Research Article

Nanoencapsulation Development for Interactive Foods

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Abstract

Nanoscience and Nanotechnology is an advance branch of science which represents new frontiers of this century. Nanotechnology has been applied successfully in the field of medicines, computer, electronics, cosmetics and very recently have found application in agriculture and food sector. It provides benefits not just within food products but also around food products. Food nanotechnology involves use of nanocapsules for improved taste, flavor, color, texture, and consistency of foodstuffs, increased absorption and bioavailability of nutraceuticals and health supplements, development of food antimicrobials, new food packaging materials with improved mechanical, barrier, and antimicrobial properties. Nanoencapsulation is one of the most feasible technique that protects and allows for controlled release of bioactive compounds at a particular time and place. It allows for targeted site specific delivery and cell absorption of bioactive compounds. Generally, nutrients that are less soluble in water (vitamins and antioxidants) are targeted for nanoencapsulation. The technologies of nanoencapsulation of foods, including spray drying, spray cooling, lyophilization, coacervation, fluidized bed coating, inclusion complexation and freeze drying are discussed in this review. In this review, research and developments, applications, techniques and trends are discussed in detail. In today's scenario, Nanoencapsulation technology has found great potential in food and agriculture sector and thus developed into a large-scale business of multimillion market and hold.

Keywords: Nanotechnology, Nanocapsules, Nanoencapsulation, Interactive foods, Nanoparticles, Food technology

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Introduction

In the vast field of the 21st century, nanotechnology is creating a very significant impact on the world's economy, industry and society [1]. The word 'nano' is derived from a Greek word, which means dwarf. The term nanotechnology was coined by late Norio Taniguchi in 1974, [2]. According to Pehanich [3], nanotechnology is the understanding and control of matter at dimensions of roughly 1 to 100 nanometers. To be more specific, nanotechnology is defined as the design, production and application of structures, devices, and systems through control of the size and shape of the material at the nanometer (10^{-9}m) scale where unique phenomenon enable novel applications [4]. Nanotechnology is an advanced branch of science that impacts a number of other fields, including medicine, cosmetics, agriculture, and food. In Food nanotechnology, nanometric materials can change the structure, texture, and quality of foodstuffs pertaining to improved taste, flavor, color, texture, and consistency of foodstuffs, increased absorption and bioavailability of nutraceuticals and health supplements. It also includes development of food antimicrobials, new food packaging materials with improved mechanical, barrier, and antimicrobial properties, nanosensors for traceability and monitoring the condition of food during transport and storage [5]. Nanotechnology has a vast potential to impact many sectors of food science & technology systems as shown in **Figure 1**.

The two approaches to attain/accomplish nanomaterials are *top-down approach* and *bottom-up approach*. In "topdown" approach, nanometre size is achieved by employing processes such as grinding, milling, etching and lithography. In contrast, self-assembly and self-organization are concepts derived from biology which are used in bottom-up nanotechnology [6-9]. The Food nanotechnology will be covered under two major heads viz. food additives (nano inside) and food packaging (nano outside).

Nano outside Food Contact Materials (FCMs), an application of nano packaging are defined as nano-outside and are materials with which food interacts either directly or indirectly during its production, processing, storage and preparation. FCMs falls into two categories viz. "Active" and "Intelligent" [10]. The former incorporate nanoparticles which have antimicrobial or oxygen scavenging properties as shown in **Figure 2**, while "Intelligent" or "smart" food packaging make use of nanosensors for sensing and signaling of microbial and biochemical changes [5, 8, 11, 12]. Nanostructures of inorganic materials have also been studied as a coating material to provide moisture or oxygen barrier properties (e.g. silicon dioxide (E551), magnesium oxide (E530), titanium dioxide (E171)). The ultimate aim of nano-packaging is to set longer shelf life. For example, Du Pont announced the release of a nano-titanium dioxide plastic additive namely "DuPont light stabilizer210", which holds the potential to reduce UV damage of foods in transparent packaging [13, 14].

