Application of Nanoprotein in Food Industry and Its Potential Toxicity related Health Issues

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ABSTRACT

Nanotechnology is one of the advanced fields of science recently and successfully used in food, medicine, and agriculture sectors. Nanoprotein molecules are useful in the food industry due to their nutritional value, non-toxicity, biodegradability, high encapsulation efficiency, and relatively low cost. Nanoproteins can increase shelf life of food products, preventing microbial contamination, and enhanced food quality. Nanoprotein molecules are able to enhance bioavailability, taste, texture, and consistency of food material. Efficiency of nanoprotein molecules depends upon size, structure, surface area, pH level, thermal exposure of the particular nano molecule. Nanoproteins can be synthesized from protein like albumin, gelatin, whey protein, gliadin, legumin, elastin, zein, soy protein, and milk protein by different techniques. These nanoparticles are used in loading and delivery of physiologically active compounds like nutraceutical. Nanoprotein formation is also helpful to combat global food security challenges which are related with increased global population, unstable world economy and climate changes. Physicochemical properties of nanomaterials may contribute toxicity. Excess usage and inhalation of ultrafine nanomolecules can accumulate in various organs and causes health problems. The main content of this review article is to point out different kinds of nanoproteins and their usage in food processing, preservation, packaging. Different techniques to obtain protein-based nanostructures and their expected toxicity impact on human health also briefly described in this article based on some currently reported data on nanotoxicity.

Keywords: Nanoproteins, Food Industry, Potential Toxicity & Health Issues

INTRODUCTION

Applications of nanotechnology have risen due to increasing demand and usefulness of nanoparticles in various fields of food science and food microbiology, including food processing, food packaging, functional food development, food safety, food borne pathogens detection, shelf-life extension of food and/or food products. Generally the range of nano particle is 10–100 nm (Mallakpour & Behranvand, 2016).
Diameter of nanoparticle size can be extended up to 1000 nanometers (Defrates et al., 2018). Protein based nanoparticles are widely used and shows promising effect in the field of food industry. Protein based nanoparticles are biodegradable, metabolizable, easily adaptable to surface modifications and have high nutritional value, availability and acceptability, greater storage stability. Preparation process and size monitoring of the nano protein particles are also very easy. Nano protein can be synthesized from water soluble proteins including bovine and human serum albumin, as well as from insoluble protein like zein and gliadin (Verma et al., 2018). Frequently used structures and systems in nanotechnology are nanoparticles, nanodispersions, nanolaminates, nanotubes, nanowires, buckyballs, quantum dots, and others. Nanotechnology is the new trend to cope with different types of barriers such as increasing rate of world population, environmental hazards, demand of nutritional and food security, global climatic change, improved food production, food quality and so on. The number of nanotechnology based commercial food and beverage markets may also expand from $6.5 billion in 2013 to approx $15.0 billion by 2020 (Naseer et al., 2018). Protein nanostructures are used to encapsulate different types of bioactive compounds such as hydrophilic or lipophilic compounds with different molecular weight, solubility, etc. Nano protein serves a high surface area to uptake active compounds and improve their absorption and bioavailability. It also shows controlled and target specific release of encapsulated bioactive compounds. Protein nanoparticle can be produced separately and attached with bioactive molecules through electrostatic, van der Waals, and/or hydrophobic interactions during the gel folding process without harming the bioactive compounds which makes nanostructure more stable than other form of nanoparticles (Bourbon et al., 2019). Protein based nanocarriers from food components efficiently encapsulate hydrophobic molecules and deliver the bioactive compounds to their appropriate location due to some functional properties of protein nanostructure such as surface activity, water-binding capacity, emulsification, foaming, gelation, and antioxidant activity (Maviah et al., 2020). Small droplet size of the nanoemulsion shows better bioaccessibility of encapsulated bioactive compounds during gastrointestinal digestion and also increases the surface area available for pancreatic lipase activity which leads to better lipid digestion. Study confirmed that reduction droplet size of β-lg nanoemulsions from 360 to 94 nm could improve bioaccessibility of the hydrophobic bioactive coenzyme Q10 from 39% to 57% (Ha et al., 2019). Nanoparticle size can be modified by altering the nozzle size and speed of spray drying method. Very high or low molecular weight protein both can hamper encapsulation efficiency of nano protein. At pH near isoelectric point (IEP) nanoparticles start aggregate and reduce its stability. Natural protein polymer is relatively cheap, easy to process, and renewable which makes it an attractive material from an economic perspective (Defrates et al., 2018). The uses of nanoparticles in the food industry can enhance functional properties of foods, provide a safe and contamination free food to the consumer level and ensure the consumer acceptability of the food (Singh et al., 2017).

