presented a purity of over 91 % and an overall yield of 71 % (*103*).

Nanofiltration application in the processing of rosemary extracts achieved an extract at a concentration that would allow a direct application as preservative and functional ingredient in the food, cosmetic, nutraceutical and medical areas (*104*). The capability of the Duramem® 200 (Evonik MET Ltd., Wembley, UK) nanofiltration membrane to separate monophenolic acids from higher molecular mass antioxidant compounds in the extracts was observed with a molecular mass cut-off of 200 Da at a pressure of 20 bar (*105*). These results open a new field of application on a variety of aromatic plants.

Anthocyanins comprise the largest group of water-soluble pigments in plants. They are highly appreciated by the food industry for their colouring properties and also because of their potential antioxidant properties (*105*). Cissé *et al.* (*106*) were able to concentrate roselle extract from 4 to 25 g of total soluble solids per 100 g, with 100 % retention of anthocyanins using ten nanofiltration flat-sheet membranes and eight tight ultrafiltration membranes.

Food safety

Quality and safety assurance in food products is of extreme importance since it is a major demand imposed by consumers. The stringent regulations imposed by governmental agencies are used to ensure food product safety and feed hygiene (*107*).

One of the major problems related to food safety concerns is the detection of microorganisms, which represent a challenge, because the initial levels of contamination are generally very low and the food supplies have limited shelf life, requiring an adequate sampling which is usually difficult to achieve (*108*). Therefore, rapid detection and determination of pathogens with a high degree of specification and sensitivity are crucial for maintaining a safe and high quality food supply (*108*).

Methods based on immunosensing or nucleic acid detection are advantageous for both pathogen recognition and for adulterous detection through the food chain. The immunosensing is based on the interaction between antigens presented on the target cells and antibodies immobilized on surfaces. The nucleic acid-based sensors detect DNA or RNA originating from target cells by exploring the complementary property of nucleic acid sequence interaction. Normally, biomolecules are conjugated to nanomaterials using covalent reactions. The nanodevice approach can use aptamers, single-stranded oligonucleic acid-based binding molecules and antibodies that can bind a wide range of targets with high affinity and specificity.

Synthetic DNA barcodes, such as nanobarcodes, have been used in the last years to detect the presence of food pathogens by measuring the reaction using a fluorescent probe under ultraviolet light. When the DNA barcodes, present in the films, conjugate with the tagged pathogens, the derived compound fluoresces, allowing to monitor the presence of a defined pathogen (*23*). The conjugation of biomolecules with nanomaterials is the foundation for nanobiorecognition, which has been explored for the detection of contamination and infectious diseases (*109*).

Amagliani *et al.* (*110*) detected the contamination with *Listeria monocytogenes* cells of 10 CFU/mL in milk samples using immobilized oligonucleotide probes to magnetic nanoparticles. However, this detection included a PCR reaction that confirmed a dose-dependent inhibitory effect.

Researchers at the University of Pennsylvania (Walnut, PA, USA) and Monell Chemical Senses Center (Philadelphia, PA, USA) have used nanosized carbon tubes (as transmitter) coated with strands of DNA (as a sensor) to create nanosensors with the ability to detect odours and tastes (*23*). Using similar technologies, electronic tongue nanosensors are being developed to detect substances in parts per trillion, which could be used to trigger colour changes in food packages to alert consumers when food is spoiled (*111*).

Boehm *et al.* (*112*) have constructed a microfluidic sensor for bacterial detection based on measuring the impedance in a fixed-volume chamber containing cells functionalized with antibodies specific to target cells. These authors were able to discriminate two bacterial strains, *E. coli* and *Moraxella catarrhalis*, in a few minutes. The sensor was able to detect $9\cdot10^5$ CFU/mL of *E. coli* cells.

Other types of technology have been developed in order to achieve the same goal, using various approaches: atomic force microscopy (AFM), which allows both qualitative and quantitative microorganism determination, using as reference pre-isolated strains of *E. coli*; the main advantage of this approach is the rapid determination of microorganisms (*113*); nanofunctionalized gold electrode applied for rapid quantification of bacteria in milk, based on the catalysis of lipid peroxidation on cell membrane of bacteria by nanoporous gold film (detection range $1.1\cdot10^3$–$2.5\cdot10^7$ CFU/mL within 1 h) (*114*); and functionalized single-walled carbon nanotubes with multivalent carbohydrate ligands on their surface, which allowed an efficient capture/detection of pathogenic *E. coli* cells (*115*). Several examples concerning this line of research are presented in a review by Heo and Hua (*116*).

