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Radiation Influence on Edible Materials

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Abstract

Innovations in the food industry are shaped both by new technologies available and by society's requirements. A good knowledge on the chemistry and biological role of the macro-nutrients (proteins, carbohydrates, lipids and also energy and water) and micro-nutrients (minerals and vitamins) is required. Food production foundations include not only the design of the food products but also the materials, mechanics, ingredients, conversion and transformation all must be taken into consideration. Edible polymers are polymeric materials that can be easily consumed by human beings or lower animals in whole or part via the oral cavity and given harmless effect to the health. An edible polymer is originated from natural products such as polysaccharides, proteins and lipids, with the addition of plasticizers and surfactants. Radiation-processing technologies are used currently for numerous applications of commercial and economic importance, but it is an emerging application with the use of ionizing radiation to enhance properties of edible polymers such as carbohydrates or proteins. This chapter aims at supplying the state of the art about the effects of ionizing radiation on edible polymers: starch and vegetal proteins and also on gelatin that comes from animal origin.

Keywords: ionizing radiation, polysaccharides, proteins, edible films, nanotechnology

1. Introduction

There is an increasing interest in green and bioactive materials that could be used in direct contact with aliments. Edible films can be produced from natural materials with film-forming ability that can be applied on food surfaces as a thin-layer edible film and can potentially extend the shelf life and improve the quality of food. Edible film as a solid sheet can be applied between food components or on the surface of the food system in order to inhibit migration moisture, oxygen, CO_2 , aromas and lipids.



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Edible polymer film is a thin layer of edible material formed on a food as a coating or placed (pre-formed) on or between food components; in this case, edible films can improve the quality of multicomponent foods. The sanitary condition of the edible packaging would need to be maintained during storage, transportation and marketing. The end result would be source reduction and or improved recyclability of the remaining elements of the packaging system.

One major advantage of using edible films and coatings is that several active ingredients (antimicrobials, antibrownings, texture enhancers and nutraceuticals) can be incorporated into the polymer matrix and consumed with the food, thus enhancing safety or even nutritional and sensory attributes [1].

Many potential uses of edible films can be described such as inhibit migration of moisture, oxygen, carbon dioxide, aromas; carry food ingredients (e.g., antioxidants, antimicrobials, flavor) and or improve mechanical integrity or handling characteristics of the food [2]. Practical uses of edible films include wrapping various products; individual protection of dried fruits, meat and fish; control of internal moisture transfer in pizzas, pies, which are based on the film's properties (e.g., sensory, mechanical, gas and solute barrier). Also, ingredients can be delivered to processors on water-soluble and edible packaging films in premeasured amounts of ingredients to food processors and foodservice operations. Future applications of the concept have been envisioned in consumer-sized pouches of dried products ready for reconstitution on water.

The functionality and performance of edible polymer mainly depend on their barrier, mechanical and color properties, which in turn depend on film composition and its formation process. Polysaccharide (cellulose, starch, dextrin, vegetable and other gums) and protein (gelatin, gluten, casein) based films can have suitable mechanical and sensory properties, while wax (beeswax, carnauba wax) and lipid or lipid derivative films have enhanced water vapor barrier properties. It was shown that the incorporation of oil between plasticized starch layers could reduce oxygen and water vapor permeability of the films [3]. The film-forming technology, solvent characteristics, plasticizing agents, temperature effects, solvent evaporation rate, coating operation and usage conditions of the film (relative humidity, temperature) can also substantially modify the ultimate properties of the film [4].

Important properties to be evaluated in an edible coating are its microbiological stability, adhesion, cohesion, wettability, solubility, transparency, mechanical properties, sensory and permeability to water vapor and gases. In order to choose the best edible film to be employed in each case, it is necessary to take in account the characteristics of the food intended to be protected. For coating a fresh fruit, for instance, it will be desired to induce low water vapor permeability to preserve texture and moderated O_2/CO_2 permeability to permit respiration. On the contrary, when it is intended to protect dried fruits and nuts, low water vapor permeability is required to maintain crispiness and low O_2 permeability to avoid oxidation.

