APPLICATIONS OF NANOMATERIALS IN THE MEAT AND SEAFOOD PROCESSING INDUSTRY

Montero, M.P.*, López-Caballero, M.E., Alemán, A., Gómez-Estaca, J. Martínez-Álvarez, Ó. and Gómez-Guillén M.C.

Instituto de Ciencia y Tecnología de los Alimentos y Nutrición (ICTAN). Consejo Superior de Investigaciones Científicas (CSIC)

C/ José Antonio Novais nº 10, 28040 Madrid, Spain.

*Corresponding author: E-mail address: mpmontero@ictan.csic.es

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ABSTRACT

The application of nanoparticles and nanostructures in meat and fishery products is relatively recent and is arousing increasing interest. Nanostructure magnifies the properties with respect to traditional compounds (which are larger), owing to the greater surface area exposed and the small size, facilitating greater accessibility in food. There are many different kinds of nanoparticles and nanostructures; for example, they may use metals, or substances of vegetable or animal origin, such as chitosan structures. Otherwise, the application of natural compounds to maintain product quality is increasingly demanded, but the sensory characteristics of the products limit their use. Encapsulation by nanovesicles (emulsions and liposomes) of these compounds is a good strategy to mitigate their sensory characteristics while allowing progressive release of the bioactive agent to exert its action. This is the case with essential oil nanoemulsions. Liposomes entrapping bioactive compounds of many different kinds could be ingredients for the design of functional food products, while they improve the nutritional value

because to their high quantity polyunsaturated fatty acid, but they can also be used in the food industry for a large diversity of technological purposes. Meat and fishery product packaging can be improved by nanotechnology in aspects concerning the improvement of physicochemical properties and the design of active and intelligent packages.

With regard to the use of nanobiosensors, often they are mere prototypes, but no doubt they will soon be implemented in industrial quality control because they present high sensitivity, repeatability, and quickness. They are mainly applied to guarantee species identification, and to detect compounds and microorganisms.

Keywords: active nanoparticles, nanoliposomes, nanoemulsions, active nanocomposite packaging, nanobiosensors

1. INTRODUCTION

Consumer rejection of chemical additives and the increasing fondness for minimally processed foods that look like fresh and natural ones necessitate the use of technologies and/or techniques that ensure the quality and stability of food while at the same time guaranteeing their safety and security. The use of nanoscale elements is attracting more and more interest every day, used alone and/or as part of packages to improve their properties or endow them with activity (by interaction of nanocomposites with food or with the environment), as part of emulsion systems, nanocapsules, etc. (Singh et al 2016). The application of nanotechnology allows new applications, and improves the properties, being especially useful in the development of the products, among the possible applications is found it is worth highlighting: the modification of the flavors and texture, the improved absorption of nutrients, and better microorganism detection system, among other properties, as well as diversification in the food packaging system.

Nanotechnology can be involved in various aspects related with both the development and the preservation of food products based on myosystems (meats, poultry and seafood). Basically, the following possibilities need to be considered: i) when adding an ingredient or additive in the nano size; in this sense, nano-sized metal structures are gaining increasing interest (Martínez-Abad et al. (2012); ii) when the ingredient or additive is modified forming a nanostructure, -for example a phospholipid that forms a liposomal nanovesicle-, which could be used to encapsulate bioactive compounds (Marín et al 2018 a,b); iii) when any of the components of the protective cover (coating solutions or films) having nano size is used to confer special properties to the packaging. This would be the typical case of exfoliated clays as nano-reinforcements in films for biodegradable packaging (Echeverría et al.,2016), or the inclusion of nano-liposomes in edible films (Montero et al 2019). These are just a couple of examples, their applications in this field being extremely versatile. Finally, nanotechnology can also be applied for nanobiosensors construction (Inbaraj and Chen 2016, Duan et al. (2017).

The use of nanoparticles in the meat and seafood processing industry is the target of this chapter, which focuses mainly on improving the quality of myosystems (including formulation and use of by-products) and their preservation. Besides it also covers aspects related to the incorporation of nanoparticles in animal feed or even in nutraceuticals/drugs. Finally, it will also deal with the use of biosensors, to the detection of compounds/analytes and microorganisms in a fast, effective manner to guarantee the quality of meat and fish products.

2. ADDITION OF NANOESTRUCTURES IN MEAT AND FISH PRODUCTS

2.1. NON-ENCAPSULATING NANOPARTICLES

Many works in the literature deal with the application of nanoparticles (NPs) not alone but incorporated in food packaging or containers, as will be discussed in Section 3. Despite this, as

many of the articles base their discussion on the activity of the particles, it is considered interesting to mention them in this section, regardless of whether the particles are included in a physical support.

Among the various kinds of nanoparticles, metal nanomaterials are mainly used for their bactericidal properties. Silver (Ag) nanoparticles are the ones that have been most studied and their use is subject to legislation in many countries, as mentioned below. Mahdi et al. (2012) found that in minced beef stored at 3 ± 1 °C the shelf life (2 days) in usual food packaging was prolonged to 7 days, while the acceptability scores in nanosilver packaging (polyvinyl chloride–polyethylene laminate trays fortified by Ag NPs) were similar. These results were associated with the thickness of the meat, and the effect was striking in *Staphylococcus aureus* compared with *Escherichia coli*. As a result of the incorporation of 0.001–10 wt% silver ions in an ethylene–vinyl alcohol (EVOH) copolymer matrix by a solvent casting technique, Martínez-Abad et al. (2012) obtained a high protein content food (chicken wings or breast and marinated pork loin) that showed low susceptibility to the films (<1 log decrease) in terms of counts of *Listeria monocytogenes* when incubated 24 and 72 h at 12 °C. In addition, vegetables (low protein content food displayed no noticeable bacterial counts for 1 and 10 wt% silver films and nearby 2 log reduction for 0.1 wt% silver films. In this work, the EVOH film (0.1% silver content) was not enough to guarantee that the products would not exceed the restriction limits recommended by EFSA (2011). Moreover, these authors stated that for Gram-positive and Gram-negative bacteria, the efficacy of antimicrobial silver is improved when incorporated in the copolymer (EVOH) rather than being present as pure silver nitrate; this could be related to the inactivation of silver nanoparticles by the nutrient broth or by the microorganisms themselves. When in solution, all the silver ions could potentially be able of being immediately inactivated, while the release continues in the polymer for some time. This increases the interval of contact between the active silver ions and the bacteria, thus avoiding proliferation more efficiently. Rai et al. (2009) reported that Ag nanoparticles display effective antimicrobial properties bring into

comparison to other salts owing to their particularly large surface area, which offers better connection with microorganisms. The nanoparticles become linked to the cell membrane and also enter inside the bacteria. The membrane of the bacteria includes sulfur-containing proteins; the Ag-nanoparticles engage with these proteins and with phosphorus-containing compounds such as DNA. Once Ag-nanoparticles enter the bacterial cell, it is formed a low molecular weight region in the midpoint of the bacterium with which the bacterium conglomerates, thus preserving the DNA from the Ag-ions. The nanoparticles preferentially disrupt the respiratory chain, and cell division finally leads to death. Furthermore, Ag nanoparticles can be incorporated in target matrices in various ways (Toker, 2013), and they are stable and have low volatility (Youssef and Abdel-Aziz, 2013).

Fernández et al. (2010) developed by in situ reduction of silver nitrate (1%) adsorbed on cellulose fibers by thermal and UV treatments silver-based antibacterial hybrid materials. These authors studied the ability of these materials to lower microbial contamination of exuded fluids during beef meat storage (4 ± 0.2 °C, 10 days, with 12 h darkness and 12 h fluorescent light) in modified atmosphere packaging (69.1% O₂, 28.2% CO₂, and 2.7% N₂). Cellulose-silver hybrid materials decreased the total aerobic bacteria, lactic acid bacteria, pseudomonad spp., and enterobacteria found in the absorbent pads around 1 log cfu/g during the storage. The total aerobic bacteria and *Pseudomonas* spp. In the presence of silver ions, counts for total aerobic bacteria and *Pseudomonas* spp. decreased considerably, while LAB were not significantly affected. Enterobacteriaceae continued under the detection threshold if silver was present. The meat color and the amount of microorganism were not noticeably changed by the silver-based materials. In beef meat exudates, the level of migrated silver ions reached 60 ppm. Regarding this, the EFSA (European Food Safety Authority) has recommended that the upper limit of Ag migration from packaging should not exceed 0.05 mg/L in water and 0.05 mg/kg in food. Therefore, assessments of silver migration would be necessary to ensure antimicrobial effectiveness in accordance with present legislation (Carbone et al., 2016). Meanwhile, the

USFDA recommends, for all materials, that manufacturers should study and elaborate a toxicological profile for each container/packaging with nanomaterials (USFDA, 2014).

In addition to silver nanoparticles, other metals are also applied at the nano level to increase the quality of food. In turkey meat, Khalaf et al. (2013) evaluated the antimicrobial activities of pullulan (composed of maltotriose units) films incorporating 100 nm silver nanoparticles (Ag NPs) and 110 nm zinc oxide nanoparticles (ZnO NPs), applied on turkey deli meat that was previously inoculated with *S. aureus* and *L. monocytogenes*, and stored for two weeks (4 °C). The effect of essential oils (rosemary and oregano) was also tested. The Ag NP- and ZnO NP-based films gradually diminished the growing of both microorganisms from the first day of storage to values close to the limit of detection, with marked inhibition for *S. aureus*, and Ag NPs and/or edible films were more active than ZnO NPs and rosemary oil, respectively. These pullulan films were also tested on raw beef, turkey breast, and ready-to-eat turkey deli meat (previously inoculated with *L. monocytogenes*, *S. typhimurium*, *S. aureus*, and *E. coli* O157:H7), under vacuum packaging during storage for 3 weeks at 4 °C (Morsy et al., 2014). Moreover, the application of these films on raw beef contaminated with *S. typhimurium* reduced the counts by as much as 4 logarithmic cycles or more. In another experiment, suspensions of zinc oxide (ZnO) (at 6 and 8 mM), containing 1% acetic acid were examined against *E. coli*, *S. aureus*, *B. cereus* and *L. monocytogenes*, (Mirhosseini & Arjmand, 2014). The results showed that suspensions of ZnO were effective against all the strains; furthermore, ZnO nanoparticles diminished initial growth when these species were inoculated in sheep meat. Silica nanoparticles (nano-SiO₂, NSLDPE) (Luo et al., 2015a) and titanium nanoparticles (nano-TiO₂, NTLDPPE) (Luo et al., 2015b) were blended with low-density polyethylene (LDPE) to produce packaging (NSLDPE and NTLDPPE, respectively) that was shown to be a promising alternative preservation process for shrimp preservation during chilled storage. These authors found that Pacific white shrimp packaged with NTLDPPE or NSLDPE preserved freshness by reducing the total viable count, the total volatile base nitrogen (TVB-N) contents, thiobarbituric acid reactive substances (TBARS), and ATP

degradation (K value) compared to the control (LDPE). The modified LDPE packaging also slowed the decrease of the water holding capacity that could might be related to the smaller degradation of muscle proteins) and the increase of the melanosis score by means of polyphenol oxidase inhibition.