Some of the advantages that have been found with these nanodevices for foodborne pathogen detection are referred to by Yang and Wang (*113*), Tallury *et al.* (*109*) and Magnuson *et al.* (*11*) as: (*i*) rapid and real-time detection, (*ii*) detection sensitivity improvement, (*iii*) simultaneous detection of multiple pathogens due to their high selectivity, (*iv*) low cost, and (*v*) portability. Consumer health can be protected considerably with these devices.

Food packaging

Research on the use of nanocomposites for food packaging started in the 1990s. Protective coating and suitable packaging handling by the food industry have become an interesting topic due to their potential capacity to increase the food product shelf life (*117*). Food processing and packaging industries spend around 15 % of the total variable costs on packaging materials (*118*). It is estimated that in the next decade, nanotechnology will have an impact of 25 % on the food packaging market, currently valued at $100 billion (*119*). Consumers' needs and demands request innovation in food and beverage packaging, which is influenced by changes in global trends, such as life expectancy and the decrease in the number of organizations responsible for food production and distribution (*120*). The most important factors in terms of food packaging are environmental conditions such as heat, light, moisture, oxygen, pressure, enzymes, spurious odours, microorganisms, insects, dirt and dust particles, gaseous emissions, among others (*23,121*). All these environmental conditions, when not controlled, can cause food deterioration (*23,121*).

The use of nanotechnology to improve food packaging material may introduce many innovations in the form of barrier and mechanical properties, pathogen detection, and smart and active packaging (*e.g.* containing nanosensors and antimicrobial activators), resulting in the increase of food safety and quality benefits for the food product (*23,100,119*).

Silver nanoparticles are the most widely used materials for antimicrobial purposes (*122,123*), due to their capacity to restrain bacterial growth. Motlagh *et al.* (*123*) studied the effect that micrometer-sized silver particles had when included in low-density polyethylene (LDPE) packages in terms of microbial and sensory factors of dried barberry. Both microbial growth inhibition (bacteria and mould) and sensorial parameters (taste, aroma, appearance, and total acceptance) were significantly increased when 1 to 2 % of silver particles were added to LDPE packages.

In order to improve barrier and mechanical properties of plastics, inorganic aluminium platelets have been self-assembled into wagon-wheel (nano-wheel) structures (*124*). Montmorillonite clay has also been widely used as nanocomponent of polymers such as polyethylene, nylon, polyvinyl chloride, and starch. These silicate nanoparticles are interspersed in polyamide films functioning as oxygen and carbon dioxide blockers, promoting an increase of the moisture of fresh meat and other food. The final package based on these nanoparticles is considerably lighter, stronger, and more heat-resistant in comparison with others (*125*).

With the increasing amount of food packaging disposal, a new field of research has been developed that considers biodegradable nanocomposite food packages. By pumping carbohydrates and clay fillers through high shear cells, films can be produced with exfoliated clay layers. These are very efficient as moisture barriers and increase the film strength significantly. Starch and chitosan are two of the most studied biodegradable matrices (*33*). However, efficient mechanical, oxygen and moisture protection are not achieved with starch-based polymers. Recently, starch/clay nanocomposite films have been obtained by dispersing montmorillonite nanoparticles *via* polymer melt processing (*126*). Nevertheless, efficient mechanical, oxygen and moisture protection were not achieved with these starch-based polymers. Mechanical characterization results demonstrated an increase of modulus and tensile strength, overcoming the problems of starch films (*126*). Recently, Arora and Padua (*127*) used montmorillonite and kaolinite clays, which showed good potential as compatible filler-polymer systems. However, new studies need to be conducted in relation to the processing technologies. Carbon nanotubes can also be used in food packaging, improving mechanical properties and having powerful antimicrobial effect (*23,120*).

Active cellulose-based packaging materials have been prepared through lysozyme binding to paper modified with anionic polyelectrolytes, which was optimized with carboxymethylcellulose or polygalacturonic acid (*128*). This type of packaging guarantees the lysozyme lytic (and therefore, antimicrobial) activity against the *Micrococcus lysodeikticus* cell wall (*128*).

The addition of nanosensors to food packages can be anticipated in the near future. Nanosensors could be used to detect chemicals, pathogens, and toxins in foods. A colour-changing film that could find its way into food packages is a polymer opal film. It belongs to a class of materials known as photonic crystals (*129*). These photonic crystals could be used to produce unique food packaging materials that change colour (*23*). Photonic crystal fibres were successfully used in apple juice to evaluate sugar composition and can be applied to other food products to determine quality parameters (*130*).