**Nanoprotein Application in Food Processing Industry:**

Nanotechnology widely used in food processing industries as anticaking agents, nano additives and nutraceuticals, gelating agents, nano encapsulation, nano carriers and can increase the shelflife of food materials, reduce wastage of food due to microbial infestation. Nanoparticle also improves food packaging, and nano biosensor used for pathogen detection (Singh et al., 2017). Protein based nanostructures are one of the most convenient and widely used metrics in food applications, food engineering and delivery applications. Preserve or increase the bioavailability of bioactive compounds such as vitamins, nutraceuticals and anti-inflammatories which leads to promoting...
health and well-being is one of the most challenging process in food industry.

**Albumin:**
Albumin (ovalbumin, bovine serum albumin and human serum albumin) is used frequently for the preparation of nanospheres and nanocapsules due to its advantageous properties such as nontoxic, biodegradable, easy to prepare, nonimmunogenic, have some reactive groups (thiol, amines, and carboxyl) those can work as ligand binding unit or surface modifier (Verma et al., 2018). Sponton et al. (2015) showed the efficacy of ovalbumin-linoleic acid nanoparticles (mean diameter of 87.5±1.2 nm) in food application (Sponton et al., 2015) Jin Feng et al. (2016) loaded curcumin with ovalbumin-dextran nanogels via the Maillard reaction and heat-gelation process which formed stable spherical structure (size diameter of≈102.31 nm) with great pH stability, storage stability (Feng et al., 2016).

**Milk Proteins:**
Milk proteins mainly whey proteins (α-lactalbumin and β-lactoglobulin, lactoferrin are the main) and caseins used frequently as encapsulation vehicles of bioactives due to their structural and functional variety. Heat-denatured β-Lg nanoparticles (< 50 nm) shows 60–70% loading efficiency EGCG bioactive compound and reduce bitterness (Shipigelman et al., 2012). Bovine lactoferrin (bLf) nanoparticles (thermal gelation, 75 °C for 20 min) were very useful for iron binding and showed near about 20% iron binding efficiency. This protein nanoparticles were stable for 76 days at 4 °C and also had thermal stability (between 4 and 60 °C) and pH stability (pH 2 – pH 11) (Martins et al., 2016) Encapsulation of bioactive compound curcumin in β-Lactoglobulin (βLG), the major whey protein nanoparticles can improve the stability, solubility and efficiency (>96%) of curcumin in comparison with its aqueous solution (Sneharani et al., 2010). β-Lactoglobulin and pectin used as a nano-capsular vehicle for ω-3 polyunsaturated fatty acids useful for enrichment of clear acid drinks (Zimet & Livney, 2009). Protein-based materials such as β-Lactoglobulin (BLG) and hen egg white protein (HEW) could be used to encapsulate α-tocopherol after salt-induced gelation of the proteins and concentrations of protein and α-TOC can regulate the encapsulation efficiency (Somchue et al., 2009). Curcumin and caffeine were successfully encapsulated in lactoferringlycomacropeptide (Lf-GMP) nanohydrogels by thermal gelation and showed high encapsulation efficiencies (>90%) and maintained spherical size and shape of bioactive compounds (Bourbon et al., 2016). Encapsulation (electrospraying and nanospray drying technology) of folic acid by using a whey protein concentrate (WPC) matrix shows greater encapsulation efficiency (Pérez-Masiá et al., 2015) . Abbas Khan et al., (2019) successfully incorporated 3,3’-Diindolylmethane (DIM) into whey protein isolate (WPI) by using combined heating-ultrasound method which showed significant encapsulation efficiency (>82%), increase stability, reduced the physicochemical limitation of DIM and maintain its activity as antioxidant, anticancer, and anti-inflammatory agent. (Khan et al., 2019).

