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Emerging Trends of Silver Nanoparticles Application in Food Formulation – Safety Assessment and Regulatory Framework

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Abstract

Nanotechnology applications in food formulation are associated with a wide range of benefits such as improved organoleptic characteristics and increased quality and safety of food products. Nanomaterials incorporated into a biopolymer matrix, are investigated as a new class of food packaging materials - known as *active packaging* - aiming to prolong self-life of the food products. Silver nanoparticles are the most common nanomaterials used in foodstuffs and food packaging formulation due to both their antioxidant capacity and antimicrobial activities against a wide range of pathogenic microorganisms. On the other hand nanotechnology used in food production conceals potential risks for the human health such as nanomaterials' toxicity and potential migration of nanoparticles into foodstuffs, thus food safety assessment is essential in order to identify and restrict these potential risks. Additionally silver nanoparticles toxicity on freshwater algae and fish underlines the possible environmental impact which is fundamental to be moderated. The scope of this mini review is to present the recent trends of silver nanoparticles application in food industry, the potential risks that might occur and the European Legislation framework regarding nanotechnology implementation in food chain.

Keywords

Nanotechnology, Silver Nanoparticles, Active and Intelligent Food Packaging, Antimicrobial Capacity, Safety Assessment, Nanomaterials Migration, European Legislation

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1. Introduction

Rapid development of nanotechnology in the food sector reveals that nanomaterials application in food production seems to be rather promising even though safety issues need to be resolved. Nanotechnology is a multidisciplinary science and technology which encloses physics, materials science, chemistry, engineering and biology, and deals with processes and phenomena that occur at the nanometer scale. A nanometer is equal to 10^{-9} of a meter; putting things in perspective the width of a human hair is 100,000 times larger. Atoms, molecules, macromolecules including biomolecules such as proteins and DNA, have dimensions in the nanometer range [1, 2]. Novel material properties and phenomena appear at the nanometer scale, which may be different than their bulk properties, since quantum mechanics and not classical physics laws describe their behavior [2].

Nanotechnology and nanoscience have found applications in many fields, including electronics, communication, catalysis, medicine and the food industry [2].

In food industry, commercially available products based on nanotechnology, have already appeared for a variety of uses [3], including silver nanoparticles (NPs) as antimicrobial [4], silica for *Salmonella* detection [5], water desalination [6], nanosized nutrients and nanoencapsulates [7]. Nanostructure

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materials have at least one of their dimensions at the nanometer range whereas NPs have all three dimensions at the nanometer range.

NPs because of their small size and high surface to volume ratio have been found to exhibit novel physicochemical properties different from the bulk materials from which they originate. Metallic NPs, especially gold and silver, are the most common ones encountered [8, 9]. Silver NPs are of paramount importance in the food industry [10, 11]. A baby mug and a cutting board, as an example, containing silver NPs, are commercially available [12].

Active packaging is important in food preservation acting not only as an inert barrier to external conditions, but also using metal and metal oxide NPs as antimicrobial agents [13].

Metal NPs such as Cu, Zn, Au, Ti and Ag can be used for this purpose, while Ag NPs seem to be the most promising since they have demonstrated bactericidal properties against a wide range of pathogenic microorganisms [14].

The properties of the NPs e.g. antimicrobial and antioxidant capacity strongly depend on their physicochemical properties such as size, shape, surface chemistry etc, which in turn depend on the synthesis methods. The metal NP synthesis routes are classified into physical (e.g. evaporation, laser ablation) and chemical methods [15]. In the later, reduction of an appropriate metal ion is the most common approach. Different organic and inorganic reducing agents, with the most common sodium borohydride alongside capping agents required for nanoparticle stabilization have been used. The involved chemicals however may be toxic and hazardous to the environment. Green synthesis using molecules derived from plant extracts has been demonstrated to be superior over chemical methods and environmental friendly [10].

Therefore, the aim of the current mini review is to present the recent trends regarding the application of silver NPs in food production along with the potential risk that might arise.

2. Current Applications of Nanotechnologies in Food Chain

2.1. Food Preservation and Packaging

Some of the main goals of the traditional food packaging are to protect the enclosed foodstuff and inform the consumers regarding the nutritional value. However, packaging with advanced functions is being investigated due to consumer demands regarding food quality and safety [16]. Fresh food, as an example, ready to be cooked (and or to be eaten) has received a lot of attraction during the last decades. This implies a minimal food processing, raising however concerns regarding foodborne diseases [14]. Food packaging materials are required to hinder gain or loss of moisture, prevent microbial contamination, act as a barrier against permeation of oxygen, water vapour, carbon dioxide, flavours and taints in addition to basic properties of packaging materials [17]. During the last decades intelligent packaging and active packaging have been emerged. The former provides communication and marketing functions, whereas the latter food protection and preservation [18]. In active packaging the food, the package and the environment interact in order to prolong food shelf-life [19]. However conventional packaging materials such as paper, plastic, glass and metal do not fulfil these requirements [17]. Among them plastic has been widely used in the twentieth century due to low cost, low weight, easy formation in various shapes, and excellent physicochemical properties [17, 18]. However, the aforementioned materials are non-sustainable and cause serious environmental problems since can not be easily degraded [17, 18].