Even though metal nanoparticles incorporated in packaging have been the main subject of study, especially in the last decade, plant-based nanoparticles such as pomegranate peel have also been studied in protein matrix. Lyophilized pomegranate peel nanoparticles (LPP NPs, 80 ± 5 nm size) were incorporated at 1 and 1.5% in freshly minced beef meat that was stored for 15 days at 4 ± 1 °C (Morsy et al., 2018). Butylated hydroxytoluene (BHT, 0.01%) was used for control purposes. The LPP NPs exhibited a high phenolic content, and in the mentioned samples the contents of thiobarbituric acid reactive substances (TBARS), peroxides, and total volatile base nitrogen (TVB-N) were lower than in the control (up to 15 days), as well as the microbial load (total viable bacteria, psychrophilic, and lipolytic bacteria). Cooking characteristics of meatballs that included LPP NPs improved during conservation. Sensory evaluation of the parameters as rancid odor and color of those meatballs were acceptable and achieved a high score for up to 15 days. In another study, benzoic acid (phenolic compound) was encapsulated in polylactic-co-glycolic acid nanoparticles (168.9 ± 0.5 nm), which were injected into chicken meat (deboned fresh and cooked) (Ravichandran et al., 2011). The nanoparticles were effective against *S. typhimurium* (1.0 and 1.6 log cfu/g decrease) and *L. monocytogenes* (1.1 and 3.2 log cfu/g decrease) on 9 and 14 days of storage, respectively, compared with control samples (1.2 log cfu/g without nanoparticles). These authors stated that the lack of an outer cell wall and the accessible entrance of the nanoparticles and phenolics compounds into the bacteria could be the reasons for the higher reduction achieved in *Listeria* compared with *Salmonella*. Moreover, the greater inhibition in cooked compared with raw samples point to the absence of common microflora destroyed during cooking, which could have hindered the inhibitory characteristics of the nanoparticles.

The antimicrobial effectiveness of citral as main compound of essential oil was improved by using nanostructured lipid carriers (NLCs) (Mokarizadeh et al., 2017). The citral interacted with the various ingredients (Mygliol, Precirol, and Poloramex), resulting in a high encapsulation efficiency, with particles that were stable for 90 days. These authors reported lower minimum inhibitory and bactericidal concentrations than those of citral emulsion for a selected group of microorganisms (*S. aureus*, *B. cereus*, *E. coli*, and *Candida albicans*); although they have not yet been applied in protein matrices, the encapsulation of essential oils in nanostructured lipid carriers could be a promising strategy to be applied in the food industry. The influence of garlic nanoparticles on the characteristics of beef burgers (premium and standard quality, raw and precooked, following the parameters established by Colombian Technical Standard (NTC) 1325 has also been studied (Torres-Vargas et al., 2016). These authors observed that the incorporation of garlic nanoparticles did not affect the structure of the precooked and raw burgers, but it produced an increase in the red tendency (a *) in the premium burgers. The cooking process showed a decrease in the L * parameter of the formulations and an increment in shear force during storage. The microbiological analysis (mesophilic aerobic organisms, fecal and total coliforms, *S. aureus*, sulfite-reducing *Clostridium* spores and *Salmonella* sp.) fulfilled the parameters required by NTC 1325.

In addition to preserving quality during storage, nanoscale materials have also been used to modify technological properties of fish proteins. Thus, Yin and Park (2014a) observed that the properties of gelation of *Alaska pollock* surimi after setting were influenced by the incorporation of nanoscale fish bone (NFB) at diverse concentrations (0%, 0.1%, 0.25%, 0.5%, 1%, and 2%). The nanoscale particles were too small to dissociate the myofibrillar protein matrix. These authors observed that the rheological parameters, as breaking distance and breaking force of these surimi gels after setting enlarged significantly as the concentration of nanoscale fish bone raised up to 1%. Both light meromyosin chains and myosin heavy chains were aggregated to a larger extent as NFB concentration augmented. Thus, the first peak temperature and storage modulus

value (G'), increased as NFB concentration increased (both related to the unfolding and aggregation of light meromyosin). Similarly, the intensity of myosin heavy chain cross-links also increased as NFB concentration increased. This fact could indicate the formation of additional ϵ -(γ -glutamyl) lysine covalent bond by means of endogenous transglutaminase (which also increased as NFB calcium concentration increased), and more calcium ions from NFB. The texture of the gel rose with up to 1% NFB, due to calcium released from NFB and endogenous transglutaminase. Moreover, these authors found that these results were affected by the particle size of the fish bone, as surimi with micro fish bone particles produced a porous and discontinuous network, where microparticles could not be inserted in the gel matrix without disturbing the myofibrillar gel network, as occurred in the case of nanoparticles (Yin et al., 2014b). Another nano-technological application in products of animal origin is the encapsulation of fish oil. Yang and Ciftci (2017) encapsulated fish oil in hollow solid lipid micro- and nanoparticles formed from fully hydrogenated soybean oil using atomization of supercritical carbon dioxide expanded lipid. The highest bioactive loading efficiency was 97.5% w/w and was obtained with a concentration of 50% w/w, and oxidative stability of the loaded fish oil improved when compared to the free fish oil and the oxidative stability of the loaded fish oil improved when compared with the stability of the free fish oil. Thus this technique is shown to be a way of obtaining solid fish oil formulations to make handling and storage reasonable and appropriate.

Animal feeding is closely related to the meat/fish processing industry. Yausheva et al. (2016) showed that the joint utilization of iron nanoparticles and arginine raised weight gain by up to 9.2% in poultry as compared to the control, and, moreover, iron nanoparticles together with a mixture of amino acids (arginine, lysine, and methionine) provided an increase of up to 20%. When applied separately, the nanoparticles and amino acids resulted in lower weight gain. Additionally, intramuscular administration of iron nanoparticles promoted an immune response that was manifested in an enhanced level of leukocytes. Iron nanoparticles have also been also

used in combination with *Lactobacillus casei* as a probiotic in rainbow trout (Mohammadi and Tukmechi, 2015). Results indicated that growth parameters as weight gain, body weight gain, specific growth rate, daily growth rate, condition factor, and food conversion rate significantly increased when the diet was supplemented with iron nanoparticles (50 µg/kg) and *Lactobacillus casei* (10^8 cfu/g), and also intestinal bacteria counts were increased by this supplemented diet. The effect of clinoptilolite coated with silver nanoparticles on meat quality attributes of broiler chickens during frozen storage has also been evaluated (Hashemi et al., 2017). The experimental groups were fed with different diets, including supplements with 1% clinoptilolite and 1% clinoptilolite coated with 25, 50, or 75 ppm nanosilver in the basal diets, and after slaughtering they were kept for 3 or 7 days at -17°C . Texture profile analysis showed that nanosilver coated on clinoptilolite did not have any negative effects on texture or sensory attributes of chicken meat. The lowest value of springiness and chewiness was for the breast muscle of broilers fed with clinoptilolite coated with 25 ppm nanosilver. Adhesiveness, cohesiveness, and gumminess values were not influenced by the treatment. At all levels the addition of nanosilver coated on clinoptilolite increased water-holding capacity (WHC) in thigh muscles after 7 days of frozen storage. In conclusion, nanosilver coated on clinoptilolite can be used as a potential feed additive in broiler diet without negative implications on meat quality characteristics.

Nanoparticles have been applied in animal medicine, including diagnosis, vaccine, food administration, etc. Of special interest is their use in fish factories as treatment for antibiotic resistance. Shaalan et al. (2016) reviewed the main applications in this field, among which it is important to emphasise the use of silver, gold, zinc oxide, and titanium dioxide nanoparticles as alternatives to antimicrobials to fight microbial resistance to antibiotics; virus-like particles chitosan and polylactic-polyglycolic acid (PLGA) for drug delivery; liposomes, immunostimulant complex, meta-nanoparticles, and gold nanoparticles for diagnosis of pathogens in fish, alone or combined with other techniques, such as loop-mediated isothermal amplification, etc.

2.2. ACTIVE CHITOSAN NANOPARTICLES

Chitosan is generally recognized as safe (GRAS). It exhibits unique properties such as good biocompatibility, low toxicity, excellent biodegradability, and a broad spectrum of biological properties, including antimicrobial, antioxidant, insecticidal, and antifungal. However, chitosan shows poor solubility at pH levels above 6.5 and can only be dissolved in acidic solutions, and this limits its application as a preservative in food. This drawback could be overcome by forming chitosan nanofibers and nanoparticles that could easily be incorporated in food and have important effects on its quality. Moreover, it is worth noting that the biological properties of chitosan nanoparticles and nanofibers seem to be better than those of chitosan. Qi et al. (2004) reported enhanced antimicrobial properties of chitosan nanoparticles that were due to their small size, as well as to the quantum size effect. The preparation of chitosan nanoparticles could also improve the penetration of chitosan in tissues, which is limited by its high viscosity in solution and also by its large particle size. Gan et al. (2005) reported that submicron-sized particles could penetrate into tissues through fine capillaries, and thus the formation of chitosan nanoparticles could facilitate the incorporation of this biopolymer in foods. However, despite the bioactive potential of chitosan nanoparticles and nanofibers, the use of these particles as natural preservative agents has scarcely been reported. Chantarasataporn et al. (2014) prepared oligochitosan particles that were formed by aggregates of individual nanoparticles with an approximate size of 100–300 nm. The oligochitosan particles were mixed with minced pork at a concentration of 0.2–0.4%, and their effect on microbial quality was demonstrated throughout refrigerated storage. Thus, the incorporation of 0.4% oligochitosan in minced pork reduced growth of lactic acid bacteria (LAB) when compared with a control sample. Furthermore, the growth of total viable bacteria, *Staphylococcus*, and Enterobacteriaceae was significantly inhibited by the presence of either 0.2 or 0.4% oligochitosan (around 20–30%). In addition, *Salmonella* sp. growth was not detected in the samples. The antibacterial effect was higher against Gram-negative bacteria than against Gram-positives. The considerable antimicrobial

effect of these chitosan particles was probably related to the positive charge on their surface, and also to their high surface area. Moreover, the oligochitosan particles showed ability to retard the production of biogenic amines, this effect being due to the ability to produce inhibition of LAB and Enterobacteriaceae. Oligochitosan nanoparticles also reduced production of secondary oxidation products and protein oxidation. This study suggests that these particles could have a considerable effect on quality and shelf life extension of processed fish or meat.