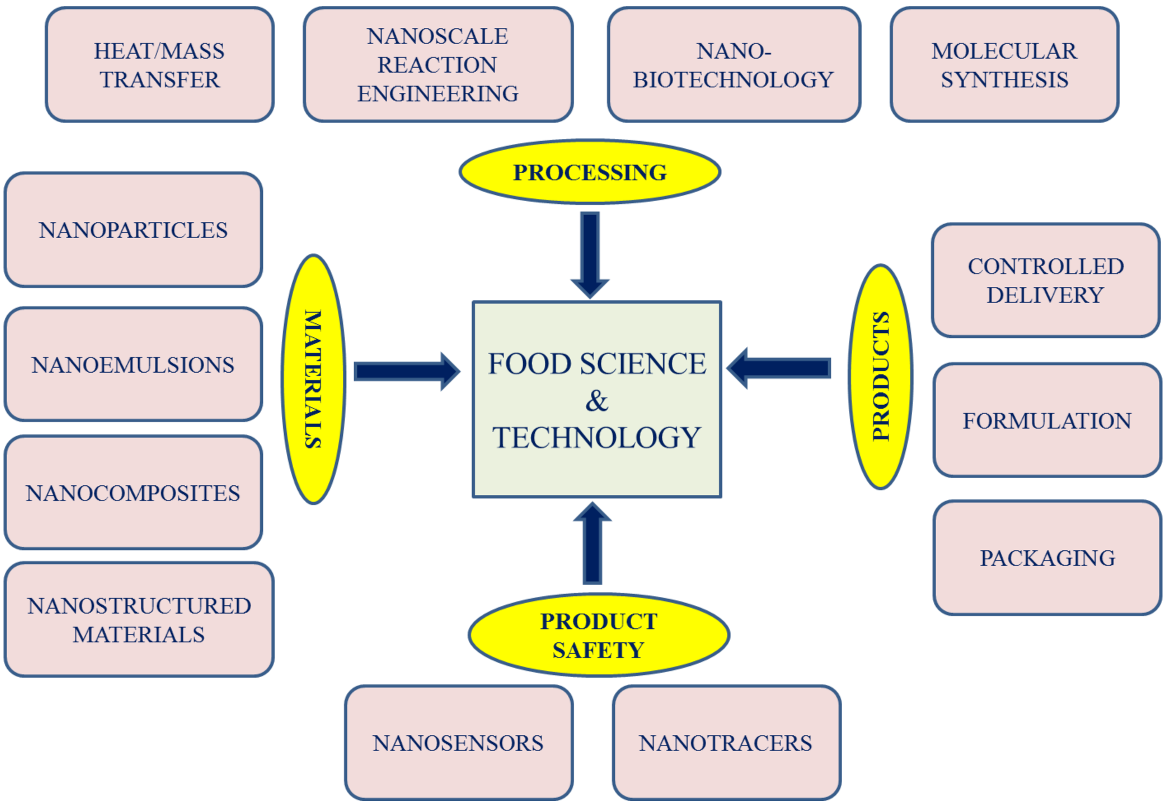

Figure 1 Applications of nanotechnology in food science

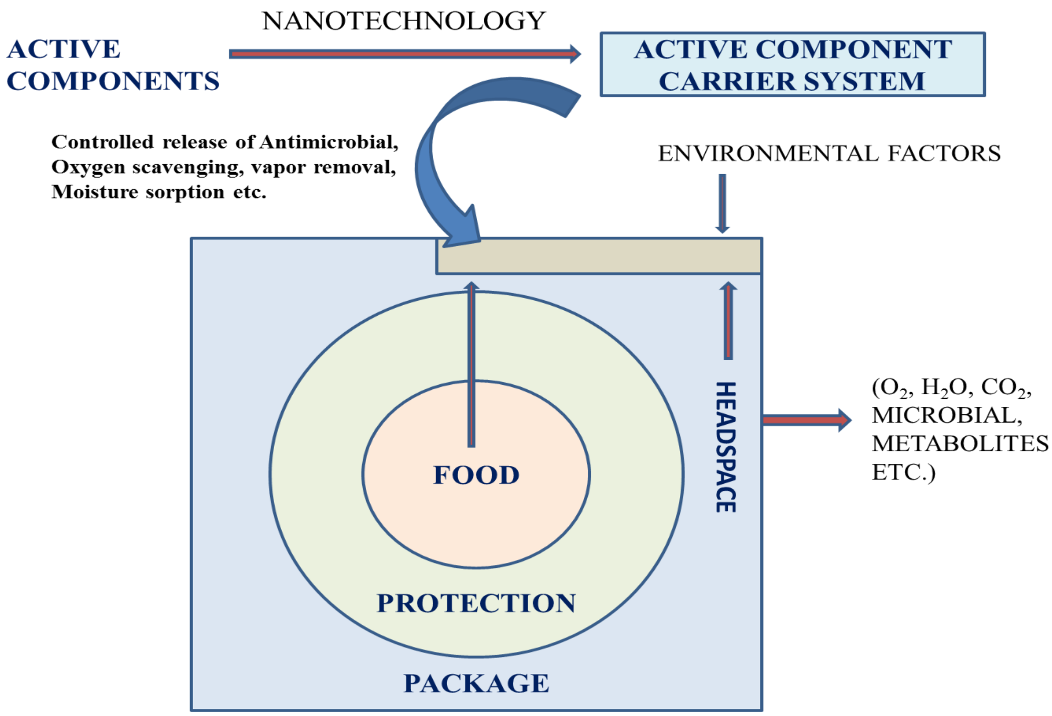

Figure 2 The active packaging concept

Nano inside

This is another area of application involving the use of nano-sized or nano-encapsulated food additives. This type of application is expected to exploit a much larger segment of the (health) food sector, encompassing colors, preservatives, flavorings and supplements. The main advantage is said to be better dispensability of water-insoluble additives in foodstuffs without the use of additional fat or surfactants, and that produce interactive desired colors and flavors by adding nanocapsules that rupture at different microwave frequencies [15], also enhanced tastes and flavors due to enlarged surface area of nano-sized additives over conventional forms thus offering the consumer a healthier option. Products of this type include low fat nanostructured mayonnaise, spreads, ice creams and SlimShake Chocolate, which incorporates silica nanoparticles that are coated with cocoa to enhance the chocolate flavor [16]. Afroz et al. [17] studied a range of food products containing nano-sized additives already available in the supplements and nutraceuticals which include bioactive compounds and are rarely utilized directly in their pure form; instead, they are often incorporated into some form of delivery system such as canola oil (nanosized micellar system) provide delivery of materials such as vitamins and minerals and a wide range of nanoceutical products containing nanocages or nanoclusters that act as delivery vehicles, e.g., a chocolate drink claimed to be sufficiently sweet without added sugar or sweeteners. Weiss et al. [18] examined a wide variety of delivery systems developed to encapsulate functional ingredients, including simple solutions, association colloids, emulsions, biopolymer matrices, and so on. Each type of delivery system has its own specific advantages and disadvantages for encapsulation, protection, and delivery of functional ingredients, as well as cost, regulatory status, ease of use, biodegradability, and biocompatibility.

Nanoencapsulation

Encapsulation represents great potential regarding the development of innovative and functional products in the food industry [19]. The term nanoencapsulation describes the application of encapsulation on the nanometer scale with films, layers, coverings, or simply micro-dispersion and it is an important field of nanotechnology that involves entrapping of bioactive agents within carrier materials with a dimension in nano-scale to form nano capsules which are within the size ranges from 10 to 1000 nm [20]. Nanocapsules differ from nanospheres when the bioactive systems are dispersed uniformly [21]. Enormous demands for production of functional food with higher nutritional value, lower dose of synthetic preservatives and better organoleptic features lead to innumerable applications of nanoencapsulation in food processing. For example, this technology has been used to enhance the stability of sensitive compounds during production, storage and ingestion, e.g. vitamins, to decrease evaporation and degradation of volatile bioactives, e.g. aromas, mask unpleasant tastes, polyphenols or limit exposure to oxygen, water or light and unsaturated fatty acids [22]. In addition to the above, encapsulation can be applied for modification of physical characteristics of the original material in order to (a) allow easier handling, (b) to help separate the components of the mixture that would otherwise react with one another, (c) to provide an adequate concentration and uniform dispersion of an active agent [23]. The encapsulating carrier material must be of food grade, biodegradable, and stable in food systems during its processing, storage, and consumption. The most suitable nano-scale carrier materials for food applications could be based on carbohydrates, proteins or lipids.

Principle of nanoencapsulation

The principle of nanoencapsulation in food processing is focusing more on food preservation and interactive foods. Nanoparticles can be incorporated into existing food to deliver nutrients, additives, developing new tastes, sensations and the texture of food components; increasing the bioavailability of nutritional components and, also could increase product shelf life [24]. In the food industry, the nanoencapsulation process can be applied for a many of reasons (**Figure 3**).

Interactive foods

Interactive foods are nanoencapulated products which allow modification of the food depending on the nutritional requirement and tastes of consumers. Interactive food also provides protective barriers, flavour and taste masking, controlled release and better dispersibility for water-insoluble food ingredients and additives. In interactive effects, color and flavor change, cooling and warming effects, foam producing, mouth popping hydrogel beads, long lasting flavour characteristics are also included. Sequential change in color and flavor of a beverage [25] and particle motion in beverage have also been reported. In Mystery Colorz Cheetos®, the tongue is dyed green or blue when cheese puffs come in contact with the saliva in the mouth. In beverages, gas infusing nanoparticles and flavor delivery system capable to develop froth or flavor has been used in instant refreshing drinks [26, 27].Wolf et al., (2007) [28], addressed utilizing encapsulated cooling and refreshing agents to give long lasting flavour and cooling effects in chewing gums. Different products have been developed based on Nanoclusters™ system, such as SlimShake Chocolate, which incorporates silica nanoparticles that are coated with cocoa to enhance the chocolate flavor. NanoCluster™, from RBC Life Sciences® Inc and Irving, TX, USA are such delivery system for food products [29].