The nanocomposites used for packaging purposes have been studied and reports have suggested an improvement of properties regarding: durability (*131*), temperature resistance (*132*), flame resistance (*133*), barrier properties (*134*), optical properties (*131*), processability due to lower viscosity (*135*), and recycling properties (*136*). With this it was possible to develop a group of nanoparticle-reinforced polymers that normally contain up to 5 % (by mass) nanoparticles. Recently, new concerns related to food packaging have arisen because studies reported on the migration of nanoparticle composites used in packaging to food (*137*), leading to a new field of investigation (*119*).

Instruments Used for Nanoparticle Characterization

The main interest in developing and using nanosized materials in food industry is the fact that they can exhibit new and improved physical, chemical and biological properties, phenomena and functionality of food products (*138*). However, characterizing these materials can be a demanding task. This is also, by itself, an emerging field with old and new characterization techniques being evolved and developed to cope with the challenges imposed by the nanosize (*139*). In these materials there is a strong relation between the dimension, shape and surface morphology and the exhibited properties, and that fuelled the research activity on the invention of characterization techniques of nanomaterials.

Traditionally, in food science, optical microscopes are used for sample observation. Nevertheless, optical aberrations and the wavelength of visible light limit the observation, with reasonable resolution, to the microscale features, rendering these instruments useless for the observation of nanosized materials and therefore other imaging techniques have to be used.

One of the most versatile imaging techniques is the scanning electron microscopy (SEM). This instrument uses a high energy beam of electrons to scan the sample surface. The interaction of these electrons with the sample atoms generates secondary electrons, scattered electrons and characteristic X-rays that contain information about the sample surface topography and composition, among others. The generated images are black and white and can reveal details down to 1–5 nm in size and achieve magnifications up to 300 000 times. SEM has been used to observe the morphology of polysaccharide nanoparticles (*140*) and to study protein nanospheres for the encapsulation of essential oils to maximize the antimicrobial properties of the oils (*141*).

Generally, when a SEM is associated with an energy dispersive X-ray (EDX) system, it can be used to analyze the surface composition and estimate the element proportion. The incident 10–20 keV beam generates X-ray emission from the sample and the energy from these emitted X-rays depends on the atomic number of the surface constituents. With this technique, the composition of nanoparticles near or at their surface can be estimated provided they contain some heavy metal ions.

Another technique using a beam of electrons is the transmission electron microscopy (TEM). In this technique the electron beams are transmitted through an ultrathin sample and interact with the sample structure as they pass through it. A TEM system is capable of resolution of the order of 0.2 nm (*142*). It has already proved to be a suitable technique to image and characterize various kinds of food nanoparticles, as in milk protein-based nanotubes (*42*), the shape of serum albumin nanoparticles (*143*), and the fabrication of protein-functionalized microtubes (*144*). More recently, it has been used to characterize the structure of gold nanoparticles deposited on β-FeOOH nanorods for detecting melamine in aqueous solutions (*145*).

One of the most suitable techniques for the quantitation of surface roughness on the nanometer scale and for visualizing the surface nanostructure is the atomic force microscopy (AFM). This is a nondestructive technique where a sharp tip of a probe (around 10 nm) located near the end of a cantilever beam is raster scanned across the surface of a specimen using piezoelectric scanners. When the tip is brought into the proximity of the sample surface the interaction forces between the tip and the surface change the cantilever deflection. Monitoring the change in this deflection when the sample surface is scanned provides the information required to create a high resolution image of the scanned area. The AFM can detect different types of forces, depending on the particular situation, including mechanical contact forces, van der Waals forces, capillary forces, magnetic forces, electrostatic forces, *etc*. It presents several advantages in relation to other microscopes, such as: (*i*) it does not require a vacuum chamber to operate, (*ii*) it can detect atomic-scale features, (*iii*) it does not need previous sample preparation, maintaining the sample native status, (*iv*) it can acquire 2D and limited 3D images, (*v*) it is possible to observe real-time processes, and (*vi*) it can be used to manipulate and research the interactions between macromolecules (*146*). AFM has been applied to investigate fine food molecular structure and molecular interaction on nanoscale. It has been successfully applied in qualitative and quantitative analysis of macromolecule structure, molecular interaction, and molecular manipulation (*147*). AFM was also used to obtain the surface morphology of chitosan-capped quantum dots and their size distribution, which can be used for the detection of waterborne bacterial pathogens (*148*).