Bio-nanocomposites i.e. NPs incorporated into a biopolymer matrix, are currently being investigated as a new class of food packaging materials [20-22]. Food Contact Materials (FCMs) that are capable of releasing nanostructures with antibacterial and antioxidant properties, improving sensory or self – life properties of food are known as *active packaging* [3]. Nanosensors incorporated into these packaging materials allow the identification of specific microorganisms, chemical compounds and environmental conditions [3]. Metallic based nanostructured materials, especially silver NPs, loaded into food packaging polymers have received considerable attention mainly due to their antibacterial activity [23].

2.2. Silver NPs Antimicrobial Capacity

Silver, a transition metal, has been known to have antibacterial properties since Hippocrates [24]. Open wound and burns were treated with silver because of its antiseptic properties [25]. The antibacterial properties of silver has been found to be increased by forming silver NPs in the range 10 - 100 nm, against Gram-positive and Gram-negative bacteria [26].

The antibacterial properties of silver NPs on *E. coli* and other Gram-negative bacteria, has been found to be size-dependent while the NPs diameter that exhibited direct impact to the bacteria has been found to be at the range 1 – 10nm [27]. Silver NPs synthesized using an *Acacia leucopholea* extract for a green synthesis approach or commercially available showed antibacterial action against *Listeria monocytogenes* that can contaminate food during processing [28, 29]. Efficient antimicrobial activity of silver NPs was also demonstrated against *Salmonella typhi* [30].

The mechanism of silver NPs antibacterial action has not been understood. Proposed mechanisms include the NP attachment at the cell surface and their penetration inside bacteria [27, 31]. Formation of free radicals followed by membrane damage [26], and silver ion release followed with subsequent interaction with thiol-based enzymes [25] have also been proposed as alternative mechanisms.

2.3. Silver NPs Antioxidant Capacity

Food quality and safety can be deteriorated by the presence of toxic compounds formed by oxidative deterioration of fats and oils in foods. Synthetic antioxidants can be toxic and of increased manufacturing costs, whereas natural antioxidants present in foods (e.g. polyphenols of the vegetables) seem to be safer.

Free radicals and oxidative stress are involved in many chronic diseases [32], whereas fruit and vegetable have been found to exert protective effect mainly due to various antioxidants they contain such as polyphenols, vitamin C and others [33]. In food free radicals can damage proteins, DNA and other small molecules [34]. Therefore using antioxidants in food systems, requires understanding both of the *in vitro* and *in vivo* antioxidant mechanism [34]. Depending on their action the antioxidants are classified into three categories [34]. Different assays for antioxidant activity have been developed and are either chemical methods or methods which measure oxidative damage in humans. The former, are based on measuring the ability to scavenge different free radicals, using different challengers such as hydroxyl (-OH), nitric oxide (-NO), and (α, α - diphenil - β -picrylhydrazyl radical) (DPPH). The latter assess protein damage, lipid oxidative deterioration, and total oxidative DNA damage [34]. Silver NPs synthesized using plant extracts have been found to exhibit increased antioxidant activity compared to plants, due to the preferential adsorption of active antioxidant material onto NP surface [34]. Bunghez *et al.,* 2012 used theornamental plants *Hyacintus orientalis L.* and *Dianthus caryophyllus L.* in order to prepare new antioxidant silver nanosystems. More specific they used the ornamental plant extracts as reducing agents for silver as well as capping agents for silver NPs formation [36]. The produced silver NPs were of sizes in the range 61 and 89 nm and their antioxidant activity was found to be superior to the one of the plant extract alone. Higher scavenging activity from biosynthesis of silver NPs compared to the plant alone was also observed using *Chenopodium murale* leaf extract [37]. Sufficient antioxidant activity compared to standard antioxidants has been also demonstrated from silver NPs synthesized using *Helicteres isora* root extract [38]. Additionally silver NPs synthesized using *Bergenia ciliate* crude extract [35] and aqueous extract of spice blend [39] have been found to show high antioxidant capacity.

