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Review

Protein–TiO_2: A Functional Hybrid Composite with Diversified Applications

Luis Miguel Anaya-Esparza [1,2], Zuamí Villagrán-de la Mora [3], Noé Rodríguez-Barajas [2], Teresa Sandoval-Contreras [1], Karla Nuño [4], David A. López-de la Mora [4,*], Alejandro Pérez-Larios [2,*] and Efigenia Montalvo-González [1,*]

1 Laboratorio Integral de Investigación en Alimentos, Tecnológico Nacional de México-Instituto Tecnológico de Tepic, Av. Tecnológico 2595 Fracc, Lagos del Country, Tepic 63175, Mexico; lumianayaes@ittepic.edu.mx (L.M.A.-E.); tesysval@gmail.com (T.S.-C.)

2 Laboratorio de Materiales, Agua y Energía, División de Ciencias Agropecuarias e Ingenierías, Centro Universitario de los Altos, Universidad de Guadalajara, Av. Rafael Casillas Aceves 1200, Tepatitlán de Morelos 47600, Mexico; noe.rbarajas@academicos.udg.mx

3 División de Ciencias Biomédicas, Centro Universitario de los Altos, Universidad de Guadalajara, Av. Rafael Casillas Aceves 1200, Tepatitlán de Morelos 47600, Mexico; blanca.villagran@academicos.udg.mx

4 División de Ciencias de la Salud, Centro Universitario de Tonalá, Universidad de Guadalajara, Av. 555 Ejido San José Tateposco, Nuevo Perif. Ote., Tonalá 475425, Mexico; karlajanette.nuno@cutonala.udg.mx

* Correspondence: david.ldelamora@academicos.udg.mx (D.A.L.-d.l.M.); alarios@cualtos.udg.mx (A.P.-L.); emontalvo@ittepic.edu.mx (E.M.-G.)

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Abstract: Functionalization of protein-based materials by incorporation of organic and inorganic compounds has emerged as an active research area due to their improved properties and diversified applications. The present review provides an overview of the functionalization of protein-based materials by incorporating TiO_2 nanoparticles. Their effects on technological (mechanical, thermal, adsorptive, gas-barrier, and water-related) and functional (antimicrobial, photodegradation, ultraviolet (UV)-protective, wound-healing, and biocompatibility) properties are also discussed. In general, protein–TiO_2 hybrid materials are biodegradable and exhibit improved tensile strength, elasticity, thermal stability, oxygen and water resistance in a TiO_2 concentration-dependent response. Nonetheless, they showed enhanced antimicrobial and UV-protective effects with good biocompatibility on different cell lines. The main applications of protein–TiO_2 are focused on the development of eco-friendly and active packaging materials, biomedical (tissue engineering, bone regeneration, biosensors, implantable human motion devices, and wound-healing membranes), food preservation (meat, fruits, and fish oil), pharmaceutical (empty capsule shell), environmental remediation (removal and degradation of diverse water pollutants), anti-corrosion, and textiles. According to the evidence, protein–TiO_2 hybrid composites exhibited potential applications; however, standardized protocols for their preparation are needed for industrial-scale implementation.

Keywords: proteins; titanium dioxide; functionalization; hybrid composites

1. Introduction

Nowadays, the development of eco-friendly materials with advanced characteristics and diverse applications is an active research area [1,2]. Hybrid compounds are composites that consist of combining inorganic–inorganic (e.g., TiO_2–Ag), organic–organic (e.g., wheat gluten–cellulose), and organic–inorganic (e.g., collagen–TiO_2) [3–5], and they can be synthesized by spin and dip coating, slot-casting, electrochemical self-assembly, and chemical vapor, atomic or molecular layer deposition methods [6]. In general, hybridization or functionalization of organic compounds by incorporating inorganic compounds is a strategy that enables the attainment of hybrid materials with beneficial properties and new functionalities [6,7]. Recently, titanium dioxide (TiO_2) has been used as a reinforcement agent to develop organic–inorganic hybrid materials with improved physicochemical, mechanical, UV- and gas-barrier, water resistance, and antimicrobial properties [1,3,8–11].

TiO_2 is an amphoteric, inert, non-toxic, biocompatible metal oxide that exhibited thermal and chemical stability for diverse applications with a relatively low cost of production [12]. The wide use of TiO_2 is to support its photocatalytic, adsorptive, UV-blocking, and antimicrobial properties [13–15]. It has been employed for environmental remediation in dye removal from aqueous media [16]. Moreover, TiO_2 is the main source of white pigments for food, pharmaceutical, and cosmetic applications in compliance with the recommended safe dosage [17–19]. Currently, there is a great interest in combining protein-based materials with inorganic compounds like titanium dioxide to fabricate protein–TiO_2 hybrid structures with improved physical and chemical properties, which open new opportunities and applications [1].

In the last decade, protein–TiO_2 hybrid composites and their potential applications have been explored [4,20–24]. Wang et al. [23] developed a soy protein isolate film combined with TiO_2 with antimicrobial properties against *Escherichia coli* and *Staphylococcus aureus*. Similar trends were reported when a marine algae protein-based film functionalized with TiO_2 was used [25]. Fathi et al. [16] informed that the sesame protein–TiO_2 hybrid film showed photocatalytic degradation of the methylene blue dye under UV-light radiation. Qingyan et al. [26] made gelatin film reinforced with TiO_2 with improved mechanical and UV-protective properties. Fan et al. [27] fabricated a collagen–chitosan–TiO_2 porous scaffold for wound-healing purposes. Meanwhile, He et al. [27] developed an active packaging with marine alga (*Gracilaria lemaneiformis*) protein isolate and TiO_2 for cherry tomatoes preservation, while a whey protein–TiO_2 hybrid film was used to prolong the shelf life of chilled and lamb meats [28,29]. Furthermore, the incorporation of TiO_2 in diverse protein-based materials (collagen, gelatin, soy, hey, marine alga, kefiran, zein, sesame, sodium caseinate, and wheat gluten) had a positive impact on the technological (mechanical, water resistance, and gas-barrier) and functional (antimicrobial and UV-protective) properties, which exhibited potential uses for various applications [16,17,20,24,25,30,31].

This review summarizes the advantages and limitations of protein-based material functionalization by adding TiO_2 nanoparticles, offers and provides an overview of their photocatalytic and antimicrobial properties, environmental remediation, potential food and non-food packaging, pharmaceutical, cosmetics, textile, and biomedical applications.