**Gelatin Protein:**
Gelatin used as a base (biodegradable) for any nanoparticulate formulations which is nontoxic, sterilizable, inexpensive, easy to crosslink, low antigenicity and also non contaminated with pyrogens (Verma et al., 2018) (−) Epigallocatechin gallate (EGCG) was encapsulated in the electrosprayed gelatin capsules which showed almost 100% encapsulation efficiency and prevented EGCG degradation (Gómez-Mascaraque et al., 2015).

**Gliadin Protein:**
Hydrophobic nature of gliadin protein and its solubility permits the nanoparticles for its controlled and sustained release. Legumin protein form nanoparticles after aggregation and chemical crosslinking with glutaraldehyde (Verma et al., 2018). The gliadin-lecithin nanoparticles used to encapsulate curcumin which improve efficiency, thermal and UV light stability, and antioxidant activity of the bioactive compound (Yang et al., 2018).
Zein Protein:
Zein consists of rich prolamine protein which is frequently used for films and coatings and generally recognized as safe (GRAS) polymer for human application. Nanoparticles from zein proteins also used to encapsulate several drugs. Dispersive solid-phase extraction (DSPE) used to formulate zein nanoparticles (Verma et al., 2018). Zein (core) and β-Lg (shell) nanoparticles were used to encapsulate poor water-soluble bioactive flavonoid (Chen et al., 2014). Soya protein isolates based nanoparticles can improve (2–3 times) the intestinal transport through the cell layers compared to non-encapsulated vitamin-B12 (Zhang et al., 2014).

Soy Protein:
Nanoparticle prepared from soy protein by addition of desolvation agents or glycinin fraction of defatted soy flour extraction (coacervation method). Nanoparticle from soy protein used as encapsulating matrix for nutraceutical encapsulation and pickering stabilizer for oil-in-water emulsion (Verma et al., 2018).

Collagen-Based Nanomaterials, Silk-Based Nanomaterials, Silk Fibroin-Based Nanomaterials, Elastin-Based Nanomaterials are also very popular recently for their significant performance (Zhang & Wang, 2019).

Nanoproteins Application in Food packaging Industry:
Nanosensors (made from nanoparticles) are now frequently used in food packaging system which helps to detect gases, chemical contaminants, aromas, temperature and light intensity, pathogens, microbial metabolism, food contaminants, flavours or colours, toxins and physical, chemical, biological modifications of the food products. Food packaging industry recently use nanotechnology for its significant antimicrobial activity, prevention of microbial spoilage and provides barrier against microbes (Nile et al., 2020). Nanotechnology use to develop new sensing and food packaging materials which successfully reduce the barrier, improve mechanical properties of food packaging and active functional packaging. Bio-nanocomposites prepared from incorporation of nanomaterials (e.g. nanofillers) within biodegradable polymers significantly protect food from microorganisms, moisture and gases (O2 and CO2) (Mustafa & Andreescu, 2020). Whey protein isolates based nanocomposite with zein nanoparticles can improve mechanical properties, packing effectiveness, moisture barrier it improves moisture barrier. Soybean protein isolates also used to prepare nanocomposites (Oymaci & Altinkaya, 2016). Gelatin nanocomposite films based on cellulose nanocrystals can be used for edible packaging. It can also decrease the moisture affinity of gelatin, and improve food packaging applications at relatively low cost (George & Siddaramaiah, 2012). Regenerated silk nanofibrils used to prepare biomimetic membrane material for smart food packaging and maintain storage food temperature (Mustafa & Andreescu, 2020). Nanocomposite cast films and coating materials prepared from whey proteins can be used as biodegradable packaging solutions, alternative to fossil-based packaging materials and also increase sustainability of the packaging materials (Rana et al., 2017). Siying Tang et al. (2019) prepared ZnO nanoparticles incorporated soybean protein isolate films and showed improvement in tensile strength, microbial inhibition capacity, thermal stability, oxygen barrier of packaging films (Tang et al., 2019). RungsineeSothornvit et al., showed that whey protein isolate (WPI) based composite films with nano-clays (cloisite Na+, cloisite 20A, and cloisite 30B) were effective against Gram-positive bacteria, Listeria monocytogenes (Sothornvit et al., 2009).