Chitosan-sodium tripolyphosphate (CH-TPP) nanoparticles have recently been incorporated in shrimp muscle to inhibit microbial growth during refrigerated storage (Chouljenko , et al., 2017a). CH-TPP nanoparticles of around 140 nm penetrated into meat shrimp by vacuum tumbling. The aerobic plate counts in shrimp including these nanoparticles remained constant during 24 days of storage. Furthermore, the CH-TPP nanoparticles showed an interesting antioxidant capacity, suggesting that they could be used as natural antioxidants in fish or meat products rich in polyunsaturated fatty acids. In a similar work, Chouljenko, , et al. (2017b) reported antimicrobial and antioxidant activity of similar nanoparticles incorporated in shrimp meat that was frozen for 120 days, without affecting color and texture. CH-TPP nanoparticles have also been used to improve the quality of frozen shrimp. For example, Solval et al. (2014) used glazing solutions containing CH-TPP nanoparticles of around 180 or 234 nm to glaze frozen shrimp, and they observed reductions of lipid oxidation, total aerobic counts, yeasts, and molds after 30 days of storage at -20°C .

Owing to their properties, chitosan nanoparticles have been used as nano-sized vehicles for encapsulation of various compounds of interest to the food industry, such as essential oils. Essential oils are natural antimicrobial and antioxidant agents with interesting applications in medicine, cosmetics, and food. However, the use of essential oils in food is restricted by their potential interactions with food components, high volatility, polarity, and also negative effects on the sensory characteristics of products. Encapsulation of essential oils in chitosan

nanocapsules could be useful to improve their solubility and stability, to mask their taste and odor, to reduce evaporation during storage, and also to allow their controlled release. Essential oil–chitosan nanoparticles could be incorporated in fish or meat products with the target of improving their overall quality and prolonging their shelf life. However, there is little information on the preservative effects of essential oils encapsulated in chitosan nanoparticles. Ghaderi-Ghahfarokhi et al. (2017) investigated the effect of the incorporation of cinnamon essential oil-loaded chitosan nanoparticles in the formulation of beef patties during cold storage. They observed that the incorporation of 0.1% cinnamon essential oil-loaded chitosan nanoparticles decreased growth of total mesophilic viable bacteria and prolonged shelf life for 4 days. Moreover, the growth of lactic acid bacteria, Enterobacteriaceae, *Staphylococcus aureus*, yeasts, and molds in beef patties that included the nanoparticles was lower than in the control. The incorporation of the nanoparticles also retarded lipid oxidation and improved the color and acceptability of the patties. The size of these nanoparticles was 235 nm, and the zeta potential and polydispersity index (PDI) were 25.1 mV and 0.33, respectively (at pH = 4.0). The release mechanism of the essential oil was simulated, and a sustained release was observed, together with polymer matrix degradation in the course of time. In a similar work, the incorporation of thyme essential oil (TEO)-loaded chitosan nanoparticles (CS-NP-TEO) of around 121 nm in beef burgers reduced lipid oxidation and growth of Enterobacteriaceae during 8 days of storage (Ghaderi-Ghahfarokhi et al., 2016). The incorporation of CS-NP-TEO in the burgers also improved their acceptability and sensory quality. Moreover, Sotelo-Boyás et al. (2017) prepared thyme essential oil-loaded chitosan nanoparticles and nanocapsules with potent antimicrobial activity against various bacterial species, including *Bacillus cereus*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Escherichia coli*. They studied the release kinetics of the bioactive substances thymol and carvacrol, and observed that the maximum release times for thymol and carvacrol were 360 and 390 minutes, respectively. The size of these particles was 6.4 nm (nanoparticles) and 9.1 nm (nanocapsules). The interesting properties chitosan nanoparticles

and nanocapsules loaded with thyme essential oil suggest that they could be incorporated directly in food or in polymeric active package films with the aim of prolonging shelf life.

Chitosan nanoparticles could also be useful carriers of flavors, antioxidants, minerals, vitamins, antimicrobials, or enzymes, with interesting applications in fish and meat products. The incorporation of nanoencapsulated vitamins would enhance the nutritional value of these foodstuffs. Also, encapsulation of vitamins would reduce the negative effect of high temperatures, oxygen, light, or humidity during processing and/or storage of foodstuffs, thus increasing their stability. However, no information is available about the use of nanoencapsulated vitamins in processed fish or meat products to date. Nanoencapsulated vitamins could also be introduced in feeds to reinforce the innate immunity system of fish. Nanoencapsulation would protect vitamins from harsh conditions in the intestinal tract and would permit their controlled release. Alishahi et al. (2011) used pellets including vitamin C-loaded CH-TPP nanoparticles to feed rainbow trout, and observed that innate immunity indices were higher than those of control groups. Chitosan nanoparticles could also be useful to enhance the immunity of shrimp. For example, Rajeshkumar et al. (2009) incorporated DNA-loaded chitosan nanoparticles in feed for aquaculture shrimp and reported significant protection to shrimp against white spot syndrome virus. Similar nanoparticles in feed were used by the same authors to protect farmed sea bass against *Vibrio anguillarum* (Rajesh Kumar et al., 2008).

2.3. ACTIVE NANOEMULSIONS AS INGREDIENT

Nanoemulsions are a class of emulsions (a mixture of at least two immiscible liquids wherein one phase is dispersed as small droplets within the other phase) with nanoscale droplet diameters. Specifically, oil-in-water Nanoemulsions are composed of lipid nanodroplets (with a size between 10 and 100 nm in diameter) dispersed in an aqueous solution (McClements, 2011). However, some authors consider a larger drop size (Wu et al., 2016; Ozogul et al., 2016; Yazgan et al., 2017).

Droplet size is one of the most important parameters for characterizing the physical properties of an emulsion. When the droplet size decreases, the viscosity of the emulsion increases, and it becomes more stable and lighter (McClements, 2011).

The methods used to form nanoemulsions are generally divided into high-energy methods (ultrasonic devices, high-pressure homogenizers, microfluidizers) and low-energy methods (phase inversion temperature, phase inversion composition, emulsion inversion point methods) (McClements & Rao, 2011). Among the various researchers that evaluate the application of nanoemulsions in seafood and meat products, high-energy methods, especially ultrasonication, are the ones most often employed to produce nanoemulsions (Wu et al., 2016; Ozogul et al., 2016; 2017a,b; Yazgan et al., 2017; Noori et al., 2018). Only a few research works have used other methods to obtain the corresponding nanoemulsions, such as phase inversion temperature (Moraes-Lovison et al., 2017) or the emulsion-ionic gelation technique (Wu et al., 2016). Low-energy methods are often more effective for achieve small droplet sizes, but they are more limited with regard to the types of oils and emulsifiers that can be used (McClements & Rao, 2011).

The application for which the emulsion (micro or nano) is used in the myosystems is mainly for the preservation of meat and fish products and therefore to extent the useful life. For this, in the emulsions are added to various bioactive compounds, many of which can make undesirable odors or colorations in the final product and, being encapsulated in this way remain protected and their effect on sensory properties diminished. The application for which the emulsion (micro or nano) is used in the myosystems is mainly for the preservation of meat and fish products and therefore to extent the useful life. For this, in the emulsions are added to various bioactive compounds, many of which can make undesirable odors or colorations in the final product and, being encapsulated in this way remain protected and their effect on sensory properties diminished.

Nanoemulsions in seafood products

Nanoemulsions are widely used in the food industry for the encapsulation of bioactives (polyphenols, carotenoids, lipids, vitamins) to improve their solubility in water, to protect them from interaction with external agents, and to enhance the bioavailability of these compounds in the human body (McClements et al., 2007). However, when they are used in direct applications in seafood or meat products, research has focused mainly on increasing the shelf life of these products. For example, Ozogul et al. (2016) studied the influence of nanoemulsions prepared with various oils on the sensory, chemical, and microbiological qualities of farmed sea bass (*Dicentrarchus labrax*) stored at refrigeration temperature. The results showed that the use of nanoemulsions extended the fish shelf life by two days when compared with the control, and improved the microbiological and chemical properties during the storage period. At the end of the investigation, sea bass fillets treated with nanoemulsions were described as having a neutral odor, whereas the control was characterized as having an acid and sour odor. In another study,, a sunflower oil-based nanoemulsion also improved the quality and shelf life of both sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) fillets during storage at cold temperature. In this research work, treatment with nanoemulsion reduced bacterial growth and improved the values of chemical parameters and the sensory properties of the fish samples. The influence of nanoemulsions on the preservation of Indo-Pacific king mackerel (*Scomberomorus guttatus*) fillets were also investigated (Joe et al., 2012), but in this study the storage temperature was 20 °C. The application of nanoemulsion allowed an extension of shelf life of 48 h compared to the control and antibiotic treatment. Thus, nanoemulsion could be used to improve storage of fish at cold temperatures and in areas with poor refrigeration.

As mentioned before, in recent years, increasing interest has been shown in the use of essential oils as natural antimicrobial agents for food preservation. However, their application may be limited owing to their low solubility in water, the high volatility of some of their constituents,

and their strong flavor and odor, which could affect the sensory properties of the food product. One strategy used to overcome these disadvantages is the encapsulation of essential oils. Various works have demonstrated that encapsulation in nanoemulsions improves the bioactivity of essential oils because it increases diffusion through biological membranes, owing to their nanoscale and lipophilic nature (Donsì et al., 2011). Nanoemulsions could also protect essential oils against evaporation, oxidation, or thermal degradation, and increase their stability, allowing a reduction in the dose of essential oils required to ensure their bioactivity and minimize their impact on the sensory properties of food (Donsì et al., 2011). Furthermore, nanoemulsions can be considered as self-preserving antimicrobials because water is bound by their structure, restricting the access of microorganisms to water (Al-Adham et al., 2000). In this context, Wu et al. (2016) prepared coatings with emulsions and nanoemulsions based on chitosan particles loaded with citrus essential oil, and investigated the effect of these coatings on the shelf life of silver pomfret fillet stored under refrigeration. Treatment with nanoemulsions was more effective than conventional emulsions, extending the durability of fish samples from 12 days to 16 days, while the control sample had a shelf life of only 8 days. The nanoemulsion coating was effective in inhibiting the growth of microorganisms and changes in the chemical properties of the silver pomfret. Furthermore, the fish treated with a nanoemulsion coating had the highest sensory scores at the end of storage. On the other hand, jujube gum-based nanoemulsions containing nettle essential oil as edible coatings were used to preserve beluga sturgeon fillets during storage under refrigeration. Physical, chemical, microbiological, and sensory characteristics of the fish samples were evaluated. The results of this work showed that the shelf life of the fish samples was extended by the use of the corresponding edible coating. A coating formulated with 12% jujube gum and 3.5% nettle essential oil allowed the lowest microbial growth, weight and cooking losses, pH changes, and sensory deterioration in beluga sturgeon (Gharibzahedia & Mohammadnabiba, 2017). Two other studies evaluated the effect of the application of nanoemulsions on the shelf life of rainbow trout fillets during cold storage.