2. Proteins: Applications and Limitations

Proteins are biological molecules composed of α-amino acids connected by peptide bonds, which can be obtained from plant-derived or animal origins [32]. For example, zein and gluten are cereal proteins [4,33], meanwhile, other proteins can be obtained from legumes such as soy [19]. Collagen and gelatin are extracted from meat, fish, and poultry by-products [1,34], whereas whey protein is a by-product of dairy manufacturing [29]. They exhibited interesting technological and functional properties for diversified applications, associated with their composition and structure [2]. Moreover, they are non-toxic, abundant, readily available, biodegradable, low-cost, and biocompatible to combine with enzymes, microorganisms, and organic and inorganic compounds [6,32,35]. Most of the applications described in the literature for protein-based materials are focused on developing packaging materials for food and non-food purposes, or biomedical applications such as wound-healing materials, as shown in Table 1.

Table 1. Potential applications of some protein-based materials.

Protein Source	Application	Ref.
Yellow pea protein isolate	Food and non-food packaging	[36]
Whey protein	Food and non-food packaging	[37]
Corn zein	Food and non-food packaging	[38]
Soy protein isolate	Food and non-food packaging	[39]
Rice bran	Food and non-food packaging	[40]
Wheat gluten	Food and non-food packaging	[41]
Gelatin	Food and non-food packaging	[42]
Gelatin	Biomedical	[43]
Keratin	Biomedical	[44]

Acquah et al. [36] fabricated a yellow pea (*Pisum sativum*) protein-based film with potential food and non-food packaging applications. It exhibited moderate water solubility (36.5%), good mechanical properties (elongation of 65%, a tensile strength of 0.65 MPa, and elastic modulus of 6.65 MPa), as well as good thermal properties (glass transition of 95.5 °C), but high moisture uptake (82%) due to its hydrophilic nature (contact angle of 60°), affecting its quality as a packaging material. Agudelo-Cuartas et al. [37] mentioned that whey protein-based films showed great potential for packaging purposes (good mechanical properties); however, their high-water solubility (59%) and water vapor permeability (1.4×10^{-10} $\text{g·m}^{-1}\text{·s}^{-1}\text{·Pa}^{-1}$) limit their uses in foods with high water content (e.g., meat). According to Guo et al. [38], the protein-based film's mechanical properties are influenced by storage conditions (temperature and relative humidity). They found that tensile strength and elongation at break of a zein film were negatively affected when relative humidity and temperature increased from 34% to 80% and from 5 to 35 °C, respectively. They argue that the available –SH groups in the protein structure decreased gradually during storage by water absorption, implying new and weak interactions.

Su et al. [39] reported that soy protein isolate film exhibited good biodegradability and gas-barrier properties against oxygen and carbon dioxide when relative humidity was low, which are suitable featuring for the development of packaging materials. Wang et al. [40] suggested that modification of protein structure by alkaline conditions could be an alternative to improve the technological properties of protein-based films. They reported that the formation of protein aggregates in a rice bran film treated at pH 11 improved their physical, mechanical, and thermal properties, associated with an increase in the β-sheet content and non-covalent interactions, due to the modification of the protein structure.

Additionally, gelatin-based films exhibited great potential for fabricating food packaging or wound-healing materials; however, due to their hygroscopic nature, they needed to be combined with a crosslinking or plasticizer agent (organic or inorganic) to improve their water resistance and thermal stability [42,43]. It has been reported that keratin films are too rigid, and the addition of glycerol improved their flexibility and mechanical resistance, which are suitable for biomedical applications [44]. Similar trends were reported in a wheat gluten film by adding glycerol, but its thermal stability was improved and could be used for packaging purposes [41].

In general, protein-based films exhibited great potential applications; however, their functionality depends on their molecular characteristics, complexity, superficial charge, denaturation tendency, water resistance, and thermal stability [35]. Therefore, the incorporation of organic and inorganic materials in the protein matrix is a viable strategy to enhance their functional and technological properties [29,32,45]. Table 2 shows some protein-based materials functionalized with organic and inorganic compounds to form hybrid composites with potential applications.

Table 2. Application of some functionalized protein-based materials.

Protein Source	Functional Agent	Application	Ref.
Gelatin	Silver-NPs	Active food packaging.	[46]
Gelatin	Resorcinol and silver-NPs	Active food packaging.	[47]
Caseinate/gelatin	Tannins	Active food packaging.	[48]
Sodium caseinate	ZnO-NPs and REO	Active food packaging.	[49]
Whey protein	Montmorillonite and citric acid	Active food packaging.	[50]
Whey protein	Organic acids and nisin	Active food packaging.	[51]
Furcellaran/whey protein	Yerba mate extracts	Active food packaging.	[52]
Yellow pea protein isolate	Whey protein isolate	Active food packaging.	[36]
Fish protein isolate	Gelatin and ZnO-NPs	Active food packaging.	[53]
Soy protein hydrolysate	Silica	Environmental remediation.	[54]
Soy protein isolate	Tragacanth, silica, and lycopene	Environmental remediation.	[55]
Silk fibroin	Ag NPs	Biomedical.	[56]
Egg white protein	Silk fibroin	Biomedical.	[57]

NPs: nanoparticles; ZnO: Zinc oxide; REO: rosemary essential oil.

According to the evidence, the incorporation of organic and inorganic compounds improves the technological (water and thermal resistance, mechanical, and adsorptive) and functional (antimicrobial activity and biocompatibility) properties of protein-based materials, associated with their ability to form intramolecular bonds through covalent and non-covalent interactions with the functional groups (–NH_2, –OH, –COOH, and –SH) of the protein structure [6,29].

Additionally, usage of TiO_2 as a functional agent to enhance the technological properties of diverse protein-based materials has been widely explored in the last years, mainly for the chemical and physical interactions between protein structure and TiO_2, which could be developed using diverse methodologies.