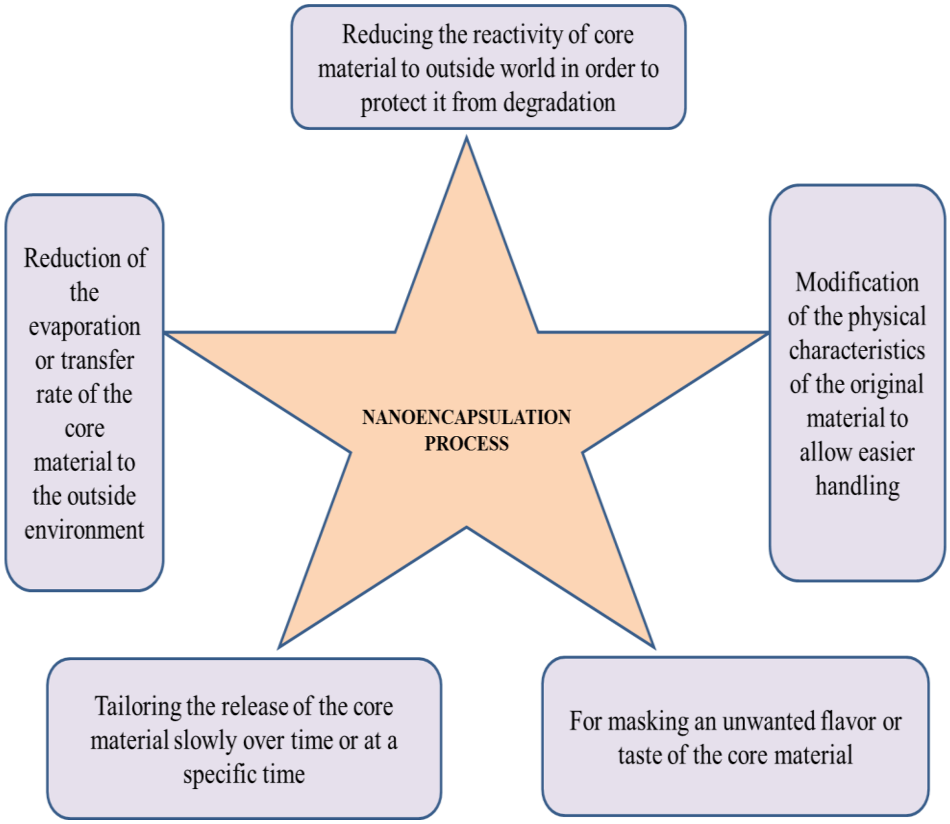

Figure 3 Applications of nanoencapsulation process

Protection of bioactive compounds

In Encapsulation technology, the bioactive components are completely enveloped, covered and protected by a physical barrier, and don't allow any protrusion of the bioactive components [30]. There is a multitude of possible benefits of encapsulated ingredients in the food industry. There is a multitude of possible benefits of encapsulated ingredients in the food industry. Encapsulation targets to achieve stability of the bioactive compounds during processing and storage in order to prevent undesirable interactions with food matrix, since it either slows down the degradation processes (e.g., oxidation or hydrolysis) [31] or completely prevent it until the product is delivered at the target sites [32]. Thus, the bioactive component would be kept as fully functional. Also, this technology may act as barriers between sensitive bioactive materials and the surrounding, and thus, allow differentiation of taste and aroma, mask bad tasting or smelling, stabilize food ingredients or increase their bioavailability.

Increase of bioavailability

In comparison to micro-size carriers, nanocarriers provide more surface area and thus enhance solubility of water insoluble food additives, improve bioavailability and facilitate controlled release and targeting of the encapsulated food ingredients [33, 18]. Generally two controlled release mechanisms can be observed during delivery of bioactive compounds [34]. (i) Delayed release, which is a mechanism by which the release of a bioactive substance is delayed from a bounded "lag time" up to a point when its release is desired. The delayed release mechanism is used for flavor release in ready-meals, color release in beverages or protection of nutritious compounds in gastric condition followed by release in the intestine. (ii) The mechanism of sustained release on the other hand, keeps constant concentration of a bioactive at its target site and thus extends the release of the encapsulated material, including flavor or certain drugs (eg. insulin, in chewing gum). Santos et al. [35] studied the use of nanoparticles as carriers for epigallocatechin gallate, quercetin, resveratrol and curcumin administration which resulted in the enhancement of their aqueous solubility, stability, bioavailability, target specificity and bioactivities.

Nanoencapsulation as an Efficient Delivery System

Encapsulation efficiency (equation 1) is generally estimated as the percentage of a compound included in a structure with respect to the total amount used in encapsulation

$$\text{Encapsulation efficiency (\%)} = \frac{\text{mass of a compound encapsulated in a structure}}{\text{total mass of the compound used before entrapment}} \quad (1)$$

In evaluating the performance of a delivery system, encapsulation efficiency is an important parameter which can be attributed to the ability of delivery systems including simple solutions, association colloids, emulsions, biopolymer matrices, and so on to control the manner and release rate [36]. If a delivery system is designed to enhance bioavailability, the meaningful entrapment efficiency should be the amount that reaches the absorption sites in the digestive system, which would require the entrapment of bioactive compounds in particles and the protection of their degradation during shelf-life storage and before reaching absorption sites after ingestion [37]. Lobato et al. [38] studied that nanoencapsulation is an effective technique for improving the solubilisation of bixin in aqueous media and, such high encapsulation efficiency occurred probably due to the nanocapsule core which contains triglycerides, which facilitates solubilisation of bixin.

Wall Material for Encapsulation and its Characterization

A lot of substances of different types and properties may be used to coat or encapsulate solids, liquids, or gases. However, only a limited number of materials have been certified for food applications as “generally recognized as safe” (GRAS). The majority of materials used for microencapsulation in the food sector are biomolecules and these are carbohydrate polymers/polysaccharides, which are the most profuse, proteins and lipids; they are grouped by hydrophilic and hydrophobic class of wall matrix materials suitable for encapsulation in the food sector as listed in **Figure 4** [39]. The selection of the materials must have the three important characteristics:

- Excellent barrier properties
- Excellent film-forming properties, that is, excellent wettability
- Phase transition point at which the matrix undergoes phase transformation

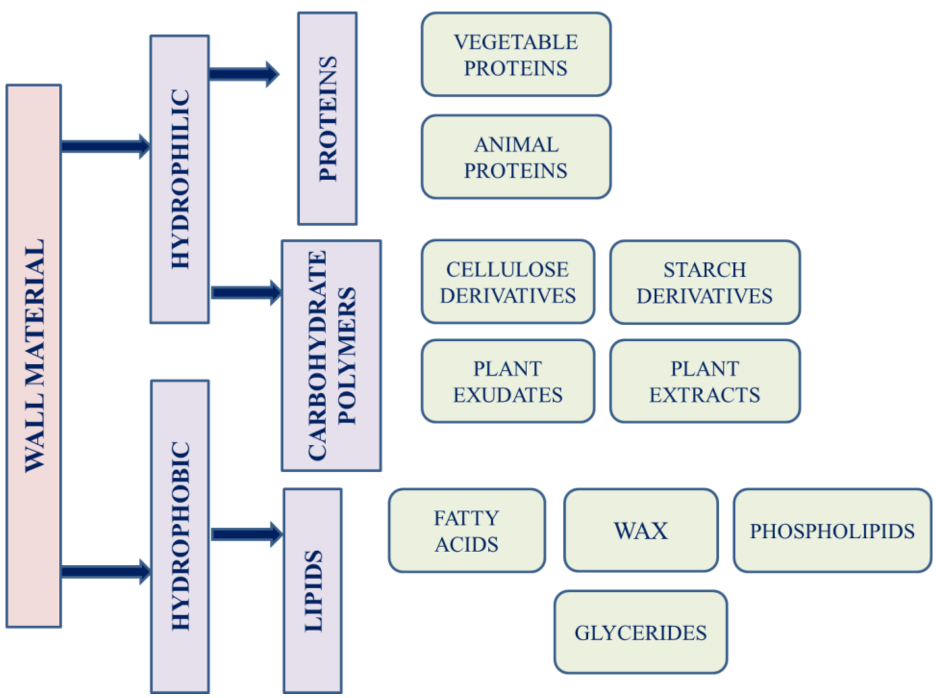

Figure 4 Hydrophilic and hydrophobic class of wall matrix materials employed for encapsulation in the food sector