Shadman et al. (2017) prepared sunflower oil nanoemulsions containing various concentrations of *Zataria multiflora* Boiss essential oils, and determined the chemical and sensory properties of the fish samples. Ozogul et al. (2017b) used nanoemulsions based on different herb oils (rosemary, laurel, thyme, and sage) and evaluated the microbiological quality, chemical properties, and sensory properties (raw and cooked) of the fish fillets. Both studies found that the quality of the fish fillets was improved by using nanoemulsions.

The lipid oxidation process can also be retarded by the use of nanoemulsions. Ozogul et al. (2017a) found that the application of nanoemulsion in combination with vacuum packing allowed a lower loss of polyunsaturated fatty acid (PUFA) content during storage of sea bass fillets compared to the control. This could be attributed to the effect of the vacuum and nanoemulsion on prevention of lipid oxidation. In some of the investigations that studied the preservative effect of nanoemulsions in seafood products, the effect on lipid peroxidation was also evaluated. Some of these works found that the use of nanoemulsion also retarded the lipid oxidation process in fish samples (Yazgan et al., 2017; Gharibzahedia & Mohammadnabiba, 2017; Shadman et al., 2017).

Nanoemulsions could also be used in the aquaculture industry. The main used is for avoid infection in fish. Mishra et al. (2014) highlights the therapeutic potential of neem nanoemulsion against *Pseudomonas aeruginosa* infection in the freshwater fish *Labeo rohita*. The nanoemulsion formulation reduced the toxicity effect of neem oil, leading to a treatment with negligible toxicity for the fish. The effect of neem nanoemulsion for controlling *P. aeruginosa* infection in *L. rohita* was similar to that of the commercial antibiotic kanamycin.

Nanoemulsions in meat and poultry products

In meat and poultry products, the researches have been carried out using essential oils encapsulate in nanoemulsions to preserve the self-life. Noori et al. (2018) compared the effect of conventional emulsion and nanoemulsion-based coatings containing ginger essential oil on

the safety and quality of chicken breast fillets during storage under refrigeration. Similarly, to the above-mentioned research (Wu et al., 2016), the nanoemulsions allowed a better preservation of foods compared to the emulsions. A nanoemulsion-based edible sodium caseinate coating containing 6% of ginger essential oil improved the shelf life of chicken breast. The positive effect on appearance was again another important benefit of application of the nanoemulsion. Samples coated with ginger essential oil nanoemulsions showed lower color difference in comparison with the fresh chicken fillets than those coated with emulsions. Furthermore, when nanoemulsion was used in the formulation of the coating, the corresponding sample showed the highest total score during storage. However, in this study the nanoemulsion treatment did not have a significant effect on lipid oxidation of chicken fillets. On the other hand, Moraes-Lovison et al. (2017) developed nanoemulsions encapsulating various amounts of oregano (*Origanum vulgare*) essential oil. In this research, the nanoencapsulation of essential oil did not significantly improve its antimicrobial properties as initially expected. However, when the nanoemulsion was incorporated in chicken pâté previously inoculated with *Escherichia coli*, the contamination on the last day of analysis was significantly lower than with direct oregano essential oil treatments.

The results of all of these studies confirm the potential utility of essential oil nanoemulsion for improving the shelf life of poultry meat or fish products.

2.4. NANOLIPOSOMES

Liposomes are vesicles of spherical shape surrounded by one or more phospholipid bilayers that are used for encapsulation and delivery of a diversity of hydrophilic and hydrophobic substances. In the field of the food industry, liposomes can be used as flavor encapsulation maskers to hide bitter tastes, and also to enrich functional foods with healthy and bioactive compounds, such as antioxidants, vitamins, minerals, peptides and enzymes, that could be useful for disease prevention (Mozafari et al., 2008; Singh, 2016). Liposomal encapsulation improves chemical and

physical stability of the active compounds, prevents their interactions with other food constituents, and favors their targeted delivery inside the organism (da Silva Malheiros et al., 2010). Liposomes are produced from the nanometer to the micrometer size, and their structural and physicochemical properties depend strongly on the method of preparation and lipid composition (Akbarzadeh et al., 2013).

A variety of lipids can be used for liposome preparation. Specifically concerning liposomes for the food industry, the high content in polyunsaturated fatty acids and residual tocopherol, both being naturally present in the soy lecithin or semi-purified phosphatidylcholine, provide a beneficial role in lipid metabolism and high antioxidant activity (Taladrid et al., 2017). The use of natural phospholipids reduces considerably manufacturing costs and meets food legislation (Laye et al., 2008). However, the unsaturated fatty acid composition decreases the bilayer thermal stability and confers higher susceptibility to lipid oxidation (Wang and Wang, 2008), causing rancidity and shelf life reduction when included in a food system. The encapsulation of antioxidant compounds in liposomes contributes to prevent lipid oxidation. However, liposomal stability, encapsulation efficiency and particle properties will depend on the type of antioxidant and its concentration (Tan et al., 2013).

Liposomal suspensions can be stable for several weeks, however they usually become unstable by aggregation, oxidation and/or hydrolysis, that could lead to sedimentation and drug leakage (Sharma and Sharma, 1997). Freezing (Chen et al., 2010), freeze-drying (Sebaaly et al., 2016) or spray-drying (Gültekin-Özgüven et al., 2016) have been proposed to enhance liposomal stability during long-term storage. However, they normally induce an increase in the particle size, especially the freezing method. Lyophilization may cause damage in the vesicle membrane by the production of ice crystals followed by aggregation during freezing and subsequent desiccation, as well as phase transition changes during liposome reconstitution (Chen et al., 2010). Polyalcohols and carbohydrates have been used to prevent freezing- or freeze-drying-

induced damage of the bilayers (Stark et al., 2010). Various natural extracts with antioxidant capacity (pomegranate peel extract, shrimp lipid extract and collagen hydrolysate) were successfully entrapped in soy phosphatidylcholine nanoliposomes together with glycerol. Freeze-drying caused the particle size to increase from 75.7–81.0 nm up to 199–283 nm, however a considerable amount still remained near the original nanoscale (Marín et al., 2018a). Noticeable changes in the liposome bilayer caused by the freeze-drying process and long-term storage were found to depend on the encapsulation efficiency and the type of the loaded compound (Marín et al., 2018b). Of particular relevance to the food industry is the fact that the incorporation of liposomes in the dry state makes easier the adjusting of the moisture content in the formulation of meat and fish restructured products and pâtés. This effect is highly advantageous for texture optimization in functional foods with a low or intermediate moisture level. Besides their bioactive potential, liposomes can also be used in the food industry for a variety of technological purposes, providing controlled release of antimicrobials and antioxidants for food preservation during storage, improving industrial processes, or avoiding health risks.

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Nanoliposomes in meat processing

There is a growing demand for healthy and safe food products with additional benefits beyond basic nutrition. In this context, the fortification of meat pâté with iron salts encapsulated in liposomes has been proposed as a dietary strategy to prevent anemia in young iron-deficient women (Navas-Carretero et al., 2009). In a previous study in rats fed with fortified meat pâté it was observed that iron bioavailability from ferrous gluconate was similar to that of a commercial liposomal-encapsulated ferric pyrophosphate preparation (Navas-Carretero et al., 2007). Meat-based products are rich in proteins with high biological value, vitamins, minerals, and other bioactive compounds, such as taurine. The main negative aspect is their high content in cholesterol and saturated fatty acids, especially in red meat, which has been largely related to cardiovascular risk. The nutritional quality of pork meat was improved by the incorporation of polyunsaturated omega-3 fatty acids (42% eicosapentaenoic and 16% docosahexaenoic) encapsulated in nanoliposomes (Ojha et al., 2017). Diffusion of the encapsulated oil into the

pork meat slices was enhanced by ultrasound treatment to overcome the low rates of diffusion into the solid food matrix.

Lipid oxidation may negatively affect the shelf life and quality of meat products, particularly in the case of ground meat. There is a growing tendency to replace synthetic antioxidants with other natural derivatives from animals or plants, which, owing to their non-toxic nature, provide greater safety for consumer health. Nanoliposome technology was proposed to mask the fishy smell of marine fish skin peptides (FSP) incorporated as a natural antioxidant in a pork patty model (Bai et al., 2017). These authors found that the FSP inhibited the lipid oxidation of the patties during refrigerated storage; however, the TBARS values were significantly higher than in the control sample. Furthermore, the amount of volatile basic nitrogen was not efficiently reduced by peptide encapsulation. As a positive aspect, patties with liposomes showed lower cooking losses than patties treated with conventional Butylated hydroxy Anisole (BHA) antioxidant. The authors concluded that liposomes made from lecithin were not good candidates for encapsulation of antioxidant peptides to be applied as meat preservatives.

Meat biopreservation by using bacteriocins, which are heat-stable antimicrobial peptides of bacterial origin, represents an innovative approach to the control of spoilage and pathogenic microorganisms. In particular, nisin and pediocin are very interesting in terms of food safety since they are generally recognized as safe substances (GRAS). However, bacteriocins may be less effective in meats because they are degraded by proteases, and their adsorption to sensitive microorganisms could be influenced by the presence of various salts. Encapsulation in nanoliposomes has been reported to provide bacteriocins with higher antimicrobial efficiency and stability (da Silva Malheiros et al., 2010). Pediocin AcH was encapsulated in multilamellar vesicles of phosphatidylcholine to afford protection and more even distribution of this antimicrobial in heated slurries of beef tallow and beef muscle (Degnan and Luchansky, 1992). The results of this work showed increases of around 28% in the recovery of pediocin activity

when pediocin was encapsulated as compared to the free form. Furthermore, dairy-based slurries showed greater enhancement of pediocin activity than meat-based slurries (Degnan et al., 1993). Besides the action of proteases, nisin could also be inactivated by enzymatic reaction with glutathione, a low molecular weight sulfur compound present in meat tissues. To maximize the activity of nisin in meat during cooking and storage, it was encapsulated in dipalmitoylphosphatidylcholine liposomes, which were prepared without the use of organic solvents (Boualem et al., 2013). Encapsulated nisin incubated with raw beef did not produce any antimicrobial effect; however, the release of nisin after cooking was found to form clear inhibition zones. It was concluded that nisin was protected inside the vesicle and was released from the melted liposomes during cooking.