Proteins as components of edible films (gelatin, zein, casein) has usually better performance than polysaccharides (chitosan, starch, pectin); lipids have excellent water vapor barrier but do not form stand-alone films, and can be used as coatings, such as waxes on fruit surfaces. Besides the barrier efficiency, edible films and coatings have to be also sensory acceptable. In the food industry, there are commonly used starches, dextrins, alginates, protein and lipid materials edible films as encapsulating materials [5]. A wide variety of foods are encapsulated: flavoring agents, acids, bases, artificial sweeteners, colorants, preservatives, leavening agents, antioxidants, agents with undesirable flavors, odors and nutrients, among others. In order to protect the viability of the probiotic bacteria, for instance, several types of biopolymers such as alginate, chitosan, gelatin, whey protein isolate, cellulose derivatives are used for encapsulation and several methods of encapsulation such as spray drying, extrusion, emulsion have been reported [6].

Active edible films represent one of the current and future trends in the development of new polymers for selected applications, particularly food packaging [7]. The concept of active packaging is described as a type of packaging that alters conditions surrounding the food to maintain product quality and freshness, improve sensory properties or enhance product safety and shelf life.

Edible and biodegradable polymer-based films offer alternative packaging without the environmental cost. In that case, they must fulfil the food packaging requirements as help to contain, protect, preserve, distribute and describe food from fresh to highly processed ones. The potential of biopolymers for packaging application is often limited due to their poor processability, so edible polymers originated from natural products are added to plasticizers and surfactants. A careful selection of the plasticizer allows biopolymer films with improved processability and high or low permeabilities to be manufactured [8].

Shit and Shah in 2014 distinguished the edible polymers into any of the four categories: (1) hydrocolloids, (2) polypeptides, (3) lipids, (4) synthetic and composite edible polymers [9].

In the following sections, the interaction of ionizing radiation with polysaccharides and proteins, which are considered the most important edible materials, as well as the role that radiation plays on the development of new edible materials will be addressed.

2. High-energy radiation and polymers

Gamma rays and electron beam are two commonly used ionizing radiation sources in industrial process. Gamma rays, 1.17 and 1.33 MeV, are emitted continuously from radioactive source such as cobalt-60, whereas electrons are generated from an accelerator to produce a stream of electrons called electron beam. The energy of electrons depends on the type of machines and can vary from 200 keV to 10 MeV. High-energy radiation using conventional gamma or e-beam sources is an adequate tool for the modification of polymer materials through degradation, grafting and cross-linking [10].

The absorbed dose of radiation is expressed in units of gray (Gy). The Gy was adopted by the International Commission of Radiation Units and measurements (ICRU) in 1975 as the special name for the standard international (SI) unit of absorbed dose, being 1Gy = 1 Joule/kg of matter. When processing food products very seldom the employed dose exceeded 20 kGy, and most of the cases remained below 10 kGy [11]. The adsorbed radiation dose is a consequence of the

radiation exposure time and the distance between the product and the radiation source in the irradiation camera.

The long molecular chains of polymers can be broken by the absorption of a quantum of energy above the energy of the covalent bond of the main carbon chain, which typically is in the range of 5–10 eV. The energy of beta and gamma photons of 1 to 10 MeV surpasses by many orders of magnitude this minimum value, representing a high probability of affecting all kinds of polymers, naturals and synthetics alike [12]. Usually, there is a competition between cross-linking and chain scission reaction. If chain scission reaction predominates the material degrades.

Radiation, as a non-selective, highly efficient tool of ionization, may form excited sites, ions and free radicals in almost all kinds of materials. Radiation treatment of polymer mixtures, even if they are (partially) incompatible, gives a chance for bridge-forming bonds [13].

The key effect of radiation is the production of reactive oxygen species (ROS), which affects biomolecules (e.g., lipid, protein, polysaccharides). One of the important radiation-induced free-radical species is the hydroxyl radical, which indiscriminately attacks neighboring molecules often at near diffusion-controlled rates. Hydroxyl radicals are generated by ionizing radiation either directly by oxidation of water, or indirectly by the formation of secondary partially ROS. These may be subsequently converted to hydroxyl radicals by further reduction ("activation"). Secondary, radiation injury is therefore influenced by the surrounding antioxidant status and the amount and availability of activating mechanisms. The biological response to radiation may be modulated by alterations in factors affecting these secondary mechanisms [14].