2.4. Active and Intelligent Food Contact Materials (FCMs) Containing Nanosilver

Food containing vessels enriched with or made by silver are known since ancient times [38]. Silver's antimicrobial ability has allowed it to be incorporated in various food related products such as inner liners of refrigerators [41], cutting boards [42] and food packaging materials [43].

Active FCMs containing nanosized materials with antibacterial and antioxidant properties, improve the organoleptic characteristics and prolong self-life of foodstuffs [3].

Silver NPs can be easily incorporated into polymer FCMs used for food packaging [40]. Environmentally friendly biodegradable polymers enriched with silver NPs have been investigated during the last decade [14].

Cellulose, a natural carbohydrate, has been used as silver NP carrier, exhibiting antibacterial action against pathogens such as *Salmonella spp., Staphylococcus aureus* and *Listeria monocytogenes*, both for beef meat and fresh-cut melon [44, 45]. Silver NPs incorporated into hydroxypropyl methylcellulose matrix also demonstrated antibacterial capacity against *Escherichia coli* and *Staphylococcus aureus* [19].

In another study, active FCMs with silver NPs were incorporated into pollutant films and have been found to be effective against microorganisms such as *Escherichia coli O157:H7*, *Staphylococcus aureus*, *Salmonella Typhimurium* and *Listeria monocytogenes* [46]. A silver NP loaded corn starch was also developed with antimicrobial and improved mechanical and barrier properties, which are essential for food packaging [47]. Additionally Yang *et al.,* 2010 evaluated the impact of a mixture of polyethylene with nanosilver, kaolin and TiO_2 powder as a nano-packaging material, on preservation quality of strawberry fruits (*Fragaria ananassa* Duch. cv Fengxiang) [48].

Non-degradable polymers loaded with silver NPs, have also been investigated including polyethylene (PE), poly-vinyl chloride (PVC), and ethylene vinyl alcohol (EVOH) [14].

Intelligent packaging where information regarding the quality of packed food can also be implemented by FCMs [49]. The information can be radio frequency identity tags, oxygen and carbon dioxide sensors, time-temperature indicators and many others [18]. A sensor using appropriate nanostructures interacts both with food factors and external environmental factors giving a response. This response informs the consumers about food quality and safety as well as allows food producers to monitor product processing [18]. Silver nanoplates for example have been used as time - temperature indicators providing information regarding the end of shelf life of a product as well as exposing temperature variation during transportation and storage [50]. In another application, silver NPs were used as a colorimetric probe of melamine detection in milk [51, 52].

3. Physicochemical Characterization of Silver NPs

Silver NPs can be prepared using two different approaches. In the *top-bottom* approach an appropriate bulk material breaks into small particles [10]. Physical methods like evaporation, milling and laser ablation have been used which involve expensive procedures [10, 15]. In the *bottom-top* approach chemical reduction is involved. Sodium borohydride, sodium citrate, Tollen's reagent and other organic and inorganic reducing agents are used for silver ion reduction. However a major drawback of this approach is that the chemicals involved are rather toxic and the by-products obtained not environmental friendly [10].

Cost effective and environmentally friendly synthetic routes were a subject of intense research that led to biological methods involving bacteria, fungi and plants [10]. Biomolecules such as proteins, enzymes, polysaccharides, phenolics etc found in plants have been used for reduction and stabilisation of silver ions [10]. Various plants as well as various plant parts have been already used; including tea leaf extract [53], extract of inflorescence of *Cocos nucifera* [54], apple extract [55], and banana peel extract [56].

A variety of techniques are required for nanoparticle characterization. Visual inspection of a colour change of the reaction mixture followed by UV – Vis spectroscopy implies nanoparticle formation by showing a characteristic plasmon resonance. NP size, size distribution, and shape are determined using Transmission and Scanning Electron Microscopy (TEM, SEM), Atomic Force Microscopy (AFM), Dynamic Light Scattering (DLS). Powder X-ray Diffractometry (XRD) is used to determine particle crystallinity [15]. The above techniques can also be used for measuring NPs present in foodstuffs [57].

4. Safety Issues – Risk Assessment

Nanotechnology implementation in food formulation may conceal potential risks for human health, therefore risk assessment is fundamental to be carried out in order to identify and restrict these risks. Regarding human body exposure to NPs three possible routes occur; ingestion, inhalation and / or dermal exposure, while ingestion seems to be the most common way of exposure to NPs present in foods either as nano-ingredients (food additives) or as nanosized FCMs of the packaging.

Even though nanotechnology is widely used in food production very few studies have been conducted in order to assess the hazards of foodstuff contamination with nanoparticles [58]. Additionally limited knowledge exists regarding the bioavailability and toxicity of NPs present in foods [58]. Characterization of nanomaterials e.g. nanoparticle size, toxicity, shape, migration and ingestion rates are critical for assessing possible health risks [59]. Tiede *et al.,* 2008 have discussed some standard analysis methods of nanomaterials detection in nanofoods and FCMs [60].