Lytic bacteriophages have received a great deal of attention as natural food preservatives with GRAS status, for example to reduce the prevalence of *E. coli* O157:H7 and other food pathogens that could be present in beef products. To avoid their antibacterial activity in the presence of enzymes, evaporite materials and acidic compounds in foods, phages have also been encapsulated in soy lecithin/cholesterol liposomes with an encapsulation efficiency of 58% and mean particle size of 150 nm (Cui et al., 2017a). The phage-loaded liposomes were embedded in a chitosan film to provide increased stability. The liposomal encapsulation increased the phages stability, and the resulting complex film applied on beef pieces showed activity against *E. coli* O157:H7. The film did not change the surface color of beef compared to that of the control group, and it scored an overall better sensory evaluation value.

Essential oils have also been applied recently in meat processing and preservation, being also considered generally as GRAS substances. They present notable limitations owing to their inherent volatility, which may decrease their antimicrobial activities and impair the sensory quality of the meat. Furthermore, the adsorption of active components onto fat globules in meat products may also produce a negative effect on the essential oil antimicrobial activity. In this

respect, thyme oil encapsulated in nanoliposomes of soy lecithin and cholesterol was reported to be more efficient in controlling the growth of *S. enteritidis* in chicken when compared with free thyme oil, without negatively affecting the sensory quality (Cui et al., 2017b). In a recent work, cinnamon essential oil was encapsulated in beta-cyclodextrin proteoliposomes of nanometric size which were fixed in a support of poly(ethylene oxide) nanofibers as an antimicrobial packaging system (Lin et al., 2017). The complex nanofibers significantly extended the shelf life of beef stored at 4 °C, with a significant reduction in the *B. cereus* population and no impact on the meat sensory properties.

Polyphenols with high antioxidant activity can also be useful to decrease the formation of harmful substances, such as heterocyclic aromatic amines (HAA), in fish and meat cooked at temperatures above 150 °C. These antioxidants may lose their efficacy due to excessive high temperatures or to oxygen or light exposition, so liposomal entrapment could be a way of improving their stability and favoring controlled delivery. The functionality of grape seed extract (GSE) encapsulated in soy lecithin nanoliposomes to prevent HAA formation in fried beef patties after marination was investigated (Natale et al., 2014). The encapsulation of the GSE in liposomes did not significantly prevent the formation of HAA compared to the use of free GSE. The authors hypothesized that the very small size of the particles caused diffusion into the interior of the meat, being less effective at inhibiting the reaction of HAA formation that occurs mostly at the surface.

Liposome technology has also been applied for meat tenderization purposes. Calcium encapsulated in egg phosphatidylcholine liposomes was injected into rabbit before slaughter to accelerate the meat aging process (Kim et al., 2006). Liposome encapsulation of calcium was developed as a method for increasing the Ca^{2+} concentration in the muscle through the blood vessels without causing animal shock and death. Liposomes produced with a 2M Ca^{2+} concentration and size of 0.25–1.8 μm were found suitable for injection into rabbit before

slaughtering to produce effective meat tenderization without causing any physical shock or contamination. Bromelain can also be used as a tenderizing agent in meat processing. Soy phosphatidylcholine liposomes with high particle stability have been proposed as a potential tool to increase the availability of bromelain

Nanoliposomes in fish products

Surimi gels could be an excellent food model for enrichment with bioactive ingredients. However, the biological activities may be hampered during the thermal gelation due to excessive interactions with the muscle protein or degradation upon heating (Montero et al., 2005). Furthermore, the encapsulation using highly unsaturated phospholipids contribute greatly to enrich the healthy design of the functional surimi gels. Freeze-dried soy phosphatidylcholine liposomes entrapping various natural antioxidants (pomegranate peel extract, collagen hydrolysate, shrimp lipid extract) were shown to decrease the gel strength and to improve the stability of surimi squid gels during long-term frozen storage. The entrapped extracts, however, were unable to decrease lipid oxidation in the gel matrix. The addition of liposomes causing weaker gel matrices by disturbing protein–protein interactions was found to enhance protein digestibility (Marín et al., 2018b). The addition of various liposomal preparations (fresh, frozen-thawed, spray-dried and freeze-dried) to salt-ground fish mince was found to increase the water holding capacity of the solubilized protein, but they interfered with the thermal aggregation ability (Marín et al., 2018c). The liposome hydration state (wet or dry), rather than the surface charge or vesicle size, was the most important factor influencing the thermal gelation profile. In figure 1 you can see the appearance of a salt ground hake mince without and with the addition of fresh liposomal solution, as well as the consequent thermal gels obtained. The difference in color attributed to the liposomes can be observed and the differences in the gels decrease after the thermal treatment.

Figure 1.- Salt ground hake muscle without A) and B)with liposomes, and their respective thermal gels C and D)

The use of lipids for protein gel reinforcement has been previously reported. It has been suggested that lipid droplets do not interact with proteins in the gel network, but act as filler in the matrix pores (Mor et al., 1999). In gelatin gel systems, liposomes located into the pores of the matrix improved the release of the bioactive compounds during storage (Mufamadi et al., 2011). Nanoliposomes containing Angiotensin converting enzyme (ACE)-inhibitory peptides with an encapsulation efficiency of 53% were included in cold-induced fish gelatin gels without hindering the thermal stability and viscoelastic properties. Furthermore, the liposomal encapsulation did not alter the ACE-inhibitory activity of the peptide fraction, thus keeping the bioactive potential of the complex gelatin gel (Mosquera et al., 2016).

A functional edible wrapping based on cooked shrimp protein was developed by including ϵ -polylysine-adsorbed liposomes entrapping antioxidant and ACE-inhibitory peptides (Alemán et al., 2016). The complexation with ϵ -polylysine increased the liposome size from 164 nm to 216 nm and was effective to increase the liposome stability and the antimicrobial capacity. The complex wrapping, which has desirable sensory properties and activity against *E. coli*, *L. monocytogenes*, *Y. enterocolitica* and *S. aureus*, could be used to design a variety of functional foods, without the requirement for product reformulation or restructuring. Figure 2 shows the aspect of this film as well as an example of functional food product.

Figure 2.- A), myofibrillar shrimp protein film containing phosphatidylcholine soya nanoliposomes loaded with bioactive peptides adsorbed with polylysine B) raw shrimp tails wrapped with this film as a culinary preparation.

Natural polyphenol-rich plant extracts and essential oils have also been used to extend the shelf life of fish products, due to their antimicrobial and antioxidant potential. Thyme extract encapsulated in liposomes and incorporated in minced silver carp inhibited the growth of mesophilic and psychrotrophic bacteria during chilled storage compared to samples containing

the unencapsulated extract (Javadian et al., 2017). Liposomes loaded with 0.5% thyme extract were also able to reduce the population of *E. coli* O157:H7. The overall quality of the minced fish was significantly improved by reducing the peroxide value, the total volatile basic nitrogen, and the microbial load during storage.

The use of liposomes based on marine phospholipids has gained attention in the aquaculture field. The main problems faced by aquaculture are the survival of fish larvae and the difficulty of supplying live prey organisms for larva feeding. Liposomes with high content in long-chain n-3 PUFA (eicosapentaenoic and docosahexaenoic fatty acids) can provide an adequate lipid profile to prey organisms, with subsequent benefits for the survival, growth, and development of fish larvae (Henna Lu et al., 2011). Owing to the ability to encapsulate lipids and water soluble components, many studies have dealt with the successful use of liposomes to enrich live prey, such as *Artemia nauplii*, with a number of nutrients, namely vitamins A and C (Monroig et al., 2007), free amino acids (Saavedra et al., 2008), and essential fatty acids (Monroig et al., 2006).

3. APPLICATIONS IN PACKAGES FOR MEAT AND FISH

One important function of packaging, , is to retard food product deterioration, improving quality and safety and prolonging shelf life (Lopez-Rubio et al., 2004). This becomes especially important in the case of fish and meat due to their short shelf life. The current trend in food packaging is the design of active packages that interact with the food product improving its quality and safety (Angiolillo et al., 2017; Lopez-Rubio et al., 2004). The other tendency in the packaging sector is the development of intelligent packages, which check the storage conditions and the quality of the packaged food item (Angiolillo et al., 2017). The unique physicochemical properties of materials at the nanoscale provide high potential for the development of active or intelligent packages, which are two of the main current applications of nanotechnology in food packaging. Additionally, improvement of the physicochemical properties of polymeric materials and

enhancement of the use of biodegradable packages are also areas in which nanotechnology is being applied (Angiolillo et al., 2017; Sanchez-Garcia et al., 2010; Sharma et al., 2017). In spite of this, most of the work in this topic is related to research rather and commercial applications are scarce (Angiolillo et al., 2017). These three main applications of nanotechnology in the food packaging sector are discussed below:

Improvement of physicochemical performance of food packaging materials

Among the variety of materials available for food packaging, polymers prevail over glass, tinplate, or paper because of several advantageous properties such as low cost, light weight, ease of printing, microwaveability, thermosealability, flexibility, etc. Furthermore, the variety of existing polymers and processing technologies provides high versatility regarding package physical properties, shape, size, etc. Indeed, there are a number of packaging materials with appropriate physical properties such as gas, vapor, and aroma barrier, mechanical properties, transparency, etc., that can be adjusted to specific applications, so almost all types of foods can be found packed in polymeric packages. Thanks to this, meat and fish can be packed in bags or trays under vacuum or modified atmosphere, preserving the desired gas headspace composition. However, in spite of the improved properties, the gas barrier properties of glass and tinplate are still superior, so nanotechnology can improve the performance of conventional polymers. Another drawback of polymeric materials is that they are made from non-renewable resources (petroleum), which are generally not biodegradable and accumulate in the environment. Furthermore, recycling is not easy, as they are commonly made of multilayer and composite structures. Consequently, research on compostable or biodegradable biopolymers has gained great interest in recent years. For most biodegradable polymers it is necessary to improve some of their properties, such as gas barrier characteristics and mechanical properties. Reinforcement of these biodegradable polymers to prepare nanocomposites has already been proven to be an effective way of enhancing these properties concurrently (Gómez-Estaca et al.,