3. Possible Structural Interaction between R-Groups Amino Acid with TiO_2 Nanoparticles

A major understanding of the interactions between proteins and TiO_2 surfaces will be a potential core for many applications in bio-nanotechnology [58]. Ranjan et al. [59] in silico observed that the TiO_2 (1.09 nm) nanoparticles bind to 13 immunological proteins (Table 3), using a docking simulation program (AutoDock 4.0), a computed atlas of surface topography of proteins (CASTp) and PyMol software (version 1.5.0.4). They observed that nano-TiO_2 bound with a positively charged R-group (lysine, arginine, and histidine) and nonpolar aliphatic R-groups amino acid (proline, glycine, alanine, valine, leucine, methionine, and isoleucine) containing amino acids, most frequently with lysine and proline. On the other hand, TiO_2 had less affinity with the aromatic R-group (phenylalanine, tyrosine, and tryptophan), polar uncharged R-groups (serine, threonine, cysteine, asparagine, and glutamine), and negatively charged R-group (aspartate and glutamate)-containing amino acids. According to the authors, the affinity of TiO_2 with the amino acids depends on the ability to form stable hydrogen bonds, which depend on the binding and intermolecular energy of each amino acid. These interactions have been exploited to develop packaging, scaffolds, wound-healing, and dental implant materials with enhanced properties, and to remove and degrade water pollutants, among others.

Table 3. Some immunological proteins–TiO_2 interaction.

Immunological Protein	Abbreviation	Binding Energy	Intermolecular Energy
Intercellular adhesion molecule 1	ICAM−I	−11.63	−12.73
Mitogen−activated protein kinases	P−38	−11.73	−12.83
The nuclear factor−kB	NF−kB	−8.29	−9.39
Cyclooxygenase 2	COX−2	NR	NR
Interleukin 8	IL−8	−4.04	−5.14
Placental growth factor	PIGF	−9.36	−10.36
C–X–C motif chemokine ligand 1	CXCL−I	1.67	0.57

Table 3. *Cont.*

Immunological Protein	Abbreviation	Binding Energy	Intermolecular Energy
C–X–C motif chemokine ligand 3	CXCL−3	NR	NR
C–X–C motif chemokine ligand 5	CXCL−5	576.34	575.34
C–X–C motif chemokine ligand 20	CCL−20	−8.25	−9.34
The cluster of differentiation 35	CD 35	5420	5420
The cluster of differentiation 66b	CD 66b	NR	NR
Matrix metallopeptidase 9	MMP−9	−9.01	−10.11

Adapted from Ranjan et al. [59]. NR: No reported.

4. Preparation of Functionalized Protein–TiO_2 Materials

The functionalization of protein-based materials through the introduction of organic (ascorbic acid, cellulose, and starch) and inorganic (metallic or metal oxide) compounds is an attractive way to fabricate protein-based hybrid materials with enhanced properties, which has seen a significant increase in the last few years [6]. The most common methods for developing functionalized protein-based materials are evaporative casting, dip-coating, layer-by-layer assembly, freeze-drying, electrospinning, and electrochemical through protein denaturation by gelation-coagulation process [6,45].

4.1. Evaporative Casting Method

The evaporative casting method is generally accepted and commercially used for its simplicity, flexibility, and applicability to large-scale production. It consists of preparing a viscous solution by mixing the components, casting them in a plate, and evaporating them under controlled temperature and vacuum conditions to remove the solvent solution and form film and coatings (Figure 1). In general, it is a relatively low-cost method (one-third to half of the other methods); however, its main limitations are the difficulty in achieving a uniform distribution of the reinforcement agent, the presence of air bubbles, and possible reactions between the polymeric matrix and functional agent [60].

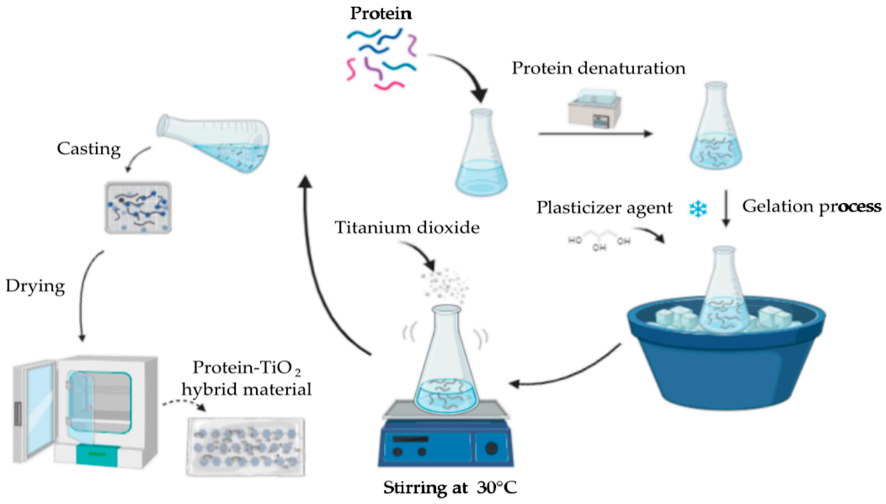

Figure 1. Schematic representation to laboratory scale of an evaporative casting method to prepare protein-based hybrid materials (adapted from Fan et al. [27], Al-Zoubi et al. [6]) (figure created with BioRender.com).

4.2. Dip Coating Method

Dip-coating is a technique widely used in many industrial fields to deposit onto any substrate. The process could be defined as depositing aqueous-based liquid phase coating solutions onto the surface of any substrate and is divided into five stages: immersion, start-up, deposition, drainage, and evaporation. It is achieved at low processing temperatures and is a low-cost method to develop thin coatings with high purity, good adhesion, high surface, and uniformity. However, this methodology requires high sintering temperatures and thermal expansion mismatch [61–63].

4.3. Layer-by-Layer Deposition Method

The layer-by-layer deposition is a common method for coating substrates to develop functional thin films. It is a cyclical process in which a charged material is adsorbed onto a substrate, and after washing, an oppositely charged material is adsorbed on the surface of the first layer. This constitutes a single bilayer film with a thickness generally on the order of nanometers, and the deposition process can be repeated until a multilayer film is obtained. This method offers advanced composites with exceptional properties (mechanical, electrical, optical, and biological) unavailable by other means, but this deposition process is complex, and the need for multiple dipping cycles hampers its usage in microtechnologies and electronics [64,65].

4.4. Freeze-Drying Method

Freeze-drying is a process that consists of removing the solvent from a frozen suspension containing mixed components. First, the gels are frozen, transforming the gel to a solid; then, sublimation of the solvent (mainly water) is then achieved at low pressure, avoiding the formation of the vapor–liquid interface. This method is widely used for aerogel preparation with highly porous and large specific surface area structures that allow rapid disintegration. However, this procedure requires sophisticated equipment compared to the evaporative casting method [66,67].