Methods employed for manufacturing of nanocapsules

Nanoencapsulation techniques uses either top-down or bottom-up approaches to develop nanomaterials and could be done in two ways i.e., by physical means to form nanospheres or by chemical means to form nanocapsules (**Tables 1** and **2**). Top-down approaches involve the application of precise tools with force that allow size reduction and shaping of the structure for the desired application of the nanomaterial. The degree of control and refinement in size reduction processes influences the properties of the materials produced. In the bottom-up approach, materials are constructed by self-assembly and self-organization of molecules, which are influenced by many factors, including pH, temperature, concentration, and ionic strength [40].

Table 1 Classification of methods

Physical methods	Chemical methods
Spray drying	Coacervation
Spray cooling/chilling	Liposome
Spinning/rotating disc	Inclusion complexation
Fluidized bed drying	
Extrusion	
Coextrusion	
Lyophilization	

Table 2 Process description, advantages and limitations of different techniques employed for manufacturing of nanocapsules in context to various food applications

Methods	Process description	Advantages	Disadvantages	Application
Spray drying	Dissolving, emulsifying, or dispersing the core substance in an aqueous solution of carrier material, followed by atomization and spraying of the mixture into a hot chamber	Fast, relatively cheap, and reproducible	Complexity of equipment, not suitable for thermo-sensitive bioactive compounds.	Flavours, spices, polyphenols, fat, colour and vitamins,
Spray cooling/chilling	Dispersing of lipid coated active agents, followed by cool air atomization	Low cost, suitable for heat labile compounds	Special handling and storage conditions could be required	Flavours, spices, polyphenols and vitamins
Freeze drying	Dispersion of core into a coating material, followed by freeze drying	Suitable for heat labile compounds	High energy input, Non uniform dispersion	Flavours, probiotic, fatty acids
Fluidized bed coating	Coating material and core solid particles are atomized in a temperature and humidity-controlled chamber under of high velocity air current	Uniform dispersion	High energy input	Vitamins and acidulants
Extrusion	Dispersion of the core material in a molten carbohydrate mass. This mixture is forced through a die into a dehydrating liquid which hardens the coating to trap the core material	Advantage of extrusion is that the material is totally isolated by the wall material and that any core is washed from the outside.	Encapsulation efficiency is low, operating cost is high	Flavour and vitamins
Centrifugal extrusion	The core and the shell materials, which should be immiscible with one another, are pushed through a spinning two-fluid nozzle.	Produce capsules of a larger size, from 250 microns up to a few millimeters in diameter	-	Flavour oil
Supercritical fluid	This technique used supercritical fluid for dispersion of shell and core material	Suitable for heat sensitive bioactive compounds	High cost	Vitamins and polyphenols
Coacervation	formation of three immiscible phases; deposition of the coating; and rigidization of coating	Inexpensive process	Low shelf stability	Fatty acids, flavour and polyphenols
Inclusion complexation	Hydrophobic core entraps into hydrophilic shell (cyclodextrin)	Suitable for less hydrophilic compounds	-	Fatty acids, fat soluble vitamins and essential oils

Physical Methods

Spray drying

Spray drying is a widely used industrial process for the continuous production of dry powders. Spray drying is the process of transforming a feed (solution or suspension) from a fluid into a dried particulate form by spraying the feed into a hot drying medium. The spray drying yields fine particles. One of the most commonly applied technologies for encapsulation is spray drying. It is fast, relatively cheap, and reproducible [41]. The principle of this technique is based on dissolving or dispersing the active ingredient in solution of biopolymer. The dispersion is then atomized in a heated air chamber which rapidly removes the solvent and produces a dried particle consisting of the active ingredient embedded in a porous wall material. This method has some limitations in context to volatile or thermo-sensitive bioactives.

Spray cooling/ chilling

Spray-chilling or spray-cooling are technologies to produce lipid-coated active agents which could be dissolved in lipids, present as dry particles or present as aqueous emulsions. The difference between these two techniques is based on the melting point of lipids. In case of spray chilling, it is in range of 34–42°C and for spray cooling, temperature is higher than 42°C. The spray cooling is a technique with possibility to achieve high yields and it can be run in both continuous and batch processing modes. In case of spray-chilling, the particles are kept at a low temperature in a set-up similar to the fluidized bed spray granulation [42-43].

Fluidized bed coating

Fluid bed coating is an encapsulation technique where a coating is applied onto particles in a batch processor or a continuous set-up. The particles are suspended by an air stream at a specific temperature and sprayed with an atomized, coating material. The coating material might be an aqueous solution of cellulose or starch derivatives, proteins and gums [44].

Extrusion

Encapsulation by extrusion involves dispersion of the core material in a molten carbohydrate mass. This mixture is forced through a die into a dehydrating liquid which hardens the coating to trap the core material. Isopropyl alcohol is the most common liquid used for the dehydration and hardening process [45]. The advantage of extrusion is that the material is totally isolated by the wall material and that any core is washed from the outside. It is mainly used for visible flavor pieces, vitamin C, colors and extension of shelf-life upto at least 2 years. Dry food applications include drink, cake, cocktail and gelatin dessert mixes since the encapsulated materials are soluble in hot or cold water. Numerous flavors have also been encapsulated by this method.

Centrifugal extrusion

Centrifugal extrusion processes generally produce capsules of a larger size, from 250 microns upto a few millimeters in diameter. The core and the shell materials, which should be immiscible with one another, are pushed through a spinning two-fluid nozzle. This movement forms an unbroken rope which naturally splits into round droplets directly after clearing the nozzle. The continuous walls of these droplets are solidified either by cooling or by a gelling bath, depending on the composition and properties of the coating material [46].

Lyophilization

Freeze-drying is a highly stabilizing process and is generally applied to enhance the physicochemical stability of the nanoparticles to achieve an acceptable product, especially in case of unfavorable storage conditions. However, energy intensiveness, long processing time (more than 20 h) and an open porous structure are the main drawbacks of freeze-drying [47]. Nevertheless, freeze-drying is normally used for separation of nanoparticles (i.e., removal of the water from the substances) produced by other nanoencapsulation techniques. Shaikh et al. [48] encapsulated curcumin by an emulsion–diffusion–evaporation method along with freeze-drying.