2016; Sanchez-Garcia et al., 2010; Echeverría et al., 2016, 2018). Nanocomposites can be defined as materials having filler with at least one dimension smaller than 100 nm (Arora & Padua, 2010). This new generation of composites exhibits significant improvements in modulus, dimensional stability, and solvent or gas resistance with respect to pristine polymer (Sorrentino et al., 2007). Among the nanomaterials incorporated in food packaging nanocomposites, it is worth mentioning cellulose fibers and nanowhiskers, carbon nanotubes and nanofibers, clays and silicate nanoplatelets, starch nanocrystals, and chitin/chitosan nanoparticles, for mechanical, thermal stability, and/or barrier performance improvements (Sanchez-Garcia et al., 2010; Sharma et al., 2017). Other developments do not necessarily include compositing of the polymers and nanomaterials, as multilayer structures can also be designed in which one of the layers is on the nanoscale. One example would be the coating of a very thin layer of nanocellulose onto conventional polymeric films, or the application of ultrathin metal coatings (Gokularaman et al., 2017; Li et al., 2015). Thanks to the application of nanotechnology to improve the physicochemical performance of packaging materials, the quality of packed meat or fish will be improved. In this manner, packages with better mechanical, thermal, and barrier properties will protect the packed product more efficiently. Furthermore, packaging materials from alternative eco-friendly polymers will be available for these packaging applications. As an example, Lloret et al. (2016) developed a polyamide/nanoclay nanocomposite that preserved dry-cured ham as efficiently as a high-barrier commercial polymer, whereas unmodified polyamide film failed to preserve product color during the 90-day refrigerated storage period as a consequence of its higher oxygen transmission rate.

Development of active packages

The traditional concept of the food package as a passive barrier to protect food from the effect of the environment has evolved to the active packaging concept, as a result of the novel consumer trends toward minimally processed and convenient food products with a prolonged

shelf life. Therefore, active packages interact with the packed product or its surrounding atmosphere with a beneficial outcome. There are a number of active packages with applications in many different food products (Lopez-Rubio et al., 2004), but oxygen scavengers, antioxidant, and especially antimicrobial active packages stand out as the most important developments for poultry, meat, and fish owing to their inherent characteristics (Gómez-Estaca et al., 2014; López-Carballo et al., 2012; McMillin, 2017). Focusing on nanotechnological applications, developments can consist in nanostructuring of the active compound that is incorporated into the active packaging system (film, pad, tray, etc.) or incorporation of a non-nanostructured active compound into a nanocomposite whose properties allow modulation of its release, as compared to unmodified polymers. Encheverría et al (2016) observed that the addition of clove essential oil increased the degree of exfoliation of the clay in the isolated soybean protein film, observing that exfoliated and interspersed nanostructures coexist in these materials.

With regard to oxygen scavengers based on nanotechnology, Xiao-e et al. (2004) developed nanocrystalline TiO_2 films with the ability to deoxygenate a closed environment under continuous UV irradiation. However, there are no applications of this packaging material on mycosystems, which would be especially interesting for poultry or white fish.

The most studied nanoparticles used as antimicrobials are metals (Ag, Au, Zn, Cu) or metal oxides (TiO_2 , ZnO, SiO_2 , MgO) (Angiolillo et al., 2017; Espitia et al., 2016; Sharma et al., 2017). Among them, silver nanoparticles are one of the most studied, and various mechanisms have been proposed to throw light upon their antimicrobial property: adhesion to the cell surface with consequent alteration of membrane electric charges; structural modification of membrane lipopolysaccharides; DNA damage; or binding to groups such as sulfur, oxygen, or nitrogen (Angiolillo et al., 2017; Rai et al., 2009). Most of the processes for the synthesis of silver nanoparticles involve the formation of colloidal nanoparticles or their incorporation in other materials (Rai et al., 2009; Sharma et al., 2010). Nanosilver-based active packages have proven

their antimicrobial effectiveness *in vivo*. As an example, Azlin-Hasim et al. (2016) developed composite Ag/PVC (polyvinylchloride) films that exerted antimicrobial action when applied to chicken breast fillets. Fernández et al. (2010) developed absorbent pads by silver nanotechnology, reducing spoilage-related microflora of packed beef meat. Prior to commercialization of silver-based food packaging systems, it is mandatory to reduce the migration below the limit allowed (0.05 mg Ag⁺/kg food). Indeed, particulate migration into foods does not occur in many applications, so the potential risk to consumers is reduced (Wyser et al., 2016). Other metal-based nanoparticles have also shown good results. Low-density polyethylene films loaded with TiO₂ and ZnO nanoparticles showed antimicrobial activity against *E. coli* O157:H7 in calf minced meat (Marcous et al., 2017). Zinc oxide has also shown good results for packing pork or poultry (Akbar & Anal, 2014; Suo et al., 2016). In spite of these positive results, overall toxicological data indicate that more research is required before metal-based nanoparticles can be tagged as toxic or safe (Sharma et al., 2017). In any case, application of these kinds of antimicrobials seems to be restricted to active packaging applications, which may make it possible to retard the release and adjust it to an effective antimicrobial dose at the food surface, where microbes grow, without reaching a toxic dose.

Apart from metal and metal oxide nanoparticles, nanocomposites including nanoclays have been developed in order to retard the release of the antimicrobials incorporated. This is the case with a bentonite nanoclay/EVOH composite developed to retard the release of an added volatile antimicrobial (carvacrol), without significant drawbacks affecting the other physicochemical properties studied thanks to the good dispersion of bentonite (Cerisuelo et al., 2012). This material may be applied to fresh fish preservation, as a food packaging material based on an active PP/EVOH/PP film in which carvacrol was added showed its effectiveness as an antimicrobial when applied on refrigerated salmon (Cerisuelo et al., 2013). In another work, Alboofetileh et al. (2016) developed an alginate/clay nanocomposite incorporating marjoram essential oil and showed a good antilisterial effect *in vitro*, as well as a delay of *L.*

monocytogenes, total viable count, and psychrotrophic growth when applied to the preservation of rainbow trout slices at refrigeration temperature. In another work, an active nanocomposite film based on soy proteins and montmorillonite incorporated with clove essential oil exerted antimicrobial and antioxidant activity on refrigerated bluefin tuna fillets, without diffusion of the clay's own metals to the muscle (Echeverría et al 2018) showing that the presence of clay facilitates the controlled release of clove essential oil, while the presence of clove essential oil seems to promote the exfoliation of clay (Echeverría 2016). Figure 3 shows the looks of the active nanocomposite film as well as the appearance of the tuna fillet coated by this film. Regarding antioxidant activity, Busolo and Lagaron (2015) developed polyethylene films based on a resveratrol-containing clay that delayed lipid oxidation of fresh meat packed in direct contact with it.

Figure 3.- A) Film made with soy protein, clove essential oil and montmorillonite nanocomposite. B) TEM of nanocomposite film based on soy protein isolate with 10%

of montmorillonite and 0,5 ml clove essential oil. C) Appearance of tuna fillet D)
Appearance of a tuna fillet (*Tumnus thymus*) coated with this film.

Development of intelligent packages

Intelligent packages are those capable of interacting with the packed food, recognizing any of their characteristics, and applying various mechanisms to record and convey information, with the final objective of boosting consumer information about quality and safety (Sharma et al., 2017). There are a number of intelligent packaging developments, including indicators (gases, temperature, freshness), data carriers (barcodes, radio-frequency identification systems), and sensors, these last being the ones with the highest potential for future developments (Ghaani et al., 2016). A sensor is a device or system with control and processing electronics, an interconnection network, and software (Patel and Beveridge, 2003). Nanotechnological applications are mostly important in the first part of the system, that is, the receptor, which is the sensing part of the sensor and is represented by a sampling area where the surface chemistry occurs. In this context, the unique chemical and electro-optical properties of nanoscale particles made them of special interest (Bouwmeester et al., 2009). The main nanotechnological developments for meat and fish include O₂ and CO₂ indicators/sensors, detection of spoilage and pathogenic microorganisms, freshness indicators, and time temperature indicators (Sharma et al., 2017).

Many food products, including fish and meat, are preserved under vacuum or modified atmosphere with limited oxygen access. In these specific cases, an O₂ indicator will give the consumer information about the quality of the food, as inefficiency in the packaging system or damage of the package would induce changes in the headspace gas composition. With regard to the application of nanotechnology, developments based on TiO₂ or SnO₂ nanoparticles for oxygen detection have been described (Lee et al., 2005; Mills & Hazafy, 2009). Carbon dioxide is

also considered as an active packaging gas because high levels reduce the metabolic rates of microbes. Therefore, detection of a decrease in its original concentration will give the consumer valuable information about the quality of the packed meat or fishery product (Puligundla et al., 2012). There are various systems for CO₂ determination for food packaging applications (Puligundla et al., 2012; Sharma et al., 2017), and future developments applying nanotechnology should be expected.

Another group of intelligent packaging systems is bacteria detection systems, notably those consisting of antibodies conjugated to nanomaterials such as quantum dots. This system is based on antibody antigen interaction, boosted by the unique optical and electrical characteristics of nanoparticles in conjunction with other properties such as spacious and simply functionalized surfaces (Sharma et al., 2017). An example of a quantum dot-based system with potential applications in meat packaging was developed by Yang and Li (2006). This system was able to detect the presence of *E. coli* O157:H7 and *S. Typhimurium*, but more research is needed prior to establishing these techniques for food packaging applications (Sharma et al., 2017). An indirect measure of microbial contamination is provided by freshness indicators. In this case the intelligent device is able to react with a microbial metabolite, giving visual information about the freshness of the product. Smolander et al. (2004) developed a food packaging material consisting of plastic film or paper coated with a very thin silver or copper layer that reacts to the presence of sulfide volatiles produced by fresh meat undergoing spoilage, inducing a color change. Another important group consists of systems that indicate the presence of volatile amines, which are indicators of fish freshness (Kuswandi et al., 2012; Ma et al., 2018; Maynor et al., 2007). Time temperature indicators can also be considered as “freshness indicators”, as temperature abuse during distribution and storage of foods largely determines their physical and microbial spoilage (Angiolillo et al., 2017). Zhang et al. (2013) developed a time temperature intelligent system constructed from plasmonic nanocrystals that can be specifically tailored to

react in a specific temperature range and thus be used to track perishable food products, indicating product quality through sharp-contrast multicolor changes.