The chemical behaviour of nanostructures is also a key aspect that needs to be fully characterized and understood. Some of this information can be acquired using specific spectroscopy techniques. Of the several types of spectroscopy techniques available, we will describe only two in their most basic configuration that have been broadly used for the characterization of nanomaterials: Raman spectroscopy and ultraviolet-visible spectroscopy.

Raman spectroscopy can be used to study vibrational, rotational, and other low frequency modes in a system. To obtain that information a monochromatic laser is used to interact with phonons or other types of excitations in the sample resulting in inelastic scattering (also called Raman scattering). As a consequence, the energy of laser photons is shifted up or down and the detection of this energy shift provides the information about the phonon modes in the system, which is very specific for the chemical bonds in molecules. It can then be used to fingerprint the molecule present in the nanomaterial. Most of Raman systems have the detection wavenumber range between 500 and 2000 cm^{-1}. The recent development of surface-enhanced Raman spectroscopy, a new variant, where metal nanoparticles are added to the analyte, enhancing the Raman scattering by several orders of magnitude, allows the development of inspection tools for food safety applications (*149,150*).

Ultraviolet-visible (UV-VIS) spectroscopy also uses propagation of light through the sample to obtain the absorption spectra. A UV-VIS spectrometer uses a light source to create reference and a sample beam, a monochromator and a detector. The source beam propagates through the sample and when the wavelength corresponds to one energy level, the energy is absorbed. The detector will then record the ratio between the reference and the sample intensity. This technique was used to characterize ZnS quantum dots doped with Mn^{2+}, which can be used for sensing and identification of waterborne bacterial pathogens (*148*).

The size of nanoparticles can also be assessed using a non-invasive technique called dynamic light scattering (DLS). The constituent particles or molecules of a suspension have a Brownian motion imposed by the solvent molecules that are also in movement due to their thermal energy. If this suspension is illuminated with a laser, the intensity of the scattered light will fluctuate at a rate that is dependent on the particle size. The analysis of the intensity fluctuations will give the velocity of the Brownian motion and the particle size (*151,152*).

Another well-established technique for nanoparticle characterization is X-ray diffraction (XRD). This technique is broadly used in materials science to retrieve information about the crystalline structure, quality and grain size dimension. In this technique, a beam of X-rays is steered to the sample and its crystalline structure will diffract the beam of light into specific directions. From the angles and intensities of these diffracted beams, the mean position of the atoms in the crystal, their chemical bonds and also their disorder can be determined. This technique was used to characterize $MtCu^{2+}$/LDPE nanocomposites, which have a potential use in food packaging (*153*).

Nanotechnology in Food Stability and Consumer Safety

The application of nanotechnology in the area of food science and technology is appealing because of all the benefits and new openings it promises, namely the stability of the new products. In the last few years, many researchers have demonstrated that encapsulation of bioactive compounds in colloidal carrier systems is realistic and can overcome issues associated with slow and low uptake and thermodynamic instability (*25*). Graveland-Bikker and De Kruif (*42*) described the stability of the α-lactalbumin nanotubes under a variety of conditions. The application of nanotechnology to improve food packaging material may introduce many innovations in the form of barrier and mechanical properties, detection of potential pathogens, and smart and active packaging with food safety and quality benefits (*23*).