Supercritical fluid (SCF)

Supercritical fluid methods have attracted increasing attention for encapsulation of bioactives in the recent years [49-

51] and represent a promising method for encapsulation of heat sensitive food bioactives. The method utilizes carbon dioxide as a widely used fluids whose supercritical region could be achieved at moderate temperatures and pressures (Tc ¼ 304.2 K, Pc ¼ 7.38 MPa). The process was initially applied by Hu et al. [52], to form spherical lutein entrapped zein nanoparticles of size range of 200 to 350 nm.

Chemical Methods

Coacervation

Coacervation is one of the most easily implemented techniques for production of carbohydrate-based delivery systems. The common driving force for this method is based on electrostatic attraction between oppositely charged molecules i.e., force may be induced between a charged bioactive component and an oppositely charged carbohydrate (simple coacervation). Alternatively, a bioactive may be trapped within a particle formed by electrostatic complexation of positively charged (e.g. chitosan) and a negatively charged (e.g. pectin and alginate) biopolymers (complex coacervation). This technique has been applied for both non-polar and polar bioactive molecules [**53]**.

Inclusion complexation

It is the only method of encapsulation that takes place on a molecular level. It is accomplished using cyclodextrins, typically B (is it B or beta ß)-cyclodextrin and constitutes 7 glucose units linked via ß1-4 linkage and has a hollow, hydrophobic center with a hydrophilic outer surface. In solution, water molecules held in the centre of cyclodextrin are replaced by less polar molecules. The technique has been used to improve the thermal stability of linoleic acid by its encapsulation in α- and β-cyclodextrins [54].

Nanoencapsulates in food technology

Another major area of current nanotechnology applications involves nanoencapsulation of food ingredients and additives to provide protective barriers, flavor and taste masking, controlled release, and better dispersability for water-insoluble food ingredients and additives [55] as shown in **Figure 5**. A number of nutraceuticals and nutritional supplements containing nano-ingredients and additives (e.g., vitamins, antimicrobials, antioxidants) are currently available. These products typically claim enhanced absorption and bioavailability of nano-sized ingredients in the body.

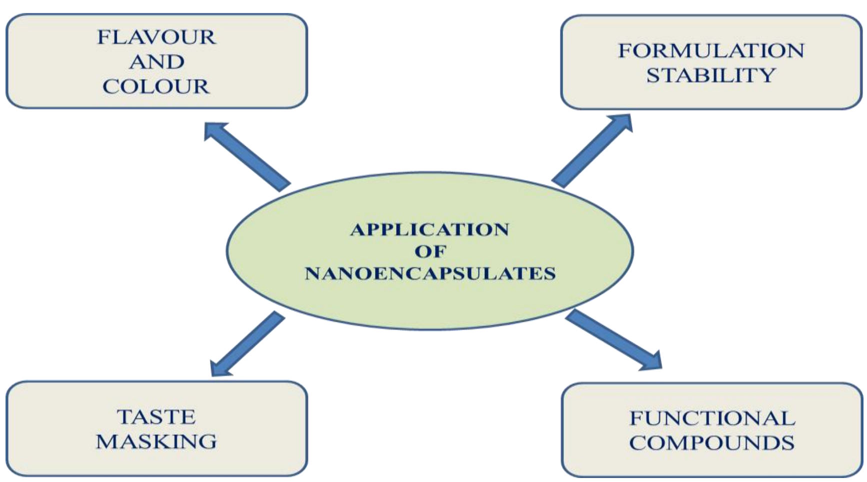

Figure 5 Application of nanoencapsulates in food technology

Flavour and taste masking

In a variety of food and beverage applications, it is desirable to regulate the release of flavour during specific phases of the consumer experience. Nanoencapsulated flavour have been used in food products for the purpose of protecting flavour from oxidation, loss, heat stress and undesirable interaction within the food systems, thus improving the shelf life and quality of the product. Other studies include encapsulated flavour; improve flavour release in chewing gum by reducing interaction of flavour and base materials [56]. Wampler and Soper [57] investigated the heat stability of flavour improve in hard candy which are produced by coacervation technique. In certain studies, flavour encapsulation also protects their interaction with yeast in bakery products where yeast is used as a leavening agent [58]. Flavour encapsulation improves dispersibilty of flavouring compounds in dry mix beverages [59, 60].

Masking undesirable taste and aroma attributes is a common objective in many different applications and encapsulation techniques have been utilized to create barriers around the offending source material. Such as, cyclodextrins, commonly used to encapsulate aroma compounds have been used in canned fish products to encapsulate offending aroma compounds [61]. Various encapsulating materials such as gelatin, whey protein, cyclodextrin, zein protein, chitosan and ethyl cellulose [62] are used to encapsulate fish oil, which contains high levels of polyunsaturated omega-3 fatty acids, and to reduce the fishy taste and smell perception as well as to prevent oxidation. Bohannon [63], used encapsulated caffeine in icing and breakfast bars without bitterness detection.

Functional foods

Encapsulation is not a new idea; it is technology that has been in use for over 50 years in the pharmaceutical, biological, nutritional, and food science fields. Now it has become increasingly important in the development of fortified and functional foods in recent years. To improve the bioavailability, the functional ingredients (including vitamins, antimicrobials, antioxidants, probiotics, prebiotics, peptides and proteins, carotenoids, omega fatty acids, flavorings, colorants and preservatives) are sometimes incorporated into the delivery system (e.g. nanostructures). The most successful applications lie in the encapsulation and delivery of conventional food products e.g., "4Bs" – breakfast cereals, bakery goods, bars and beverages [64] and more recently applications are extended for advanced delivery of nutraceuticals such as polyphenols, probiotics, omega fatty acids [65]. The utilization of encapsulated polyphenols can overcome the drawbacks of their instability, removal of unpleasant tastes or flavors, as well as improve the bioavailability and half-life of the compound *in vivo* and *in vitro*.

Depending on the type of nanoparticles, incorporated EGCG (epigallocatechin-3-gallate) can be partially or completely sequestered in the nanoparticles, resulting in high stability [66]. Hu et al. [52], encapsulated EGCG into food grade peptide/chitosan nanoparticles, and they found that the apparent permeation rate across the $CaCO_2$ monolayers was increased more than twofold by nanoencapsulation. The increased solubility and bioavailability and improved sustained release by nanoencapsulation may elevate the bioactivities of quercetin. Polyphenolic compounds such as catechins oxidize in neutral and alkaline pH environments. EGCG has been observed to rapidly oxidize; the oxidation of EGCG results in the formation of dimerized products, which possess a greater superoxide radical scavenging activity than EGCG itself and have powerful iron chelating properties [67]. Nanoencapsulation of EGCG with milk proteins confers protection to EGCG from oxidative degradation compared to nonencapsulated EGCG [68]. Nanoencapsulation protects trans-resveratrol against light-exposure degradation and hence increases its stability [69]. Resveratrol has antioxidant, anti-inflammatory and anticarcinogenic properties.