4. BIOSENSORS FOR DETECTION OF COMPOUNDS OR MICROORGANISMS

Among the techniques for evaluating quality as for what concerns quality control, food safety, and traceability of food products, and specifically of seafood meats and derived products, in recent years the potential use of biosensors stands out. They allow interaction with the chemical compound to be determined with high sensitivity in a fast and economic way.

The biosensors most commonly used or that stand out as interesting for their application are of various kinds, depending on which compound, or even microorganism, they are intended to detect. In many cases they have not yet been implemented and are mere prototypes, but soon we will be surprised by the total implementation of these devices in commerce because their responses are extraordinary in terms of sensitivity, achieving lower detection limits, speed, and cost.

Regardless of the compound to be determined, there are several types of biosensors. In this chapter we will refer to nanosensors. Nanoscale materials have unique properties such as having a greater surface area, increased surface reactivity, electrical conductivity, and magnetic properties (Inbaraj and Chen 2016), and therefore could be used to design detection devices that improve sensitivity and reduce detection time and cost. In addition, nanomaterial is very suitable for functionalizing; it can attach to a wide diversity of biomolecules that give it functionality to detect or recognize biological analytes or bioagents on the surface or nanomaterial. For this purpose it should have suitable charges and structure orientation.

The following describes some types of nanobiosensors developed as potential substitutes for traditional methods to quickly and sensitively analyze certain compounds or microorganisms in

meat, fish, or seafood and derived products that lead to quality loss. Obviously, the classification is not strict, because there can be overlaps, depending on the systems.

Nucleotide or peptide sequence-based nanoparticle sensors

This type of sensor has immediate application in the identification of species; therefore its most immediate use may be for detection of meat adulteration, and for identification of microorganisms, focusing on identification of pathogenic organisms in fresh or processed meats or seafood.

Basically it consists in coupling a fragment of oligonucleotide (very characteristic of the species in question) to a nanocrystal, often nanoparticles of gold, which are stabilized in turn by covering them with a solution. When it comes into contact with the sample to be identified, the oligonucleotide fragment, if it is of the selected species, will be recognized and a conformational change will be produced favoring the fluorescence signal of the fluorophore that has at one end the oligonucleotide with which it was labelled previously (Ali et al., 2012).

In this regard, Ali et al. (2012) fabricated a sensor that has been successfully tested to detect adulteration with meat such as beef, mutton, chevon, or chicken in pork meatballs. For this purpose, a 27-nucleotide AluI fragment of pork cytochrome b (cytb) gene was joined to 3 nm Ø citrate–tannate-coated gold nanoparticles. The biosensor was applied to evaluate the amount of pork meat in meatball formulations (in both raw and cooked). Two variations of the sensor were made, as described by Ali et al., 2012, 2014). The differences mainly lie in the detection method, either by fluorescence (Ali et al., 2012) or by colorimetry (Ali et al., 2014). The sensor was sensitive to detect 1% of pork meat in meatballs (raw and cooked), elaborated from formulations containing pork and beef previously mixed in different proportions. Recently, a group of researchers modified this probe design with thiol groups, giving a stronger bond with gold nanoparticles (Ardhiyana et al., 2017). As in previous works, it was confirmed that the

biosensor does not react with DNA samples belonging to other species such as beef and chicken, the limit of detection being 16.04 ng DNA/ μ l.

It has also been applied for the determination of pathogens frequent in meat and fishery products, such as *L. monocytogenes* (Yang et al., 2017), using a conjugated DNA fragment or a specific peptide on which a protease of the microorganism acts (Alhogail et al., 2016).

Nano-gold particle electrode modified by L-cys and glucose oxidase complex

Umuhumuza and Sun (2011) manufactured a sensor to measure glucose in pork meat, using the *longissimus dorsi* muscle, to evaluate the effectiveness of this system in comparison with the traditional measurement determined by HPLC. Glucose is a measure of meat quality and determines its quality/freshness. In broad outline, this sensor consisted of a gold nanoparticle electrode to which L-cys was bound, forming monolayers through the thiol groups, complexing the polyglutamate oxidase enzyme, and leaving free the carboxylic and amino groups that could interact with biological molecules. When the sample of *longissimus dorsi* pork muscle preserved for 5 days at 4 °C was passed through the electrode, the values obtained showed a similar evolution and data very close to those obtained by HPLC, and without significant differences in almost all the points analyzed. Therefore, this type of nanosensor stands out as a quick and reliable method, of great interest for implementation in the industry as a method for detecting glucose in meat.

Sulfide nanoparticle amperometric sensor

The objective of this nanosensor is to use a simple, reliable, fast method to estimate residues of chloramphenicol in meat (beef, pork, chicken), therefore easy to implement, as a new alternative to traditional methods (chromatographic, immunological, etc.), which are time-consuming and expensive (Kim et al., 2010). An amperometric immunosensor was used to determine chloramphenicol, based on covalent immobilization of anti-chloramphenicol

acyltransferase antibody on a system of cadmium nanoparticles that act as an electrode, modified with dendrimers of polyamidoamine and gold nanoparticles (Au NP), which are applied to improve the sensitivity of the dendrimer, and a conductive polymer (poly 5,2':5',2''-terthiophene-3'-carboxyl acid (poly-TTCA)) (Kim et al., 2010). The system thus assembled contained a high quantity of amine groups available in the poly-TTCA/Au NP/dendrimer layer and free carboxylic groups in cadmium sulfide nanoparticles. The immunosensor for chloramphenicol exhibited a low detection limit, 45 ± 5.0 pg/mL, an analogous result to that obtained by HPLC, and to those obtained by chemiluminescence immunosensor (100 pg/mL–10 ng/mL) (Park and Kim, 2006). These authors also studied whether the immunosensor detected other antibiotics (ampicillin, streptomycin, tetracycline, neomycin, in 0.1% BSA (bovine serum albumin)), but it did not respond to significant concentrations.

Quantum dot sensors

Quantum dot sensors consist of a crystalline cluster with a nano-sized quantum-confined semiconductor. They have unique properties, such as their small size, energy-dependent emission, and excellent optical characteristics. They also present easy surface functionalization, which makes them very suitable for the detection of analyte through labeling or bioimaging due to changes in light emission properties (Rowland et al., 2016; Pérez-López & Merkoci, 2011). They can be synthesized from various materials: cadmium sulfide, cadmium selenide, cadmium telluride, etc.

In the last few years there have been many studies related to this type of nanobiosensor, with various adaptations in their functionalization, mostly intended to be used for the determination of residues of toxic substances in meat and fish products and illegal use of substances such as clenbuterol and ractopamine in animal feed.

Zhang et al. (2016) described a sensor that consisted of zinc sulfide quantum dots and polyaniline that was deposited on a gold nanoparticle electrode together with antibody against

clenbuterol that resulted in an amperometric immunosensor for this substance. The biosensor has a detection limit of 5.5 pg/mL. It was also verified that ractopamine and salbutamol (related species) did not interfere. A somewhat less sensitive nanobiosensor (10–12 g/mL) was also developed previously by Tran et al. (2015) to determine clenbuterol. In this sensor the fluorescence resonance energy transfer (FRET) effect was used to fabricate a system based on cadmium telluride quantum dots. Attempts have also been made to detect residual ractopamine in meat by means of optical biosensors. A system to detect ractopamine was developed for this purpose, supported by the fact that fluorescent polymer dots (positive readout) in close proximity to gold nanoparticles produce a strong fluorescence quenching (Shi et al., 2015).

This type of compounds with hormonal effect in animals such as clenbuterol, ractopamine, etc, are not the only ones for the determination of which this type of nanobiosensor has been applied. Among other examples can be mentioned, Hu et al. (2017) developed one for the determination of sulfaquinoxaline, which is a type of sulfonamide, an antibiotic whose presence in foods of animal origin is not allowed or is allowed only in limited concentrations, depending on the country. The detection limit for this antibiotic was 1 ng/mL in standard solution and 8 mg/kg in the samples tested; the products tested being chicken and shrimp meat.

Carbon-based nanomaterials

Their use in biosensors is highly appreciated because of their biocompatibility and high electrical conductivity using photophysical and fluorescent properties, and their very diverse functionalization. They present many different properties, depending on their structure, metal semiconductor, and polymeric nanomaterial, owing to their good surface effects, small size, and quantum tunnel effect (Afreen et al., 2015).

As in the previous case of quantum dots, most of the studies found in the literature for determinations in meat products focus mainly on the determination of trace or non-permitted compounds such as clenbuterol. These nanosensor systems present various functionalization

strategies; for example, Liu et al. (2017) have been developing a fluorescence resonance energy transfer (FRET) system consisting of carbon dots and gold nanoparticles for recovered fluorescence detection of clenbuterol in pork meat. The limit of detection for clenbuterol was 3 nM, and the results obtained with the sensor were similar to those obtained by HPLC analysis (for example, 20.9 nM versus 18.75 nM), demonstrating that it could be used as a fast, reliable, cost-effective method for determination of clenbuterol traces in pork meat samples.

Xanthine oxidase immobilization systems

Special attention should, perhaps, be paid to xanthine oxidase immobilization systems with nanobiosensors; at any rate, much development work has been done on them in recent years. Devi et al. (2012) described an amperometric xanthine biosensor based on immobilization of xanthine oxidase on a zinc oxide/chitosan/multiwalled carbon nanotube/polyaniline composite film. Subsequently, Dalkiran et al. (2014) described an amperometric sensor based on immobilization of xanthine oxidase on a wall of chitosan/carbon nanotubes modified with glassy carbon electrode. Devi et al. (2013) described another amperometric biosensor, a modification of the previous one, for the determination of xanthine, based on covalent immobilization of xanthine oxidase in a chitosan/Fe-NPs-Au film. They studied both the reuse of the electrode enzyme and its stability, and they tested fish samples in chilled storage (4 °C) during their shelf life. The authors observed that with this modification they improved the sensitivity with respect to that reported in Devi et al. (2013), and they attributed the good stability, repeatability, and reproducibility to the covalent immobilization. However, the electrode showed a 25% loss after 100 uses. Later, an immobilization of xanthine oxidase covalently bound to a graphite electrode coated with the copolymer poly GMA-co VFc (glycidyl methacrylate and vinylferrocene) was developed by Dervisevic et al. (2015). The results of xanthine in fish samples stored at 4 °C were very accurate. Albeda et al. (2017) manufactured an amperometric nanosensor of hypoxanthine, based on an electrode consisting of a nanocomposite of graphene/titanium dioxide in which

xanthine oxidase was immobilized. for evaluation in pork meat. Development of a glassy carbon electrode coated with chitosan-polypyrrole-gold nanoparticles and with subsequent immobilization of xanthine oxidase was carried out by Dervisevic et al. (2017). Various samples of fish, beef, and chicken were tested in the electrode with precise and repetitive results. In general, the lowest detection limits oscillate around 9.0 μM in the sensors described, although some of them show a somewhat higher limit, and the analysis ranges are very variable.