Nanoproteins Applications to Improve Food Safety and Self life:
Edible nano-coatings on food material can prevent moisture and gas exchange, retain colour, antioxidants, enzymes, anti-browning agent which helps to increase the shelf life of manufactured food even after the opening of packet. Nanoencapsulation of functional component slow down chemical degradation
process by modified the properties of the interfacial layer which are surrounding them. Bio-nanocomposite film prepared from fish skin gelatin and silver-copper bimetallic nanoparticle can successfully reduce the growth of both gram-positive and gram-negative bacteria and preserve food material against spoilage bacteria (Arfat et al., 2017). Nanotechnology can help to maintain authenticity, traceability and safety of manufacturing food products. It also used to detect antibiotics residues, foodborne pathogens (Vibrio, Salmonella, E. coli, Listeria, S. aureus), pesticide residues (agricultural industry) and several mycotoxins in food and poultry industry (King et al., 2018). Gas content and non-invasive detection methods are able to detect and monitor the gas content, excess moisture, and oxygen content of a packaged material. Those methods are very helpful because presence of oxygen inside the packaging can reduce shelf-life of food through creating healthy environment for microbial growth. Nanotechnology used to prepare biofilms which provide effective measure to evaluate the quality and safety of food even after the production process has been done (Bajpai et al., 2018) Mosaad A Abdel-Wahhab et al. (2018) encapsulated cinnamon essential oil (COE) within whey protein and made emulsion droplets (diameters = 235 ±1.4 nm) to reduce toxicity of fumonisin B1 and aflatoxin B1 (fungal metabolities occur in food stuffs). Study showed whey protein encapsulation process maintained antioxidant effect of COE and enhanced the growth of gut microbes to decrease viability of mycotoxins in rat model (Abdel-Wahhab et al., 2018). Whey protein isolate encapsulated lutein (hydrophobic carotenoid) improves stability (at 4°C for near about 1month), water solubility during storage of lutein (Zhao et al., 2018). Angélica I. S. Luis et al., (2020) prepared zein protein based nanoparticles (diameter= 150 nm) for encapsulating eugenol and garlic essential oil. Study showed this zein based nanoparticles enhanced efficiency (>90%), stability (90 days) of the bioactive compounds and had a bactericidal activity against various fish pathogenic bacteria such as Aeromonas hydrophila, Edwardsiella tarda, and Streptococcus. (Defrates et al., 2018); (Luis et al., 2020). Li KK et al., (2013) Prepared nanoparticles from sodium caseinate (SC), zein solutions and loaded thymol. Study confirmed the significant antimicrobial activity of thymol-loaded nanoparticles and its effectiveness to prevent Staphylococcus aureus growth at 37°C (Li et al., 2013). Nanotechnology based food pathogen detection methods are now very popular to prevent health hazards and ensure food safety. Nanotechnology (magnetic beads) used to detect various enterotoxins produced by pathogenic bacteria such as Staphylococcus aureus even when present in very low amount (Alfadul & Elneshwy, 2010).

Various Techniques of Nanoprotein Formation:
Nanoeencapsulation Techniques: In this technique bioactive compound present within a unique polymer membrane cavity. Moreover nanocapsules are vesicular systems. Nanoeencapsulation technique and delivery system can affect efficiency of the nanoparticle through modification of physicochemical properties such as particle size, size distribution, surface area, shape and solubility. Nanospheres are matrix systems where the bioactive compound is uniformly dispersed.