4.5. Electospinning Method

Electrospinning is a simple method to produce ultra-thin fibers with high surface area, highly porous structure, and small pore size. In this method, the mixed solution is pumped through a capillary conductive needle to form a droplet; under suitable conditions, solvent evaporation occurs, and the compound contracts into solid polymeric materials instead of fibers. It has the advantages of mild experimental conditions, low cost, easy operation and function, and a wide range of raw materials. The spinning process is controllable, and the parameters can be adjusted according to the different requirements in various research fields. However, electrospinning with raw materials that have a low molecular weight is difficult [68].

4.6. Electrochemical Method

Electrochemical methods are widely used for the preparation of thin films and coatings through anodic or cathodic techniques. Both processes are commonly used to prepare coatings by electrodeposition which include: electrophoretic process (EPD) using deposition of charged particles in a stable colloidal suspension on a conductive substrate, acting as one of the two oppositely charged electrodes in the EPD cell, and the electrolytic process (ELD), which starts from solutions of metal salts. They exhibit some advantages like low-cost, ability to coat complex shapes, speed, uniform coating thickness, rapid deposition rates, and the ability to coat complex substrates; however, it is difficult to produce crack-free coatings, it requires high sintering temperatures, and the bonding strength between coating and substrate is not strong enough [61,69].

5. Applications of Protein–TiO_2 Hybrid Composites

Protein-based materials exhibited a wide range of applications. However, most of their potential uses are limited by their poor physicochemical properties [35]. Thus, their functionalization with TiO_2 is a viable alternative to improve the technological and functional properties of protein-based materials such as gelatin, wheat gluten, kefiran, zein, and soy and whey protein isolates for several applications [49] (Figure 2), as discussed below.

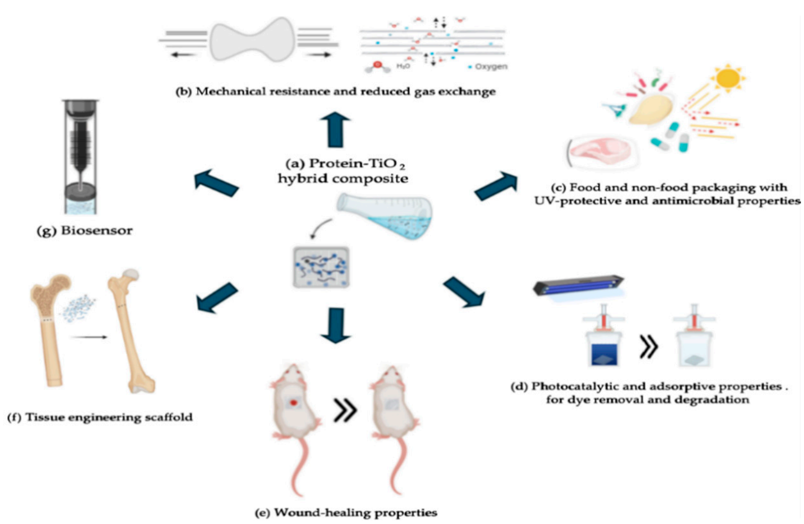

Figure 2. Protein–TiO_2 hybrid composites (**a**) with enhanced mechanical and reduced gas exchange (**b**) and their applications: as food and non-food packaging with UV-protective and antimicrobial properties (**c**), photocatalytic activity for dye removal and degradation (**d**), wound-healing material (**e**), tissue engineering scaffolds (**f**), and for the development of biosensors (**g**) (adapted from Lin et al. [3], Fan et al. [27], Alizadeh-Sani et al. [28], Emregul et al. [70], Ferreira et al. [71]) (figure created with BioRender.com).

5.1. Gelatin–TiO_2 Hybrid Composite

In the last years, the number of applications of gelatin-based materials has considerably increased. Gelatin is a protein obtained from the hydrolysis of collagen from mammalian sources, mainly pork and cattle. It is non-toxic, biodegradable, and biocompatible [72]. However, its main disadvantage for industrial applications (e.g., food packaging) is its hydrophilicity [73]. Therefore, the incorporation of TiO_2 into the gelatin matrix is a viable strategy to improve its technological and functional properties [74]. The most common method for the preparation of gelatin–TiO_2 hybrid composites is evaporative casting for films and coatings and freeze-drying for aerogels. Furthermore, the nanoparticles used are commercially available with sizes ranging from 10 to 25 nm in its anatase phase, in some cases in its rutile phase, using concentrations ≤1% in weight of total solid content, as shown in Table 4.

Table 4. Effect of TiO_2 incorporation on gelatin matrix properties.

Application	**Method/Presentation**	*** Composition**	**TiO_2 Specifications**	**Relevant Results**	**Ref.**
Food and non-food packaging	Evaporative casting/Film	Gelatin (4 g 100 mL^{-1}), glycerol (30% *w*/*w*)	Commercial SM: Hydrothermal (TiO_2): 0.5% *w*/*w* Size: 25 nm CP: Anatase	TiO_2 enhanced the physicochemical and antimicrobial properties of gelatin film.	[1]
Food and non-food packaging	Evaporative casting/Film	Fish gelatin (2.3% *w*/*v*), chitosan (1% *w*/*v*), glycerol (1% *w*/*v*)	(TiO_2:Ag): 0.4% *w*/*w*	Hybrid films showed improved physicochemical and antimicrobial properties.	[3]
Food and non-food packaging	Evaporative casting/Film	Gelatin (4 g 100 mL^{-1}), glycerol (25% *w*/*w*)	Commercial (TiO_2): 1% *w*/*w* Size: <10 nm CP: Anatase	TiO_2 improved UV-barrier, thermal, mechanical, and water-related properties of gelatin film.	[12]
Food and non-food packaging	Evaporative casting/Film	CMC (1 g 100 mL^{-1}), gelatin (1 g 100 mL^{-1})	Commercial (TiO_2:Ag): 0.4% *w*/*w* Size: 20 nm	TiO_2 improved the technological and photocatalytic properties of gelatin film.	[18]