3. Radiation and polysaccharides

Carbohydrates or saccharides are polyhydroxy aldehydes or ketones that have the empirical formula $(CH_2O)_n$. Most of the carbohydrates found in nature occur as polysaccharides of high molecular weight. Polysaccharides are polymers of monosaccharides linked with glycosidic bonds containing usually no more than two kinds of residues. The most abundant monosaccharide is the six-carbon sugar D-glucose; it is the primordial monosaccharide from which all others are derived. D-Glucose is the most important fuel molecule for most organisms, and also the basic building block of the most abundant polysaccharides. The monomeric units contain many hydroxyl groups, which can engage in intra- and inter-molecular formation of hydrogen bonds. This hydrogen bonding keeps the chains together and contributes to the high tensile strength of the polymeric material. Other forms of functionalization can also occur.

Polysaccharide films are made from starch, alginate, cellulose ethers, chitosan, carageenan, or pectins and impart hardness, crispness, compactness, thickening quality, viscosity, adhesiveness and gel-forming ability to a variety of films. These films usually exhibit good gas permeability properties, resulting in desirable modified atmospheres that enhance the shelf life of the product without creating anaerobic conditions. Additionally, polysaccharide films and coatings can be used to extend the shelf life of muscle foods by preventing dehydration, oxidative rancidity and surface browning, but their hydrophilic nature makes them poor barriers for water vapor.

Starch contains only D-glucose as monomeric units and occurs in two forms, α -amylose (a long unbranched chain) and amylopectin (highly branched). Films produced from pure starch are generally brittle and difficult to handle, although there are some exceptional reports that starches such as cassava can make transparent and colorless flexible films without any previous chemical treatment [15].

Other polysaccharides like pectin, pullulan and chitosan, coming from different origins, present different capability of acting as edible active coatings [16].

The poor mechanical properties and water stability of starch have restricted its industrial applications. However, they can often be functionalized with carboxyl groups, phosphate groups and/or sulfuric ester groups, and the combination of plasticizers and surfactants can bring the enhancement of properties of the starch films [17, 18].

The development of biodegradable materials based on starch has become an attractive option and the production of starch-based plastics is gradually obtained considerable importance in the world. Chemical modifications (e.g., cross-linking) or using a second biopolymer in the starch-based composite have been studied as strategies to produce low water-sensitive and relatively high-strength starch-based materials [19–22].

Particularly, citric acid showed to cross-link starch and improve the tensile strength, thermal stability and decrease the dissolution of starch films in water and formic acid [23].

Starch is considered easily depolymerizable by radiation treatment. Gamma and electron beam irradiation are used to induce radiation degradation of chitosan, alginate, carrageenan, cellulose, pectin, for recycling these bio-resources and reducing the environmental pollution. These carbohydrates, when degraded by radiation, present various kinds of biological activities such as promotion of plant growth, anti-microbial activity, phytoalexins induction, biocontrol elicitors and also can be added to aquaculture and animal feed to enhance immunity of animals [24, 25].

The radiation starch or dextrin degradation is a clean and safety tool. The process is very efficient and can be easily controlled by choosing a proper irradiation dose. A dose of 4.4 kGy, for instance, was enough to decrease the molar mass of wheat starches by one order of magnitude [26]. Other works describe the action of gamma radiation on potato, bean and maize starches [27–29].

Physical and structural characteristics of rice flour and starch obtained from gamma-irradiated white rice were determined. Pasting viscosities of the rice flour and starch decreased continuously with the increase in irradiation dosage. Gamma irradiation had no significant effect on the amylopectin branch chains, but produced more branch chains when the irradiation dosage was less than 9 kGy. It might be deduced that gamma irradiation caused the breakage of the amylopectin chains at the amorphous regions, but had little effects on the crystalline regions of starch granules, especially at low-dosage irradiation [30].

Several other reports were published about the beneficial use of gamma radiation on the improvement of properties of different biopolymeric materials. Ionizing radiation was employed for the synthesis of polysaccharide derivatives to be used as oral delivery system for a colon-specific drug carrier [31]; other works mention the increase of mechanical and barrier properties of polysaccharide materials and the induction of grafting of methylcellulose-based films [32].

Some authors reported the development of edible films from potato peel. High-pressure, gamma rays and ultrasound were applied to potato peel solutions to break down biopolymer particles in the solution small enough to allow for biopolymer film formation. Film properties, including moisture barrier and tensile properties, color and microstructures, were investigated from the films formed with different concentrations of plasticizer (glycerol) and emulsifier (soy lecithin). The authors concluded that the concentrations of both plasticizer (glycerol) and emulsifier (soy lecithin) were important variables in producing biopolymer films from potato peel [33].