Emulsification Technique:
Nanoemulsions are colloidal dispersions comprising two immiscible liquids with droplet sizes between 50 and 1,000 nm. Nanoemulsions are mostly used in two form liquid states (directly) and dried powder form by using spray drying and freeze drying after emulsification. Nanoemulsions have high kinetic stability and are prepared by using high-energy emulsification methods (high shear stirring, high-speed or high-pressure homogenizers, ultrasonicator, microfluidizer) (Ezhilarasi et al., 2012).

The coacervation technique:
It is a promising encapsulation technology due to its very high encapsulation efficiency (up to 99 %), stabilization of bioactive compounds in
food ingredients against oxidation, degradation, and controlled release. It also masks undesirable flavour. The nanocapsules between 100 and 600 nm are mainly produced via coacervation technique but the major problem of this technique is commercializing the coacervated food ingredient due to the usage of glutaraldehyde for cross-linking (Wang et al., 2018).

**Inclusion Complexation:**
In this technique a supra-molecular association of a ligand (encapsulated ingredient) encapsulated within a cavity-bearing substrate by hydrogen bonding, van der Waals force, entropy-driven hydrophobic effect. This technique is very useful due to high stability, encapsulation efficiency, mask odors and flavors and preserve aromas mainland it mainly used to encapsulate of volatile organic molecules, β-cyclodextrin and β-lactoglobulin (Ezhilarasi et al., 2012).

**Nanoprecipitation Technique:**
Nanocapsules (100 nm and below) produced by this technique are useful due to its miscibility with the aqueous phase. This technique is limited to water-miscible solvents. Solvent Evaporation Technique (nanocapsules sizes below 100 nm), and Supercritical Fluid Technique (has low critical temperature and minimum use of organic solvent) are also frequently used techniques for nano encapsulation (Ezhilarasi et al., 2012).

**Electrospray Technique:**
This technique helps to incorporation of bioactive compounds into the nanostructure with high efficacy. In this process liquid jet stream produced by application of high voltage in solution. Nanoparticles produced from electrospraying has high stability, encapsulation efficiency, reproducible, and no need of heat application (Bourbon et al., 2019). Generally electrosprayiong used for gliadin and elastin peptide base nanoparticles (Verma et al., 2018).

**Spray drying:** In this technique feed in a fluid state (emulsion, dispersion, solution) transfer into a powder through spraying into a hot drying gas which helps to produce uniform spherical particles. Spray drying process can manipulate size, bulk density, and flow properties of nanoparticles (Bourbon et al., 2019).

**Freeze Drying:**
This process is also known as lyophilization. The freezing process can stabilize nanoparticles mainly through four stages (freezing, sublimation, desorption stage, storage). This technique is useful for dehydration of heat-sensitive materials and able to maintain most of the nanoparticle size below 400 nm. It improves stability of bioactive compounds, shows encapsulation efficiency near 70 % and can alter nanoparticle size due various freezing temperatures (Ezhilarasi et al., 2012).

**Ionotropic gelation:**
In the Ionotropic gelation process two ionic species (polymer) electrostatically interact under certain conditions and form nanoparticles. Efficiency of this technique is very high (near about 100%). This method is relatively inexpensive and less time consuming (less than 10 h) (Pedroso-Santana & Fleitas-Salazar, 2020).

Among gelation mechanisms denaturation of globular protein, thermally-induced gelation, acid-induced gelation, ionic gelation, enzymatic gelation, water-in-oil heterogeneous Gelation methods are most frequently used (Bourbon et al., 2019). Other commonly known nanoencapsulation and delivery techniques are spray cooling, fluidized bed coating, extrusion, liposome encapsulation, cyclodextrin encapsulation (Arpagaus, 2019).