4.1. Migration of Silver NPs from FCMs into Foodstuff

Prediction and quantification of any potential migration of NPs present in a plastic nano-packaging matrix requires information such as the viscosity of the polymer and the size-dependent diffusion of the particles [61–64]. Therefore migrating tests regarding food nano-packaging is important to be conducted prior to safety assessment of the nano-packaging material [65].

Migration of silver NPs into chicken meat from a PVC nanocomposite was found to occur using inductive coupled plasma mass spectroscopy [65]. Simulation models of silver nanoparticle migration into chicken breasts was also developed and evaluated experimentally [59]. Studies from commercially available nanosilver plastic food containers demonstrated also silver nanoparticle migration [66].

4.2. Silver NPs Toxicity

Human body can be exposed to NPs by oral ingestion, inhalation or dermal contact. Oral ingestion arises either from NPs migrating from food packaging or incorporated into the food itself [64]. However, NP effects through the gastrointestinal route are not known [67]. To determine nanoparicles toxicity certain physicochemical properties must be studied e.g. NP size, shape, solubility, surface reactivity etc [68]. It has been shown that NPs can cross cellular barriers and cause oxidative damage to the cell [67]. Smaller particles generally are expected to be more toxic [64]. Silver NPs biological activity is related to silver ion release, surface and photocatalytic properties [69].

Silver NPs once in the gastrointestinal route can pass into the blood [70, 71]. Reactive oxygen species can be readily produced by silver NPs and interact with proteins, enzymes and DNA [70]. NPs for example interact with proteins leading to protein unfolding, and thiol cross-linking, which in turn produce hazardous biological by-products [72]. This depends on particle size, shape, curvature, surface charge, free energy and surface coating [70, 72].

A few studies have been published over the last decade regarding silver nanoparticle toxicity on various cells, including rat liver cells [73], human fibroblasts [74], human macrophages [75], human lung carcinoma [76], and mammalian cell lines [77].

Silver NPs can be hazardous to the environment through water, soil and air dusting [72]. Inhalation of airborne NPs and therefore through the respiratory tract is another important route for human exposure [78]. Oxidative stress, peribronchial inflammation and gene mutations are a few of the respiratory effects caused by nanoparticle exposure [78]. Toxicity of silver NPs on freshwater algae, fish, and *Daphnia* has been demonstrated as an example of nanoparticle environmental impact [79].

5. European Regulatory Framework

Regarding nanomaterials application in the food industry, legislation is not yet completely developed. However when nanotechnology is incorporated in foodstuffs, these products have to comply with the Horizontal European Legislation on food safety along with the European Legislation on Novel Foods. Regarding the Horizontal European Legislation on food safety Directive 2001/95/EC on general product, provides the safety requirements and underlines the responsibility of the producers for safe food products, ensuring consumers' safety [80]. EC Regulation 178/2002 is the fundamental regulation containing all general principles ruling food and feed safety [81]. Regulation (EC) No 1907/2006 (REACH Regulation) on chemicals, provides information regarding the manufacture and control of chemicals in order to prevent any hazards on humans or the environment [82], while EC Regulation 258/97 on novel foods and novel food ingredients, provides the requirements for safe food production after nanotechnology application [83].

According to Directive 94/36/EC silver is accepted in Europe as a coloring agent (E-174) with no restrictions [84].

Additionally, the European Food Safety Authority (EFSA) recommends that silver ions (e.g. silver zeolites, silver zirconium phosphates and silver-containing glasses) migration from FCMs into food matrices should not exceed 0.05 mg Ag^+ /kg food [85].

Due to increasing concern about the safety of consumers exposed to nanofoods, EFSA released the first guidance for safety assessment of nanoscience and nanotechnology application in the food chain [86], underling the fact nanomaterials' characterization such as migration, distribution, both *in vitro* and *in vivo* toxicity test should be obligatory in food industry.

6. Conclusions

Nanotechnology applications in food formulation are associated with a wide range of benefits such as improved quality and safety of food products. Silver nanoparticles are the most common nanomaterials used in food industry due to their antimicrobial activities against both Gram-positive and Gram-negative bacteria. Additionally silver NPs synthesized using plant extracts have been found to exhibit increased antioxidant activity, due to the preferential adsorption of active antioxidant material onto NP surface. On the other hand nanotechnology used in food production conceals potential risks for the human health thus safety assessment is required along with more adequate European Regulatory framework.

Conflict of Interest

All the authors do not have any possible conflicts of interest.

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