Table 4. *Cont.*

Application	Method/Presentation	* Composition	TiO_2 Specifications	Relevant Results	Ref.
Food and non-food packaging	Evaporative casting/Film	Gelatin (4 g 100 mL^{-1}), glycerol (15% *w*/*w*)	SM: Sol–gel (TiO_2:Ag): 1% *w*/*w* Size: 10–20 nm CP: Anatase Body-centered tetragonal crystal structure	TiO_2 improved the technological properties of the gelatin film.	[20]
Food and non-food packaging	Evaporative casting/Film	Agar (1.5 g 100 mL^{-1}), gelatin (4 g 100 mL^{-1}), glycerol (35% *w*/*v*)	Commercial (TiO_2): 0.5% *w*/*w* Size: 10–20 nm CP: Anatase-Rutile	The hybrid film showed a marked UV-protective effect and improved water resistance.	[21]
Food and non-food packaging	Evaporative casting/Film	Gelatin (15 mg·mL^{-1})	(TiO_2): 0.5% *w*/*w* Size: 12.2 nm CP: Anatase Crystal structure	The film exhibits antibacterial activity.	[26]
Food and non-food packaging	Evaporative casting/Film	Gelatin (3 g 80 mL^{-1}), PVA (3 g 80 mL^{-1}), glycerol (30% *w*/*w*)	Commercial (TiO_2:4A zeolite): 1% *w*/*w*	Functionalization improved the physicochemical and antimicrobial properties of the gelatin–PVA film.	[74]
Food and non-food packaging	Evaporative casting/Coating	Gelatin (3 g 80 mL^{-1}), PVA (3 g 80 mL^{-1}), glycerol (30% *w*/*w*)	Commercial (TiO_2:4A zeolite): 1% *w*/*w*	The hybrid film effectively extended the shelf life of white shrimp.	[75]
Food and non-food packaging	Evaporative casting/Film	Gelatin (8% *w*/*w*), sorbitol: glycerol ratio 3:1 (40% *w*/*w*)	Commercial (TiO_2): 1% *w*/*w* Size: 10–15 nm CP: Anatase-Rutile	The hybrid film showed antimicrobial properties.	[76]
Food and non-food packaging	Evaporative casting/Film	Gelatin (1 g 100 mL^{-1}), CMC (1 g 100 mL^{-1})	Commercial (TiO_2:Ag): 0.4% *w*/*w* Size: 21 nm CP: Anatase	Hybrid films showed improved physicochemical and antimicrobial properties.	[77]
Food and non-food packaging	Evaporative casting/Film	Gelatin (4% *w*/*w*), agar (1.5% *w*/*v*), glycerol (35% *w*/*w*)	Commercial (TiO_2): 2% *w*/*w*	Gelatin–TiO_2 effectively delayed fish oil oxidation.	[78]
Biomedical	Freeze-drying/Hydrogel	Sodium alginate (2% *w*/*v*), gelatin (0.5% *w*/*v*), β-tP (1% *w*/*v*)	SM: Electrochemical anodization (TiO_2): 0.1% *w*/*v* Size: 110 nm CP: Anatase Nanotubes	Hybrid hydrogel had adequate porosity and mechanical resistance.	[79]
Biomedical	NI/Scaffold	NI	SM: Biometic (TiO_2): NI Size: 30–35 nm CP: Anatase	Hybrid scaffold promoted osteointegration and enhanced bone regeneration.	[71]
Biomedical	NI/NI	Gelatin (2 mg·mL^{-1})	Electrochemical anodization (TiO_2): NI Size: 100 nm CP: Rutile Nanotubes (20 nm × 350 nm) Low crystal structure	Hybrid material could potentially be used for orthopedic and dental applications.	[80]
Biomedical	Electrochemical deposition/coating	Hap (NI), Gelatin (100 mg 100 mL^{-1}), GO (2 mg mL^{-1})	Hydrothermal (TiO_2): NI Crystal structure	The hybrid coating showed excellent biocompatibility with MC3T3-E1 cells.	[81]
Biomedical	Freeze-drying/Hydrogel	Gelatin (2 g 100 mL^{-1})	(TiO_2): 0.5% *w*/*v*	The hybrid composite had better wound-healing properties than gelatin film.	[73]

Table 4. *Cont.*

Application	Method/Presentation	* Composition	TiO_2 Specifications	Relevant Results	Ref.
Biomedical	Polymer blend/Biosensor	CMC:Gelatin (3.75 mg), solution of superoxide dismutase (4733 U, 1 mg), glutaraldehyde (0.005 M)	SM: Hydrothermal (TiO_2): 0.1% *w/w* Size: 50 nm CP: Anatase	The biosensor exhibited high analytical performance, high sensitivity, and fast response time for superoxide radical detection.	[70]
Pharmaceutical	NI/Capsule	Gelatin (NI)	Commercial (TiO_2): 3.5% *w/w* Size: 177.2 nm CP: Anatase Crystalline structure	The capsules could be printed gray by UV-laser.	[82]
Metal corrosion resistance	NI/Coating	Gelatin (8 wt.% in 20 wt.% acetic acid)	Commercial (TiO_2): 3% *w/w* Size: 10–25 nm CP: Anatase Purity: >99% Density: 3.9 $g \cdot cm^{-3}$	Gelatin–TiO_2 composite improved the corrosion resistance of steel material.	[83]
Hydrogen production	NI/microspheres	Gelatin (5 g 100 mL^{-1})	SM: Sol–gel Titania precursor (10 mL of tetra-n-butyl titanate in 50 mL of ethyl alcohol) Size: 50–100 nm CP: Anatase High crystallinity and purity	Gelatin improved the adsorptive properties of TiO_2.	[84]

* Material composition was based on the best-reported results. NI: No information; CMC: carboxymethyl cellulose; PVA: polyvinyl alcohol; GO: graphene oxide; β-tP: β-tricalcium phosphate; Hap: hydroxyapatite; SM: synthesis method; (TiO_2): concentration of titanium dioxide; CP: crystallite phase.

5.1.1. Food and Non-Food Packaging Applications of Gelatin–TiO_2 Hybrid Composite

The potential use of gelatin-based materials functionalized with TiO_2 nanoparticles as food and non-food packaging material has been extensively explored [26,76]. Nassiri and Nafchi [76] developed a bovine gelatin film reinforced with TiO_2 nanoparticles with antimicrobial properties against *S. aureus* and *E. coli*, associated with the physical and chemical interactions of TiO_2 with the bacteria cell membrane. Incorporation of TiO_2 at low concentrations (5% *w/w*) decreases the water vapor (from 8.90 to 1.61 × 10^{11} $g \cdot m^{-1} \cdot s^{-1} \cdot Pa^{-1}$), and oxygen permeability (from 214 to 95 $cm^3 \cdot \mu m/m^2 \cdot day$) of protein-based film. Similarly, Qingyan et al. [26] informed that gelatin–TiO_2 film exhibited antimicrobial activity against *E. coli* (54% inhibition of viable cells) and *S. aureus* (44% inhibition of viable cells) under UV-light irradiation (365 nm) after 120 min of exposure. The above, associated with the photocatalytic properties of TiO_2 and its ability to generate reactive molecules (hydrogen peroxide, hydroxyl radical, and superoxide anions) with antimicrobial properties by affecting the cell viability. Moreover, the addition of TiO_2 (1% *w/w* of total solid content) in the gelatin film promoted an increase in its mechanical and thermal properties. It decreased water solubility, moisture uptake, water vapor permeability, and transparency due to the formation of hydrogen and Ti–O–C bonds and electrostatic interactions between protein and inorganic nanoparticles [12].