Nanocapsules have been used to mask the taste and odor of tuna fish oil (source of omega-3 fatty acids) from (e.g. ''Tip Top-up'' brand) bread by George Weston Foods, Australia. The nanocapsules break open only when they reach the stomach and hence the unpleasant fish oil taste can be avoided [16]. Krishnan and Prabhasankar [70] reported the health trend of pasta fortified with omega-3 fatty acids. Encapsulation technique developed Ultra Rice®. It is a reconstituted, nutrient-fortified rice premix made by extrusion, to resemble the shape, size, and appearance of rice kernels.

Nanotechnology will enable modification of junk foods like ice cream and chocolate so as to reduce the amount of fats and sugars making them more suitable for consumption. This is possible by using nanoparticles to prevent the body from digesting or absorbing these components of the junk food. In this way, the nano industry could market vitamin and fibre-fortified, fat and sugar-blocked junk food as health promoting and weight reducing [71].

The nanoencapsulated designer probiotic bacterial preparations may act as de-novo vaccines with the capability of modulating immune responses [72]. Nanoencapsulated *Bifidobacteria* with starch by spray coating exhibited an affordable and industrially convenient encapsulation process [73]. The bioavailability of lycopene can be increased by fortifying nanoparticles of lycopene in tomato juice, pasta sauce, and jam [74]. Nanosized micelles using milk protein, casein, has been employed as a vehicle for delivering sensitive health promoting ingredients including vitamin D2 [75].

Commercial success in this area has been achieved by omega-3 fatty acids, and certain beneficial probiotic bacteria species, lycopene, vitamin D_2 and β-carotene [16]. Maintaining nutraceuticals in a stable state throughout the production process is invariably challenging. The nanoscale production of nutraceuticals with maximized nutrient content and increased stability throughout the processing chain, will be of significant interest to food processors and hence will ultimately be of benefit to consumers [55].

Immobilized enzymes

Encapsulation may also be used in food processing applications such as fermentation process and metabolite production processes involving immobilization of cells or enzymes. Regardless of the use of encapsulated enzymes used in food products to improve product quality, such as extending shelf life of bread and tortillas or flavor of cheese, delivery of enzyme to the application must be done at the correct time; the following case studies highlight the use of enzyme encapsulation to deliver unique benefits. Horn [76], studied the oil encapsulation, controlled release and protection of alpha amylase during baking and thus retard staling. On the other hand encapsulation of lipase extends the shelf life by eliminating rancidity. Various encapsulated enzyme activities are used in dairy products; they serve various purposes such as ripening of cheese, contributing flavor and texture. The use of liposomes to encapsulate and deliver enzymes offers improved stability [77].

Techniques used for capsule analysis

The exact knowledge of size and size distribution system is a prerequisite for nanoparticulate drug delivery system, since these parameters lead to modification of physico-chemical as well as biopharmaceutical behaviour, like affecting drug release kinetics as well as transport phenomenon across biological barriers [78]. Several techniques are applied in the size determination of nanoparticulates in food and agricultural samples [79-80], such as hydrodynamic chromatography (HDC), electron microscopy (EM), light scattering analysis and other techniques (**Figure 6**). In chromatography, compounds can be separated based on their charge (weak/strong cation or anion exchange chromatography; [IEC]), molecular mass (Size exclusion chromatography [SEC]), hydrophobicity/polarity (reversed-phase HPLC), hydrophobic interaction chromatography), and specific characteristics (affinity chromatography), depending on the type of materials in the stationary phase [81-83]. HPLC allows the separation of pigments, carbohydrates, vitamins, additives, mycotoxins, amino acids, proteins, lipids, chiral compounds, and triglycerides in fats and oils [84-85].

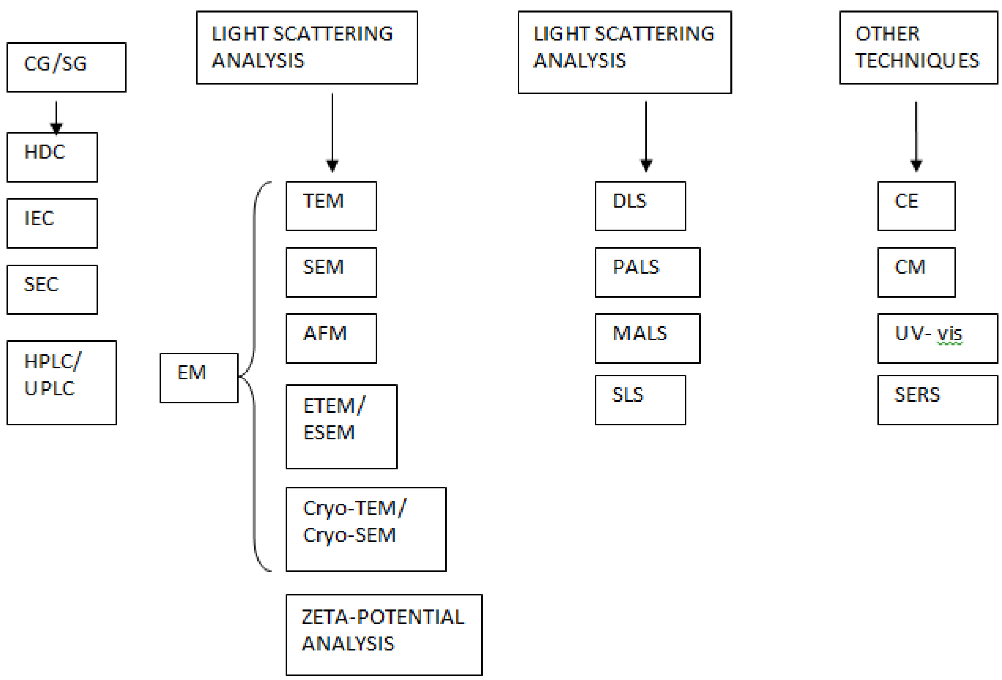

Figure 6 Techniques for the measurement of nanomaterials (NMs)/nanoparticles (NPs) in food and agriculture products. AFM, atomic force microscopy; CE, capillary electrophoresis; DLS, dynamic light scattering; EM, electron microscopy; ETEM/ESEM, environmental TEM or SEM; HPLC/UPLC, high- or ultra-performance liquid chromatography; MALS, multiangle light scattering; PALS, phase analysis light scattering; SEM, scanning EM; SERS, surface enhanced Raman scattering; SLS, static light scattering; TEM, transmissionEM; UV-vis, ultraviolet-visible

Hydrodynamic chromatography (HDC) is also a very efficient technique to separate Nanoparticles in food and agricultural samples based on their hydrodynamic radius [83]. HDC coupled with an ultraviolet–visible (UV-vis) detector has been used for the size characterization of colloidal suspensions and biomolecules in food and biological samples [81, 84, 85]. Electron microscopy (EM) techniques are widely used to determine the size, shape, and other elemental properties of Nanoparticles/Nanomaterials in food matrices. Though electron microscopy shows single particles and does not give statistically averaged values. Transmission EM (TEM) is one of the indispensable technique for the characterization of Nanomaterials< 200nm.