4. Radiation and proteins

All proteins are polymers and their monomeric units are α -amino acids. Twenty chemically different amino acids are incorporated in proteins. Proteins in foods serve dual roles as nutrients and structural building blocks. The concept of protein functionality has historically been restricted to non-nutritive functions—such as creating emulsions, foams and gels—but this places sole emphasis on food quality considerations and potentially overlooks modifications that may also alter nutritional quality or allergenicity. Foegeding proposed recently a new model that addresses the function of proteins in foods based on the length scale(s) responsible for the function. Properties such as flavor binding, color, allergenicity and digestibility are explained based on the structure of individual molecules, placing this functionality at the nano/molecular scale. At the next higher scale, applications in foods involving gelation, emulsification and foam formation are based on how proteins form secondary structures that are seen at the nano- and microlength scales, collectively called the mesoscale. The macroscale structure represents the arrangements of molecules and mesoscale structures in a food. Macroscale properties determine the overall product appearance, stability and texture. For applications in food products, protein functionality should start with the identification of functional needs a0 scales. Those needs are then evaluated relative to how processing and other ingredients could alter desired molecular scale properties, or proper formation of mesoscale structures. This allows for a comprehensive approach to achieving the desired function of proteins in foods [34].

Proteins have long been empirically used to make biodegradable, renewable and edible packaging materials. Numerous cereal and vegetable proteins, such as corn zein, wheat gluten and soy proteins, and animal proteins (such as milk proteins, collagen, gelatin, keratin and myofibrillar proteins) are commonly used to form agricultural packaging materials.

Lacroix and Vu, among others, had described the methods to produce protein-based films and coatings, the properties of them and their applications in food systems. They focus on selected

proteins originated from animal and plant sources consisting of caseins, whey proteins, collagen and gelatin, plasma proteins, myofibrillar proteins, egg white proteins, soy protein, wheat gluten and zein [35–37].

Soy protein isolate (SPI), a protein with good biocompatibility, biodegradability and processability, has a significant potential in the food industry, agriculture, bioscience and biotechnology. Up to now, several technologies have been applied to prepare SPI-based materials with equivalent or superior physical and mechanical properties with petroleum-based materials [38].

Among protein-based films, those made of gelatin are specially important and can be produced from a variety of origins, with or without the addition of other components as plasticizers, surfactants or mixture with others substances [39–42].

Fish gelatin is a potential alternative to current mammalian (beef and pork) gelatin. However, its physical and thermal properties limit its use in many applications. The treatment of microbial transglutaminase as a cross-linking agent could be a practical way to increase the use of fish gelatin films in various applications [43].

Edible protein film characteristics can be enhanced with chemical and enzymatic methods, combining with hydrophobic material or some polymers or using a physical method, and the resulting film properties will depend on modification methods and conditions [44].

The enzyme and chemical modifications are efficient in lowering water vapor permeability. Composite edible protein films in combination with lipids can result in better functionality than the films produced with only proteins, especially with respect to their barrier properties. Of the lipids, waxes produce the best water vapor barrier properties, but produce fragile and/ or brittle films.

The preparation of gelatin and glycerol mixture with microbial transglutaminase as the crosslinking agent was described [45]. A composite casein-gelatin was prepared using also transglutaminase [46]. In some cases, however, the chemical/enzymatical cross-linking was not satisfactory [47].

Application of radiation has been extended to the modification of proteins. Depending on the adsorbed radiation dose or radiation exposure time, various effects can be achieved resulting in the polymerization (cross-linking) or depolymerization of protein molecules. Most food proteins, however, undergo irradiation-induced cross-linking and subsequent improvement on the film properties [48].

Radiation-induced lipid oxidation can be readily detected by our body's olfactory cells rancidity, off-flavor and, sometimes on the positive aspect, aroma; similarly, it is easy to notice discoloration that results from the oxidation of pigments. In contrast, protein oxidation occurs undetected by sensory organs; instrumental analysis is therefore required. Yet, proteins are very susceptible to reactive oxygen species (ROS) and impart both desirable and undesirable consequences when oxidatively modified. For example, oxidant-initiated disulfide bond formation among gluten molecules is responsible for the desirable rheology and sponginess of bread. And functional myosin (or actomyosin) aggregates produced by the reaction of cysteine, lysine and tyrosine residues with low concentrations of free radicals promote protein gel networks in processed muscle foods, hence the products' firmness and mouthfeel.