Azizi-Lalabadi et al. [74] made a hybrid film composed of gelatin and polyvinyl alcohol, reinforced with TiO_2 nanoparticles previously embedded in 4A-zeolite. The enhanced physicochemical (optical, gas-barrier, and water resistance) were attributed to the interaction of the N–H functional group present in the protein structure, with TiO_2 through hydrogen bonds. Moreover, the hybrid film exhibited antimicrobial properties especially against Gram-negative bacteria (*E. coli* and *P. fluorescens*). Moreover, the hybrid film effectively extended the shelf life of white shrimp (up to 12 days) compared to uncoated samples (6 days), without significant changes in sensory attributes [75]. Likewise, Riahi et al. [1] fabricated an active gelatin–TiO_2–grape seed extract film for food packaging purposes and found that water contact angle, water vapor permeability, mechanical properties, and UV-protective effect improved in a dose-dependent response with an optimum TiO_2 concentration of 0.5% *w/w*, which was attributed to the chemical interaction of TiO_2 and C=O groups in the protein structure. On the other hand, the hybrid film exhibited antimicrobial activity in strain- and TiO_2 dose-dependence, where the Gram-negative bacteria were less susceptible than Gram-positive. At low concentrations of TiO_2 (<3% *w/w*), the hybrid film showed a bacteriostatic effect against *E. coli* and *L. monocytogenes*, while at 5% *w/w* exhibited bactericidal action.

Pirsa et al. [77] evaluated the antioxidant and antimicrobial properties of a carboxymethyl cellulose–gelatin film reinforced with TiO_2:Ag-doped nanoparticles. The hybrid film exhibited better mechanical properties (greater flexibility) in comparison with the control group. Moreover, it showed antioxidant activity and antibacterial effect against *E. coli* and *S. aureus* in a TiO_2:Ag concentration-dependent response. Furthermore, the carboxymethyl cellulose (CMC)–gelatin–TiO_2: Ag exhibited good photocatalytic degradation of ethanol, benzene, and ammonia [18]. Furthermore, the incorporation of TiO_2: Ag-doped nanoparticles improved the antioxidant, mechanical, UV-barrier, water resistance, and mechanical properties of a *Rhinobatos cemiculus* gelatin film in a dose-dependent manner. At a low concentration of TiO_2, it can disperse uniformly and insert in the amorphous region of soy protein isolate (SPI), leading to a major interaction between both components; however, at high concentrations of TiO_2, it could cause agglomerations interfering with the organization and interaction of protein and TiO_2 [16].

Similar results were reported in a fish gelatin–chitosan film functionalized with TiO_2:Ag nanoparticles, where the improved antibacterial activity (*E. coli*, *S. aureus*, and *Botrytis cinerea*), optical, water-related, and mechanical properties were in a TiO_2:Ag dose-dependent response [3]. The addition of TiO_2:Ag-doped nanoparticles did not alter the typical structure of biopolymers, but instead promoted stronger intramolecular hydrogen bonds formation [16,85]. On the other hand, it has been reported that the improved UV-protective effects, water-related, and mechanical properties of a fish gelatin–agar–TiO_2 film could be negatively affected by a high concentration of TiO_2 (>0.5 g 100 mL^{-1}), mainly by an inhomogeneous dispersion and saturation of nanoparticles in the protein structure [21].

Additionally, Vejdan et al. [78] informed that a gelatin–agar bilayer film functionalized with TiO_2 nanoparticles effectively delays fish oil photo- and auto-oxidation up to 18 days. They reported that hybrid film containing 2% of TiO_2 could control fish oil oxidation due to the enhanced UV-protective and oxygen-barrier properties associated with the physicochemical characteristics of TiO_2.

According to the results, incorporation of TiO_2 into the gelatin-matrix improved its mechanical, thermal, UV-protective, gas-barrier, and water-related properties with antioxidant and antimicrobial performance, both desirable characteristics for the development of food and non-food packaging materials.

5.1.2. Biomedical Applications of Gelatin–TiO_2 Hybrid Composite

Gelatin–TiO_2 hybrid composites have been used for biomedical purposes. Lai et al. [80] immobilized gelatin onto TiO_2 nanotubes to modulate osteoblast behavior for orthopedic and dental applications. The authors found that cell spreading, proliferation, and differentiation of osteoblasts were improved by gelatin–TiO_2 hybrid material. They argued that extracellular matrix protein-based plays an important role in bone mineralization, while TiO_2 present in the hybrid matrix facilitates osteoblast differentiation. Ferreira et al. [71] fabricated a macroporous TiO_2–functional hydroxyapatite–gelatin scaffold loaded with multipotent adult progenitor cells for bone regeneration applications in calvaria defects. They informed that a hybrid scaffold promoted osteointegration and enhanced bone regeneration with complete closure defect. The result was associated with the ability of TiO_2 to form complexes with calcium ions, promoting the adsorption of calcium-binding extracellular matrix proteins and Argine-Glycine-Aspartate specific peptide sequences.

Additionally, hydroxyapatite–gelatin–graphene oxide composite deposited on TiO_2 nanotubes by electrochemical deposition exhibited excellent biocompatibility with MC3T3-E1 cells, promoting a better cellular integration [81]. Moreover, Urruela-Barrios et al. [79] mentioned that a sodium alginate–gelatin hydrogel 3D printing functionalized with nano-TiO_2 and β-tricalcium phosphate exhibited a potential use for tissue engineering application. The hybrid material fabricated with the micro-extrusion process, exhibited adequate porosity (pore size ranged from 150 to 240 μm), and mechanical resistance (13 MPa) to promote cell proliferation and cartilages.