In TEM, electrons are transmitted through the sample to acquire animage [86]. TEM has been employed to measure milk-protein-based nanotubes, the shape of serum albumin Nanoparticles, and enzyme-functionalized peptide nanotubes [84]. TEM analysis demonstrated that the exposure of the selected microbial strains (Staphylococcus aureus, Escherichia coli, Salmonella Typhimurium and Klebsiellapneumoniae) to the composite nanofibers led to disruption of cell membranes along with depressed activity of some membranous enzymes, leading to death of bacteria. These researches stated that surface modification using NiO and TiO2 composite nanostructures is an innovative combination to enhance food safety. TEM, coupled with an energy dispersive X-ray spectroscopy (EDS or EDX) detector is used to get the elemental compositions of Nanomaterials, while at the same time, TEM image scan provide the size, morphology, and size distribution of nanomaterials with accuracy of –5% [87]. However, this technique is mostly used to localize and identify inorganic particles. This technique is not helpful in organic Nanoparticles, as carbon is the major element in the Nanoparticles and the food matrix [86].

SEM can be employed for detecting larger particles (achieving a spatial resolution of 500 nm)[87] and offers high-resolution image of a sample surface in a distinctive three-dimensional appearance. Additionally, SEM has been used to observe the morphology of polysaccharide, protein and liposomal nanoparticles [84].Atomic force microscopy (AFM) is also considered as a powerful tool to investigate the fine structural information of food materials and could detect irregularities in the polymer structure that were usually hindered during whole sample-based analyses [88]. Thus, AFM imaging provides the potential to characterize the integral heterogeneous assemblies of food macromolecules [89-90].

An environmental TEM or SEM (ETEM/ESEM) can be employed to characterize samples in wet conditions or without chemical fixation. The food and agricultural samples can be imaged in a controlled atmosphere in ETEM, whereas, in ESEM, hydrated samples can be imaged as the samples remain under high vapour pressure. Reports indicate that it is possible to image samples with 100% relative humidity by controlling the vapour pressure [87-91]. ESEM has been employed to investigate the presence of inorganic microsized and nanosized contaminants in food products [92]. Cryo-TEM/Cryo-SEM can be used to acquire high-resolution images of biological samples under high vacuum and below ambient temperature (between -100ºC and -175ºC). The lower temperature (typically the vitrified state) allows the life-like appearance of the sample and helps to obtain the micrograph of hydrated and chemically unmodified state of the sample [91].

DLS (also known as photon correlation spectroscopy) uses the scattered light to measure the rate of diffusion of Nanoparticles and provides a size distribution in terms of hydrodynamic diameter. This is suitable for sensing small aggregated proteins (< 0.01% weight) in various food samples [93]. Yegin and Lamprecht [94] reported the use of DLS for size characterization of lipid nanocapsules. Durand et al. [95] described the use of DLS (along with optical microscopy) for the size measurement of natural particles (1–3 lm) present in milk. The surface structure of the casein micelle Nanoparticles were also achieved in simple and rapid experimentation using DLS [96-98]. However, it is hard to quantify accurately the presence of any aggregates with DLS. This problem can be overcome by using the phase analysis light scattering (PALS) technique. PALS have been used to determine the isoelectric point and electrophoretic mobility of the whey protein isolate solution [99]. Static light scattering is also considered as another rapid and reproducible light scattering technique for food samples varying from 0.05 to 2000 lm. This technique is already been used for the particle size measurement of dairy products [100], casein micelles [101], lactose crystals [102], skimmed milk [103], and whole milk [104, 105].

In addition to EM, confocal laser microscopy can also be used to detect Nanomaterials in agricultural samples, specifically, in plant and microbial systems. This technique was used to detect CeO_2 and ZnO Nanoparticles inside corn plant tissue. Confocal microscope images showed NP aggregates in root epidermis, cortex, and some NP aggregates in the xylem vessels [106, 107].

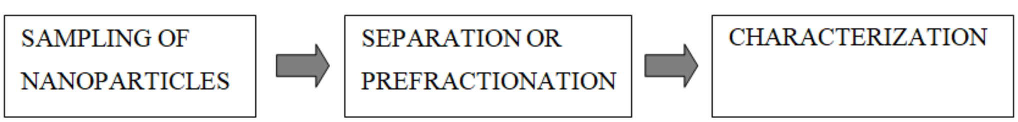

Figure 7 Different steps for measurement of nanoparticles present in food and agricultural samples

Recent advances in nanoencapsulation

The understanding of food materials and food processing at nanoscale level is important to create new and improved food products. Currently, the use of nanotechnology of any kind in food products is relatively small. Nanotechnology has the potential to influence the food science in a revolutionary way, as it could generate innovation in food texture, food taste, processability, and stability of food during shelf life [108].

Nanoparticles exhibit several features that make them of great potential for food industry, such as high surface/volume ratio, reassembling and self-reassembling capability, and the ability to create porous structures [109]. In comparison to conventional bulk equivalents, organic nanomaterials are used in food/ feed products for their increased uptake and absorption, and improved bioavailability of vitamins, antioxidants in the body (**Table 3**). A wide range of materials, for example food additives (eg, benzoic acid, citric acid, ascorbic acid) and supplements (eg, vitamins A and E, isoflavones, beta-carotene, lutein, omega-3 fatty acids, and coenzyme-Q10) are available in this category.

Table 3 Nanoencapsulates in food

Nanoencapsulates	Products	References
Nanoemulsions - Oil in water emulsion, usually 50-500nm	β-Carotene (antioxidant, Vit A precursor); α-Tocopherol (antioxidant);Nanoemulsion based ice-cream (Nestle, Unilever) Lycopene (antioxidant)-Natural food colour Phytosterols(Cholesterol absorption inhibitor); Medicine, cosmetic and as food additive	111-113
Biopolymeric Nanoparticles - Dense matrix network of sub-100nm in which active molecules are dispersed throughout	β - lactoglobulin; NanoceuticalsTM (RBC Life Sciences®); Nano Calcium/ Magnesium (Mag-I-Cal.com USA); nanoselenium-enriched Nanotea (Shenzhen Become Industry) Natural biopolymers (Chitosin, cellulose) Improves solubility, stability and cellular uptake of chitosan and glutamic acid nanoencapsulated resveratrol (polyphenolic antioxidant)	5,110, 114-117
Nanocapsules - Vesicles in which oil or liquid Ingredients are confined within polymeric membrane of (20-100nm)	Nanocapsules of Capsium, oleoresin, eugenol, lysozymes, vitamins, phytosterols; “Tip top” up bread with nano fish oil (Nu-Mega), Kraft foods-personalized flavours and colours	75, 118-120
Nanospheres – Solid colloidal particles enclosed by polymer matrix with several phases in suspension	Omega 3 fatty acids, whey protein nanospheres; Citral flavour (Key Lime Formulations); Chocola Chocolate chewing gum with nanococoa (Olala)	120
Nanoliposomes- Polymeric aggregates of lipid bilayers esp. phospholipids such as egg or soy	Lactoferrin, nisin, phosvitin, enzymes, vitamins, antioxidants, coenzyme Q10 Green tea catechins (antioxidants) encapsulated in soy lecithin nanoliposomes and incorporated into full fat cheese.	114, 121-123
Nanocochleates –Cigar shaped multi-layered structure with spiral solid lipid bilayer	BioralTM for nutrients (BioDelivery systems)	5, 124
Nanoclusters	Slim shake chocolate, Nanoceuticals (RBC Life sciences); Nanococoa (Royal Body care)	5, 114, 120
Nanomicelles- Sub 100nm spherical particles formed spontaneously upon surfactant addition after Critical micelle conc. has reached	Limonene, carvacrol, lutein, eugenol, Omega 3 fatty acids, whey proteins, essential oils; Lycopene (BASF Germany), NutiNanoTM(Solgar, USA); Novosol (Aqanova®) for nutrients; Canola Activa Oil (Sheman Ind.)	5, 114, 125-129