The ultrasmall size of nanostructures associated with the chemical composition and surface structure provides not only unique features and huge potential applications but also potential toxicological properties (*154*). Although the potential benefits of nanotechnology in several areas are well emphasized, it adversely affects the safety aspects of its application in food production, and the incorporation of manufactured nanostructures into food products is not well known. Therefore, it is imperative that regulatory frameworks for nanotechnologies applied to food are defined based on scientific arguments, mainly due to the fast emergence of nanotechnology applications in the food area and the uncertainty about its negative impacts on biological systems (*2,39,154–156*). In a recent publication, Bouwmeester *et al.* (*39*) have offered an updated overview concerning the scientific issues that need to improve the existing risk assessment methodology, good governance and regulatory framework of the application of nanotechnology in the food sector. In another publication, Chau *et al.* (*67*) suggest different criteria and recommendations that should be considered for the development of standards, definition, control measures, and regulations for nanofood products. Abbott and Maynard (*157*) point out the real challenges that need to be addressed when evaluating the unique characteristics of nanostructures present in the nanofoods. These authors suggest that in order to evaluate the real effect of the nanostructure, basic characteristics of nanoscale materials (*e.g.* particle number mass size distribution, charge) and different exposure assessments to the nanomaterials (*e.g.* occupational, consumer and environmental) may have to be considered, which are not suitable for measuring functional or structural properties when food is altered during digestion and the function may change during this process. As the application of nanostructures in consumed products is recent, the literature on its potential toxicity is quickly increasing (*2,39,67*). However, toxicity results are often obtained for individual nanostructure type and size. When considering only the nanostructure size, the interaction with the biological system may alter significantly with this unique property and affect both toxicokinetic and toxicodynamic applications. In the use of novel nanofood products, the particle size should be considered as an explicit criterion to trigger a reassessment process. The consumer safety implications from nanotechnology applications in food are related to the physicochemical nature of the developed nanostructure (*i.e.* chemical composition, size, particle form, surface functionalization and charge, porosity and aggregation tendency) and the likelihood and extent of exposure through consumption of nanofoods (*2,16,158*). According to Dreher (*159*), several aspects may be considered in order to evaluate the nanotoxicity: (*i*) exposure assessment of nanostructures, (*ii*) toxicology of manufactured nanoparticles, (*iii*) possibility to use existing toxicological databases of nanostructures, (*iv*) environmental and biological fate, transport, and persistence of manufactured nanoparticles, and (*v*) transformation and recyclability, and overall sustainability of nanostructures. Although a nanostructure might cause harmful effects beyond expectations, some studies have also demonstrated that the development and processing procedure may not necessarily produce products with such effect (*160*).

Regarding the application of nanotechnology in food packaging material, the main risk of consumer exposure is through potential migration of nanostructures into food and beverages. Up to date, there is a lack of information on the extent of such migration. Avella *et al.* (*126*) reported that the migration of metals would be minimal. Another way of exposure to nanostructures can occur through the ingestion of food matrix which contains them (engineered organic or inorganic) by design (22).

To conclude, in food industry, despite the amount of regulatory framework to control the potential risks, most current frameworks are not designed to cope explicitly with the new challenges created by the advent of nanotechnology (*57*). For nanoproducts, there are no special regulations that go beyond the general food law prescriptions. In the EU the Regulation (EC) No 178/2002 is laying down the general principles and requirements of food law, established by the European Food Safety Authority and describes procedures in matters of food safety (*161*). Several organizations are already involved in nanotechnology research, regulations, and guidelines, in which risk assessors work alongside toxicologists and food technologists among others (*22,67,162*). Regarding the impact of nanotechnology on food products and packaging materials, deficiencies in regulations were already detected by the Institute of Food Science and Technology in 2006 (*163*). However, nanostructures can be naturally present in food products, *e.g.* milk (milk proteins, casein), with a significant difference between such 'natural' nanostructures and many engineered nanostructures in their totally different biological degradation behaviour (*158*).

Conclusions

With regard to the application of nanotechnology in food industry, promising results have already been developed in several areas including food manufacturing, packaging, safety and storage. The incorporation of nanostructures into final food products will improve different properties: protection and stability of functional food ingredient, bioavailability and shelf-life improvement, development of new consumer sensation and efficient delivery of bioactive substances into biological systems.

The most widely applied nanocarriers consist of natural molecules, such as lipids, proteins or polysaccharides. The reason for their wide application is based mainly on the excellent biocompatibility presented by such carriers. Nevertheless, these vehicles are also able to overcome the harsh conditions that food products are submitted to during digestion allowing the release of intact functional ingredients in desired sites.

Recent research has taken place in the area of nanofiltration, with exciting results in the reuse of wastewater and in the recovery of compounds of interest in several areas of application**.** However, due to its novelty, there are still lots of uncertainties that need to be explained and attended so it can be more widely applied.

The application of nanosensors in food products is an emerging area of utility, and it is very relevant when considering the consumer protection and safety. The inclusion of biosensors into different packaging material may increase significantly the consumer protection against foodborne pathogens, toxins and adulteration, mainly because it can result in quick and visual detection. A wider application of such intelligent packaging may in a long term save a large number of lives. However, concerns related to the security of the nanoparticles used for food packaging (*e.g.* stability, toxicity, *etc.*) can limit their wider application because their properties are not well studied.

Nowadays, more studies are requested in several areas considering the emerging applications of nanotechnology in the food sector, nanotoxicity, regulation and potential risk evaluation and benefits to sustain its development. For regulatory consideration of a nanofood product, it is necessary to establish the nanotechnology product statutory classification.

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