Nikpasand and Reza-Parvizi [73] evaluated in vivo the wound dressing properties of a gelatin–TiO_2 hybrid hydrogel in an open and infected with *S. aureus* methicillin-resistant at 5×10^7 colony forming units (CFU) by excision-type wound-healing study in rats. They found that the hybrid composite exhibited a good wound-healing effect (wound area closure of 100% after 21 days), in comparison with gelatin-wound treatments (wound area closure of 71% after 21 days). Nonetheless, animals treated with the hybrid composite did not show wound infection by pathogenic bacteria after 14 days of evaluation and exhibited accelerated re-epithelization through fibroblast proliferation without inflammatory response after 21 days, which could be considered for wound therapies. On the other hand, Emregul et al. [70] developed a carboxymethyl cellulose–gelatin–TiO_2–superoxide dismutase biosensor supported in Pt surface for $O_2^{\bullet-}$ detection. They reported that the biopolymer blend (CMC and gelatin), provided a biocompatible environment for super oxide dismutase–TiO_2, which acts as a nanoscale electrode, enhancing the electron transfer rate through the Pt electrode. The hybrid sensor exhibited high analytical performance with a wide linear range of 1.5 nM to 2 mM, and high sensitivity and fast response time (1.8 s) for $O_2^{\bullet-}$ detection in healthy and cancerous brain tissue (coefficient of determination or R^2 of 0.991). In this context, functionalization of gelatin-based materials with TiO_2 exhibited potential biomedical applications, associated with its enhanced biological properties.

5.1.3. Other Applications of Gelatin–TiO_2 Hybrid Composite

Other investigated applications of the gelatin–TiO_2 hybrid composite include pharmaceutical (development of empty capsule shells), anti-corrosive material, and hydrogen storage. Hosokawa et al. [82] evaluated the application of UV-laser irradiation (at 355 nm) to print hard gelatin capsule shells with TiO_2, and it was found that hybrid capsules could be printed gray in a laser power-dependent response.

Additionally, Hayajneh et al. [83] studied the effect of gelatin–TiO_2 hybrid coating on the corrosion resistance of AISI 304 stainless steel, in a simulated marine environment (solution with NaCl at 3.5% *w*/*v*) through potentiodynamic polarization studies. The presence of hybrid coating improved the corrosion resistance of steel material (corrosion rate 2.63×10^{-3} mpy) in comparison with gelatin-coated (corrosion rate 10.10×10^{-3} mpy) and uncoated (corrosion rate 9.94×10^{-3} mpy) steel. The results were associated with the formation of a dense and stable network structure formed by the gelatin and TiO_2 nanoparticles.

Furthermore, Bin Liu et al. [84] used gelatin as a template to fabricate TiO_2 mesoporous microspheres for hydrogen production. They reported that the assistance of gelatin positively influenced the morphology and physicochemical characteristics of TiO_2 nanoparticles (surface area of $98.3\ m^2 \cdot g^{-1}$ and pore size of 11.9 nm), enhancing the hydrogen adsorption capacity and hydrogen storage performance of hybrid microspheres. However, its hydrogen adsorption mechanism remains unclear. According to these data, the gelatin–TiO_2 hybrid material exhibited pharmaceutical, anti-corrosive, and hydrogen production applications.

5.2. Whey Protein–TiO_2 Hybrid Composite

Whey protein is a by-product obtained from dairy processing during cheese production. It is used to develop edible films and coatings with good biodegradability and lower gas permeability for diverse applications [29]. However, the potential uses of whey protein-based materials are limited by their higher hydrophilicity due to polar residues outside the globular structure, which causes softening when they come in contact with high-moisture environments [86]. On the other hand, it exhibited good biocompatibility to interact with inorganic compounds like TiO_2 to improve its technological and functional properties [29]. The most common method for preparing whey protein–TiO_2 hybrid composites is evaporative casting. Furthermore, the nanoparticles used are commercially available with sizes ranging from 10 to 25 nm in its anatase phase and using concentrations ≤1% in weight of total solid content, as listed in Table 5.

Table 5. Effect of TiO_2 incorporation on whey protein matrix properties.

Application	Method/Presentation	* Composition	TiO_2 Specifications	Relevant Results	Ref.
Food and non-food packaging	Evaporative casting/Film	WPI (10% *w*/*w*)	Commercial (TiO_2): 0.25% *w*/*w* Size: 50–100 nm CP: Anatase Purity: >98.5%	TiO_2 improved the physicochemical properties of whey protein film.	[17]
Food and non-food packaging	Evaporative casting/Film	WPI (10% *w*/*v*), cellulose (1% *w*/*v*), glycerol (6% *w*/*v*), REO (2% *w*/*v*)	Commercial (TiO_2): 1% *w*/*v* Size: 10–25 nm CP: Anatase	Coated meat exhibited microbial stability during cold storage.	[28]
Food and non-food packaging	Evaporative casting/Film	WPI nanofibers (5% *w*/*v*), glycerol (4% *w*/*v*),	Commercial (TiO_2): 1% *w*/*w* Size: 20 nm Nanotubes Purity: >99%	The hybrid film effectively extends the shelf life of chilled meat.	[29]
Food and non-food packaging	Evaporative casting/Film	WPI (5% *w*/*v*), kefiran (5% *w*/*v*), glycerol (35% *w*/*w*)	Commercial (MMT-TiO_2): 1% *w*/*w* CP: Anatase	TiO_2 improved the physicochemical properties of kefiran–whey protein film.	[31]
Food and non-food packaging	Evaporative casting/Film	WPI (10% *w*/*v*), cellulose (1% *w*/*v*), glycerol (6% *w*/*v*), REO (2% *w*/*v*)	Commercial (TiO_2): 1% *w*/*v* Size: 10–25 nm CP: Anatase Purity: >99%	The hybrid film exhibited antimicrobial and antioxidant properties.	[86]
Food and non-food packaging	Evaporative casting/Film	WPI (5% *w*/*v*), TiO_2 (1% *w*/*w*), glycerol (5% *w*/*v*)	Commercial (TiO_2): 1% *w*/*w* Size: <20 nm CP: Anatase	TiO_2 improved the physicochemical properties of whey protein film.	[87]
Food and non-food packaging	Evaporative casting/Film	WPI (10% *w*/*v*), cellulose (1% *w*/*v*), glycerol (6% *w*/*v*), REO (2% *w*/*v*)	Commercial (TiO_2): 1% *w*/*v* Size: 10–25 nm CP: Anatase Purity: >99%	Meat treated with the hybrid film showed reduced lipid peroxidation during cold storage.	[88]
Food and non-food packaging	Evaporative casting/Film	Chitosan (1.5 g 50 mL^{-1} of acetic acid), WPI (0.5 g 50 mL^{-1} of water)	Commercial (TiO_2): 0.01 g CP: Anatase Crystalline structure	The hybrid film exhibited improved physicochemical properties.	[89]
Food and non-food packaging	Evaporative casting/Film	WPI (5% *w*/*v*), kefiran (5% *w*/*v*), glycerol (35% *w*/*w*)	Commercial (MMT-TiO_2): 1% *w*/*w* Size: 20 nm CP: Anatase	The hybrid film exhibited improved physicochemical properties.	[90]
Food and non-food packaging	Evaporative casting/Film	WPI (3% *w*/*v*), chitosan (10 g/L), ZMEO (1% *v*/*v*), glycerol (30% *w*/*w*)	Commercial (TiO_2): 2% *w*/*w* CP: Anatase-Rutile	The hybrid film exhibited antimicrobial activity.	[91]
Textile	Dip-pad-dry-cure process/Coating	WPI (3% *w*/*v*), cotton fabrics (200 g/m^2)	Commercial (TiO_2): 6% *w*/*w*	The hybrid coating exhibited improved antimicrobial activity.	[92]