**Nanoprotein Toxicity Related Safety Issues:**
Size, shape, charge distribution, surface area, chemical reactivity, and dosage of various nanoparticles can induce toxicity in human body. Physical interaction between nanoparticle and biological membrane may damage the activity of membrane, protein folding capacity, various transport processes and chemical interaction results production of reactive oxygen species which leads to oxidative damage (Naseer et al., 2018). Toxicity assessment methods can be mainly two types: In vitro and in vivo. Among

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necrosis assay, oxidative stress assay and DNA damage assays are generally used to assess toxicity. Bio distribution, clearance, haematology, serum chemistry and histopathology assessment commonly used to assess toxicity in case of in vivo methods and this type of methods normally performed on animal models (rats, mice) (Kumar et al., 2017). Composition, crystalline structure, aggregation, concentration, surface coating and surface roughness of the nanoparticles are some possible factors which can poses toxicity in the food products (Gatoo et al., 2014). Nanomolecules are able to enter the blood circulation through respiratory tract, digestive tract, mucous membranes (Deng et al., 2020). Various Human and animal studies have confirmed the adverse short and long-term effect of nanoparticles on the respiratory system and cardiovascular system due to inhalation. Nanoparticles can cross the air-blood barrier, induce inflammation, oxidative stress, atherosclerosis (Kan et al., 2018). Nanocarriers frequently used for lung drug delivery applications (Pontes & Grenha, 2020). Inhalable nanomedicines developed to prevent lung cancer, chronic pulmonary diseases, and tuberculosis (Anderson et al., 2019) but nanoparticles also shows negative impact such as multifocal granulomas, peribronchial inflammation, progressive interstitial fibrosis, chronic pleural lesions and gene mutations (Ferreira et al., 2013). Positively charged nanoparticles shows higher toxicity due to higher cell electrostatic interactions and endocytic uptake (Hühn et al., 2013). Positively charged nanoparticles accumulates due to easy separation more in tumors than negatively charged due to easy separation in the interstitial space and internalized by tumor cells (Hoshyar et al., 2016). The cytotoxicity level of nanoparticles increased due to increase in surface charge of the nanoparticles (Foroozandeh & Aziz, 2018). Protein nanoparticles which used to digest in the upper gastrointestinal tract are able to promote toxicity. Alteration in peptides that are produced from nanoparticles may alter their allergenicity profile. Absorbed indigestible protein nanoparticles interacts and results some unpredicted adverse effects. With the gut microbiota, which could have some unforeseen effects (Mcclements & Xiao, 2017). Gliadin is the main cause of celiac disease. Glutamine and proline based protein nanomolecules can induce malabsorption and villous atrophy. Gluten protein based nanoparticles and nano-carriers should be restricted to this patient with celiac disease (Malekzad et al., 2017). Unni C. Nygaard et al., (2009) demonstrate that single-walled (sw) and multi-walled (mw) carbon nanotubes with ovalbumin (OVA) increased the concentration of OVA-specific IgE, eosinophils in bronchoalveolar lavage fluid and Th2-associated cytokines in the bronchoalveolar lavage fluid leads to allergic responses in mice (Nygaard et al., 2009). Immunogenicity may plays a role as a limiting factor for wide application of protein nanocarrier (Voci et al., 2020). Internationally accepted regulatory system is required to regulate nanoparticle usage in food industry and for that huge number of human experiment is required (Singh et al., 2017).

CONCLUSION

Recently food industries are showing interest towards protein nanostructures for various food applications, food processing and food packaging. Nano Proteins can able to preserve food and enhance shelf life which ensures health aspect of the consumer. It has ability to retain colour, flavor, texture and activity of bioactive compounds. The production cost and technical barriers of the already existing nanomaterials are relatively low and much easier than developing novel technologies but commercialization cost is dependent upon application methods. The methods of formation of nano proteins from different sources are under investigation and huge number of human experiment is required. To confirm nanoparticle related toxicity lot of work is needed. Interaction between nanoparticles and food stuffs and its effect should be estimated to reveal the exact cause of nanoparticle related toxicity. The use of
nano protein in various aspects is increased day by day but internationally accepted regulatory system and appropriate food labelling are required to regulate nanoparticle usage in food industry.

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