HPLC electrochemical analyses with nanoparticulate electrodes

An electrochemical detection method coupled to HPLC to evaluate possible adulteration of meat with meat of another species has been developed by Hung et al. (2011), based on previous studies. With this method they aim to differentiate ostrich meat from other meats such as chicken, beef, and pork. The method basically consists of an HPLC coupled to an electrochemical analyzer with 3 electrodes (a silkscreen electrode plated with copper nanoparticles, an Ag/AgCl reference electrode, and an auxiliary platinum electrode). Lysine, carnosine, anserine, and glutamine are detected in the chromatographic profiles. The results indicate that ostrich meat is easily distinguishable from the others owing to the absence of carnosine, while beef and pork manifest absence of anserine, so they can be differentiated from the poultry myosystem (ostrich and chicken). Therefore this method could be suitable for incorporation as a routine method to differentiate these myosystems in processed meats.

Layered double hydroxides (LDHs)

Layered double hydroxides are structures formed by layers of positively charged metal hydroxides and they are stabilized with interlaminar anions and water. The interlaminar distance can be at the nanoscale. The negative charge on the surface of the layers, owing to the hydroxyl groups, and the interchangeable anions make them ideal systems for preparing functionalization products, hybridizing molecules, or acting as biosensors, allowing a wide range of applications to be carried out at the nanometer scale, such as catalysts, intercalation products, etc. Basically,

they consist of a magnesium hydroxide structure where magnesium is centrally arranged in the layers and hydroxides on the surface. LDHs are the result of substitution of a fraction of (divalent) magnesium cations by a trivalent cation, which generates a residue with a positive charge in the layers. Intercalation is a term frequently used to refer to the introduction of a great variety of organic species and ions between the layers. They can also be intercalated in a single step, called coprecipitation (Martínez & Carbajal, 2012).

In addition, a measurable signal must be shown, for example chemiluminescence, which has a fast response, high sensitivity, and cheap and easy implementation, so its application in food is highly useful.

An application of this system has been used for determination of biogenic amines in fish during deterioration, as well as in pork. The results were carried out with simplicity, selectivity, and sensitivity (Wang et al., 2014.) In general, the method used was coprecipitation for Mg-Al-CO₃, with Mg/Al and exfoliation in formamide solution, favoring delamination through hydrogen bonds, which leads to strong luminescence of the exfoliated nano-LDH. This photoluminescence decreases in the presence of biogenic amines, owing to displacement of the O-H ... O bond by the O-H ... N bond (Wang et al., 2014).

Nanoporous colorimetric sensor for detection of trimethylamine

Various authors coincide in using this type of nanosensor for the determination of volatile compounds such as amines. The colorimetric sensor array must contain a functional group to interact strongly with the analytes, and this functional group should be firmly coupled to a powerful chromophore (Xiao-Wei et al., 2014). Xiao-Wei et al. (2016) created a nanoporous structure as a gas sensor sensitive to the detection of trimethylamine (TMA) in Yao meat, which is a typical salted Chinese meat. TMA is one of the volatile organic compounds that allow evaluation of the freshness of meat, fish, and seafood owing to its production and release in animal tissues as a result of microbial deterioration of these myosystems. The determination in

the sensor is based on the faster diffusion of the analyte gases on the porous sensor surface. To do this, nanoporous colorimetric matrices of chemosensitive dyes were created, for which the dyes were printed on a plate of nanoporous TiO₂ film. The TMA was collected by means of a conductive gas that was deposited on the porous film, which was dyed in a known range of colors, depending on the concentration of TMA. This system was very sensitive to low concentrations of TMA and was repeatable.

Morsy et al. (2016) applied another colorimetric sensor array to monitor fish spoilage compounds, including various amines. The chemosensor contained an array of 16 chemosensitive compounds in conditions of response to standard volatile compounds that are produced by fresh fish or as a result of fish spoilage during storage. This sensor detects changes in fish muscle pH and in the thiobarbituric acid with great reliability, but not detect amines produced. During storage, it shows a linear correlation with the increase in microorganisms, responding to differences due to storage conditions (room and 4 °C). With a similar system, Xiao-Wei et al. (2014) created a colorimetric gas sensor matrix based on four highly sensitive natural pigments for the determination of freshness (microbial growth and biogenic amines) in pork meat stored at 4 °C. The results established good correlations with the data obtained by conventional analyses. Therefore, these authors consider that it is a viable method for evaluating total microbial growth and the biogenic amine index.

Aptasensors

Aptasensors are basically nanosensors in which aptamers are immobilized. Aptamers are single-stranded oligonucleotides with sizes between 70 and 100 nucleotides, with high affinity to various types of analytes or specific molecules, and capable of specifically recognizing them by means of a 3D structure. The aptamer is usually obtained by selection in the SELEX method (Systematic Evolution of Ligands by Exponential Enrichment), with high affinity and specificity for a specific analyte. Consequently, aptamers are used as probes in sensors; they have high

stability at room temperature, it is easy to mark the nucleotide sequence with other molecules (that is, chemically label the aptamer for later identification), and they can be synthesized chemically and at low cost.

The aptasensor preparation is determined by the aptamer immobilization method, because the sensitivity and selectivity of the assay will be affected by the orientation and accessibility of the receiver. To facilitate interaction of the immobilized aptamer with the ligand in solution, it is common to add a spacer by means of a suitable functional group.

Prototypes of aptasensors have been created for the detection of chemical compounds such as antibiotics or anabolic steroids that could remain in meat, and for the detection of microorganisms. The number of aptasensors for an analyte determination can vary greatly owing to many factors, such as type of sensor, labelling etc.

In the last few years several aptasensors have been developed for the determination of clenbuterol. Duan et al. (2017) tested a fluorescent method based on a specific aptamer for clenbuterol by adsorption to graphene oxide, which was used to confirm its utility as a recognition receptor with high affinity and specificity for the detection of clenbuterol in pig samples. The measurement showed good sensitivity in the range of 0.10 to 50 ng/mL and a detection limit of 0.07 ng/mL. In samples of pork meat the results fitted this range perfectly, which means the aptasensor could be applied to this type of product.

A colorimetric aptasensor was created for sensitive and selective detection of chloramphenicol based on gold nanoparticles (Au NPs) labelled with Power Vision (PV) and magnetic separation (Gao et al., 2015). The chloramphenicol aptamer was immobilized on $\text{Fe}_3\text{O}_4@\text{Au}$ magnetic nanoparticles as a capture probe (AuMNPs–Apt) to concentrate target chloramphenicol. The power vision system consists of peroxidase enzyme and conjugated antibody. The color evolution produced can be measured by ultraviolet-visible (UV-vis) spectroscopy. The responses

to chloramphenicol concentration were in a range of 0.05–200 ng/mL, with a detection limit of 0.02 ng/mL. This test was successfully applied to analyze chloramphenicol in fish and pork muscle pieces, and the results were according with those of the conventional enzyme-linked immunosorbent test (ELISA).

More recently, however, Ouyang et al. opted for another kind of aptasensor for detection of traces of tetracycline in pork and milk (Ouyang et al., 2017). The design was based on those mentioned before, using nanoparticles of Fe_3O_4 functionalized with amine and modifying the surface with lanthanide doping. The nanoparticles were conjugated with the aptamer. When the sample of pork with tetracycline (or any kind of food containing tetracycline) was added, the aptamer preferentially bonded with tetracycline to cause the dissociation of some cDNA and the liberation of some cDNA-nanosensor, producing a decrease in the fluorescent signal. The results indicate that the aptasensor is very specific for this antibiotic, as in the presence of others it only recognizes tetracycline. The detection in samples of adulterated meat and milk showed great repeatability of the method and an amplitude range in the measurement (0.01–100 ng/mL, with a limit of detection of 0.0062 ng/mL) that makes it ideal for determination in real samples to ensure their safety.

As mentioned earlier, aptasensors can also be used for the identification of microorganisms, and some of them will be mentioned as case studies. The surface-enhanced properties of Raman scattering (SERS) nanomaterials have also been used to develop sensors for the detection of microorganisms, especially pathogens. The pathogenicity of certain bacteria such as *Salmonella* Typhimurium, *Listeria monocytogenes*, etc. is well known, and also the ease with which they contaminate certain foods, especially meat products. For this reason, development of this type of sensor for these types of bacteria has begun. As nanoprobe Ma et al. (2018) used SERS (surface-enhanced Raman scattering) spiny gold nanoparticles immobilized with molecules with Raman signal and a thiolated aptamer for *S. typhimurium*. The aptasensor responds specifically

against *S. typhimurium* owing to the high affinity and specificity shown by the aptamer, with a detection limit of 4 cfu/mL in inoculated pork meat.

Shahdordizadeh et al. (2017) describe 3 kinds of aptasensors for the detection of *Staphylococcus aureus*: colorimetric, electrochemical, and fluorescent. While the colorimetric type is the simplest and the result is seen with the naked eye, the electrochemical type shows the highest sensitivity and a low limit of detection (1 cfu/mL), attributed to the sensor, based on reduced graphene, which has very good conductivity. Finally, the fluorescent aptasensor presented a good signal for *S. aureus* (8 cfu/mL) and for the toxin that it produces (6 ng/mL).

CONCLUDING REMARKS

The enrichment of meat and fishery products with natural active compounds added freely or encapsulated in diverse nanostructures, such as chitosan nanoparticles, liposomes or emulsions, is a field with great prospects for industrial and market development. These nanosystems not only may favour the controlled release of antioxidants or antimicrobial agents to enhance the quality and durability of the product, but also they have great potential in the design of versatile functional foods.

Nanotechnology can be also applied to the design of food packaging materials with improved properties, such a better physico-chemical performance or an active/intelligent activity, thus improving fish and meat quality during storage.

Most of the nanobiosensors that are being designed today are very diverse and are based on their electrical, magnetic, luminescent, etc properties of nanomaterials. In addition, they are very suitable to be functionalized, that is, capable of joining a great variety of biomolecules that enable it to detect biological analytes on the surface of nanomaterials, conferring specificity them. Among the most widely studied compounds include anabolic in meat as clenbuterol, biogenic

amines in meat, poultry and fish, as well as detection of microorganisms, in special, certain pathogens, what gives an idea of the versatility in terms of their applications

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