Chemically, protein oxidation that can result from radiation treatment in the presence of O_2 involves the initial modification of amino acid side chain groups by ROS. The radiation sensitivity of the amino acids is the highest for cysteine and decrease following the sequence: cysteine, methionine, tyrosine, tryptophan, phenylalanine, valine, leucine, histidine, glutamyl, proline, threonine, arginine and lysine. The initial modification of amino acid side chain groups by ROS leads to the conversion to carbonyl and other derivatives. Electron-deficient carbonyl groups are highly reactive with amines and thiol moieties to produce cross-links between polypeptides or segments within the same protein molecule. Disulfide and dityrosine are other covalent linkages in oxidatively stressed proteins. Protein radicals, which usually have a long half-life, are precursors of polymers as well [49].

The field of health science has pioneered protein oxidation research. Similarly, research in food science over the past two decades has established ubiquitous occurrences of protein oxidation in both fresh and processed foods, especially meat products. Most of the studies point to the negative aspect because uncontrolled oxidation leads to deleterious consequences: tissue hardening (as in frozen fish), loss of water-binding potential and off-flavor due to thiol oxidation. For that reason, developments about novel antioxidants, such as phenolic derivatives, peptides/protein hydrolysates, phospholipids and polysaccharides, and their role in food quality preservation are going on [50].

Many peptides and protein hydrolysates, being preferred targets of ROS and excellent functional compounds (water-binding, foaming, rheology and so on), are qualified as "multi-functional" natural food additives. Aside from the negative impacts of oxidation, mildly oxidized lipids give us a complex, highly desirable aroma of foods as that coming from frying. Today, the beneficial effects of limited protein oxidation are also no longer ignored. That must be taking in account whenever any protein food is submitted to oxidative processes such as irradiation.

Gamma-irradiation affects proteins by causing conformational changes, oxidation of amino acids, rupture of covalent bonds and formation of protein free radicals that can be beneficial for specific further applications. Chemical changes in the proteins that are caused by gamma irradiation are fragmentation, cross-linking, aggregation and oxidation by oxygen radicals that are generated in the radiolysis of water. For example, the hydroxyl and super oxide anion radicals that are generated by radiation of film-forming solution could modify the molecular properties of the proteins, which results in the alteration of protein films by covalent crosslinkages formed in protein solution after irradiation.

Using gamma irradiation to induce cross-linking was found to be an effective method for the improvement of both barrier and mechanical properties of the edible films and coatings based on proteins. There are plenty of examples in the literature that corroborate the efficiency of radiation application on natural films, like those made of zein, the protein found in maize [51]. Different types of gelatin-based films with enhanced properties can be produced by means of application of ionizing radiation [52, 53].

The effect of electron beam accelerator doses on properties of plasticized fish gelatin film was studied. The electron spin resonance spectra indicated free radical formation during irradiation, which might induce intermolecular cross-linking. Tensile strength for gelatin film significantly increased after irradiation (improved by 30% for 60 kGy). The vapor permeability was weakly affected by irradiation. Surface tension and its polar component increased significantly in accordance with the increase of wettability. So, the authors suggest that irradiation may change the orientation of polar groups of gelatin at the film surface and cross-link the hydrophobic amino acids. They did not observed modification of the crystallinity of the film. So, they conclude that structure changes only occurs in the amorphous phase of the gelatin matrix. It is also observed that irradiation enhances the thermal stability of the gelatin film, by increasing the glass transition temperature and the degradation temperature [54].

Cross-linked copolymers of gelatin and poly(vinyl alcohol) (PVA) with excellent water absorption and water retention abilities were successfully synthesized using Co-60 gamma radiation [55]. Also, gamma irradiation was applied on milk protein to improve characteristics of milk protein films [56–59].