The main achievements, such as harnessing the casein micelle, a natural nanovehicle of nutrients, for delivering hydrophobic bioactives; discovering unique nanotubes based on enzymatic hydrolysis of α-lactalbumin; introduction of novel encapsulation techniques based on cold-set gelation for delivering heat-sensitive bioactives including probiotics; developments and use of Maillard reaction based conjugates of milk proteins and polysaccharides for encapsulating bioactives; introduction of β-lactoglobulin–pectin nanocomplexes for delivery of hydrophobic nutraceuticals in clear acid beverages; development of core-shell nanoparticles made of heat- aggregated β-lactoglobulin, nanocoated by beet-pectin, for bioactive delivery; synergizing the surface properties of whey proteins with stabilization properties of polysaccharides in advanced W/O/W and O/W/O double emulsions; application of milk proteins for drug targeting, including lactoferrin or bovine serum albumin conjugated nanoparticles for effective *in vivo* drug delivery across the blood–brain barrier; beta casein nanoparticles for targeting gastric cancer; fatty acid-coated bovine serum albumin nanoparticles for intestinal delivery, and Maillard conjugates of casein and resistant starch for colon targeting were reported [110]. The dairy industry utilizes three basic micro sized and nanosized structures (casein micelles, fat globules, whey proteins) to build all sorts of emulsions (butter), foams (ice cream and whipped cream), complex liquids (milk), plastic solids(cheese), and gel networks (yogurt). In fact, dairy technology is a combination of microtechnology and nanotechnology, that has existed from a long time. Research into naturally occurring nanostructures in foods is mainly designed to improve the functional behavior of the food [19].

A Hungarian company has developed an ice gel for soft drinks or ice-cream containing CO_2 bubbles of 1-10 nm in diameter for effervescence. Nanotech spray is available in which Nanodroplets of 87 nm are used to enhance the uptake of vitamin B12 and other supplements for use in foods [114]. Kraft Foods and NanoteK consortium have plans to incorporate the electronic tongue (which is chemical change based biosensor) into foods to release accurately controlled amounts of the suitable molecules for the customized tailor-foods [8, 120].

Nanotoxicology

Nanotoxicology is a branch of bionanoscience that deals with the assessment of toxicological properties of nanoparticles. It determines whether and to what extent these nanoparticles can pose a threat to environment and human health. Both inorganic (silver, iron oxide, titanium dioxide, silicon dioxide, and zinc oxide) and organic (lipid, protein, and carbohydrate) nanoparticles in foods may have toxic effects. The small size of the nanoparticles may behave differently within the human body owing to their smaller size in comparison to the larger particles conventionally utilized as food ingredients. Nanoparticle’s adsorption, distribution, metabolism and excretion can determine the potential toxicity of the nanoparticle. The gastrointestinal fate of the ingested nanoparticles as well as their characterization is important to determine their potential toxicity [130]. Any nanoparticles that are not digested or absorbed in the upper gastrointestinal tract will reach the lower gastrointestinal tract where they may alter the microbiome [131].The size, shape, surface area, surface charge, crystal structure, coating, and solubility/dissolution of nanoparticles as well as factors such as temperature, pH, ionic strength, salinity, and organic matter influence nanoparticle’s behavior, fate transport and toxicity. Depending upon their composition and structure, nanoparticles may produce toxicity in cells through a variety of different mechanisms. One of the major factors contributing to the toxicity of inorganic nanoparticles is their ability to produce Reactive oxygen species (ROS), such as singlet oxygen, superoxide, hydrogen peroxide and hydroxyl radicals [132]. These ROS may then interact with lipids, proteins, or nucleic acids, thereby causing damage to cell membranes, organelles, and the nucleus [132, 133]. As a consequence, many biochemical functions required to maintain cell viability, may be adversely affected resulting in DNA mutation, cancer, and possible fatality. The mechanisms of action that govern toxicity of nanoparticles are the subjects of ongoing research as well as, new analytical methods are also required to address the special properties of nanoparticles. The insight of the mechanism involved in interaction of nano-materials and bio-systems will help in taking appropriate steps for the good of human health and environment.

Future perspectives of nanoencapsulation

Over the last few decades, nano- or microencapsulation technology has developed into a large-scale business, becoming a multimillion market with the impact of nano- or microtechnology. The technology was foreseen with the prospects to employ more than 1.5 to 2 million workers and the expected business trade to rise to about 0.5 to one trillion U.S. dollars by 2015 [16]. Since, research differs based on the companies and location, Lux anticipated a growth of 2.6 trillion US$ in manufactured products based on nanotechnology industry by 2014 and according to the U.S. Department of Agriculture (USDA), the global impact of nanoproducts was anticipated to be around 1 trillion US$ annually by 2015. A recent report suggested that there may be about 400 companies involved in the nanosize production of food materials [16].

Microencapsulation production for the encapsulation of various functional compounds is one of the emerging fields in encapsulation applied to the food industry. Various functional compounds, such as vitamins A and E, isoflavones, phytosterols, lycopene, and lutein are available [134]. In addition, nanotechnology has been applied to protect the flavour compounds under various pHs and temperatures with higher stability [135]. Some multinational companies, such as Nestle and Unilever, which are well known worldwide, are also developing functional foods using the encapsulation technologies, for example, low fatice cream with 1% fat content. In spite of various nano or microencapsulation technologies being applied in the nano-industries, various countries lack regulatory systems and a framework in relation to the nanomaterials. The foremost concern is their minute scale which can make them go deeper into the human body, thus being unable to be detected. Before various health risks from consumption of nanoproducts surface, the countries where regulations are still lacking regarding these technologies should be identified. Even though legislation is still being adapted, other actions may be taken to improve consumer trust in the food encapsulated with nano-sized particles.

Conclusion

Nanotechnology has the potential to improve foods, making them tastier, healthier, and more nutritious, to generate new food products, new food packaging, and storage. However, many of the applications are currently at an elementary stage, and most are aimed at high-value products, at least in the short term. Nanocapsules can be used to enhance food flavor and texture, to reduce fat content, or to encapsulate nutrients, such as vitamins, to ensure they do not degrade during a product's shelf life. The use of nanotechnology to manufacture of processed foods with enhanced processing, health and packaging functionalities, flavour, texture, shelf-life, transportability, reduced costs and nutritional traits will facilitate the expansion of the range, quality and quantity of processed foods, and to thereby meet the contemporary demands for both 'health' and 'convenience'. It has enabled the development of food safety and food quality aspects.

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Publication History

Received	17.08.2020
Revised	24.09.2020
Accepted	26.11.2020
Online	30.12.2020