5. Radiation and nano technology on synthetic and composite edible polymers

Two classes of biodegradable polymers can be distinguished: synthetic or natural polymers. Recent developments in biopolymer-based food packaging materials include natural biopolymers (such as starches and proteins), synthetic biopolymers (such as poly lactic acid), biopolymer blends and nanocomposites based on natural and synthetic biopolymers [60]. The combination of synthetic and natural polymers to form films are of remarkable importance in producing composite polymers with permeability or mechanical properties according to the need of a specific application that can be applied either in the form of an emulsion, suspension or dispersion of the non-miscible constituents, or in successive layers, or in the form of a solution in a common solvent [61].

Biodegradable synthetic aliphatic polyester, like polylactide (PLA), has been studied extensively for a number of applications like drug delivery system. Frequently renewable resourcegenerated monomers possess better mechanical properties and easy processability by conventional methods like thermoforming, injection and blow molding with non-toxic degradation products, which have made it superior than the other conventional thermoplastics [62].

In addition to traditional plant materials for biodegradable polymer production, it is worthy to mention the advances in synthesizing novel polymers within transgenic plants, especially those in the polyhydroxyalkanoate class [63].

Application of nanoscience and nanotechnology to the agriculture and food sector is relatively recent compared with their use in drug delivery and pharmaceuticals [64, 65].

In the food industry, nanotechnology can be utilized in order to enhance the delivery of food ingredients to target sites, increase flavor, inhibit bacterial growth, extend product shelf life and improve food safety.

Applications of nanomaterials that do not involve direct addition of nanoparticles to consumed foods, and thus more likely to be marketed to the public in the short term, are related to food packaging and food safety. These applications include polymer/clay nanocomposites as high barrier packaging materials, silver nanoparticles as potent antimicrobial agents and nanosensors and nanomaterial-based assays for the detection of food-relevant analytes (gases, small organic molecules and food-borne pathogens) [66].

Other emerging topics of nanotechnology for food and agriculture are smart delivery of nutrients, bioseparation of proteins, rapid sampling of biological and chemical contaminants and nanoencapsulation of nutraceuticals, as well as advances in technologies, such as DNA microarrays, microelectromechanical systems and microfluidics [67, 68].

Nano-scale cellulose fiber materials (e.g., microfibrillated cellulose and bacterial cellulose) are promising candidates for bio-nanocomposite production due to their abundance, high strength and stiffness, low weight and biodegradability [69].

Several reinforcing nanoparticles such as clays, silica or silver have been used for industrial applications, but cellulose nanocrystals (CNCs) are a better choice for food industry due to their biodegradable and biocompatible nature as well as their outstanding potential in improving mechanical and barrier properties of nanocomposites [70–72]. Also, cellulose nanofibers (CNF) reinforcement improved mechanical properties, except elongation of mango puree edible films [73].

The development of nanoscale systems for the encapsulation, protection and delivery of lipophilic nutrients, vitamins and nutraceuticals was recently reported [74]. A promising route to the synthesis of protein-mimetic materials that are capable of complex functions, such as molecular recognition and catalysis, is provided by peptoid nanosheets polymers structurally related to biologically occurring polypeptides [75].

The conjunction of radiation and nanotechnology for the improvement of biomaterials are drawing special interest [76]. Gamma radiation and nanocrystalline cellulose was used for the reinforcement of poly(caprolactone) composites [77]. Also, the radiation synthesis of gelatin hydrogels containing Ag nanoparticles was reported [78]. Grasielli and his group synthesized nanoparticles of seroalbumin via intramolecular cross-linking using gamma rays technology [79]. The radiation synthesis of a composite prepared from the algae polysaccharide alginate and clay nanocomposite was also described [80].

6. Concluding remarks

To meet the growing demand of recyclable or natural packaging materials and consumer demands for safer and better quality foods, new and novel food-grade packaging materials or

technologies have been, and continue to be, developed. Examples of these packaging materials include bio-based polymers, bioplastic or biopolymer packaging products made from raw materials originating from agricultural or marine sources. Under that view, the use of ionizing radiation to synthesize and or enhance properties of edible polymers based on polysaccharides or proteins appears as an emerging promising application. The use of edible coating previously cross-linked by irradiation has permitted a better control of the release of active compounds. So far, the industrial effectiveness of the processing accumulates enough evidences within the huge spectrum of possibilities that radiation technology can offer. In the meanwhile, in order to increase further applications, regulatory status and future perspectives on innovative food preservation must still be examined carefully.

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