* Material composition was based on the best-reported results. NI: No information; WPI: whey protein isolate; REO: rosemary essential oil; MMT: montmorillonite; ZMEO: *Zataria multiflora* essential oil.; SM: synthesis method; (TiO_2): concentration of titanium dioxide; CP: crystallite phase.

5.2.1. Food and Non-Food Packaging Applications of Whey Protein–TiO_2 Hybrid Composite

The potential use of whey protein–TiO_2 hybrid material for food packaging purposes has been investigated [28], as shown in Table 5. Zhou et al. [87] prepared a biodegradable whey protein film functionalized with TiO_2. It was found that technological properties such as UV-protective, mechanical, and water-resistance properties were improved in a TiO_2 dose-dependent response, associated with the intramolecular connections of protein and TiO_2 through covalent and non-covalent interactions. Moreover, the authors argued that at low concentrations of TiO_2, a reinforcement of whey protein–TiO_2 structure occurs. Meanwhile, self-assembly of TiO_2–TiO_2 interactions are detected at high TiO_2 concentrations, influencing its technological and functional properties, mainly associated with a reduction in the crystalline structure of TiO_2 by its incorporation in a polymeric matrix and its tendency to form agglomerates at higher concentrations [17,31]. Similar trends were informed in a kefiran–whey protein film functionalized with TiO_2, where an excessive amount of TiO_2 in the polymeric matrix affected its functionality because TiO_2 may act as an anti-plasticizer agent [31,90]. Moreover, in a combined chitosan–whey protein film reinforced with sodium laurate–TiO_2 nanoparticles. Zhang et al. [89] reported that sodium laurate-modified TiO_2 incorporation influenced the transparency, water vapor permeability, and mechanical and thermal properties of the hybrid film in a dose-dependent manner, and its intermolecular interaction with the available functional groups of the chitosan–whey protein matrix. Gohargani et al. [91] fabricated a chitosan–whey protein film, functionalized with TiO_2 and *Zataria multiflora* essential oil (ZMEO) nanoparticles with enhanced antimicrobial properties against foodborne pathogenic bacteria such as *L. monocytogenes*, *S. aureus*, and *E. coli.* Results were attributed to the synergistic effect of bioactive compounds present in the ZMEO and TiO_2 nanoparticles. Moreover, the TiO_2–ZMEO incorporation into the hybrid film, improved water vapor permeability, and tensile strength with a significant decrease in the film's transparency and color, associated with the physicochemical properties of TiO_2.

Alizadeh-Sani et al. [28] informed that a whey protein isolate–cellulose nanofiber-TiO_2–rosemary essential oil (REO) effectively preserved quality (microbial deterioration and sensory attributes) of refrigerated meat during cold storage. They reported that lamb meat treated with the hybrid film showed microbial stability (4.1 $\log\cdot CFU\cdot g^{-1}$ of viable cells) for 6 days at 4 °C storage without changes in sensory attributes (color, odor, texture, and overall acceptability). Moreover, the treated meat exhibited reduced lipid oxidation during storage, ascribed to antioxidant properties of REO (80% of radical scavenging) [88]. Furthermore, the TiO_2 (1% *w*/*w*) and REO (2% *w*/*w*) addition in the whey protein isolate/cellulose nanofiber hybrid film, improved mechanical (tensile strength, elongation at break, and elastic modulus) and water-related properties (moisture uptake, water solubility, and water vapor permeability), with a decrease in its transparency in a dose-dependent response in comparison with whey protein-based film, associated with the UV-scattering ability of TiO_2. Furthermore, the hybrid film showed an antimicrobial effect against foodborne bacteria (*E. coli* O157:H7, *L. monocytogenes*, *P. fluorescens*, and *S. enteritidis*) in a strain-dependent manner. It was associated with antimicrobial properties of TiO_2 and bioactive compounds (1,8-cineole, α-pinene, and camphor) in the REO; which can alter the cell membrane and finally cause cell death [86]. Nonetheless, they informed that a low content of TiO_2 migrated from the polymeric matrix to the meat product, under the Food and Drug Administration limit recommendations (<1% *w*/*w*) [88]. Similarly, Feng et al. [29] informed that a whey protein–TiO_2 hybrid film is effective in extending the shelf life of chilled meat (up to 15 days) without significant changes in its quality parameters (weight loss less than 7.87%, reduced lipid peroxidation, and microbial stability) during cold storage (4 °C). Moreover, the hybrid film exhibited enhanced mechanical, optical, and water-related properties associated with the physical and chemical interactions between carboxylic and sulfhydryl groups of some amino acids present in the protein matrix with TiO_2.

According to the evidence, the incorporation of TiO_2 into whey protein-based materials can improve the thermal, UV-barrier, mechanical, and water-related properties through physical and chemical interactions. Furthermore, whey protein films functionalized with TiO_2 exhibited antimicrobial properties for potential food and non-food packaging.

5.2.2. Other Applications of Whey Protein–TiO_2 Hybrid Composite

Ortelli et al. [92] fabricated a hybrid cotton fabric with anti-fire properties incorporating a whey protein–TiO_2 coating by the dip-pad-dry-cure process (Table 5). In general, the hybrid cotton material showed major durability (resistance to washing) and flame-resistant compared with the control group because TiO_2 acts as a physical reinforcement agent to fix whey protein to cotton fabrics in a stable way with the hydroxyl groups.

5.3. Collagen–TiO_2 Hybrid Composite

Collagen is a large, coherent, covalently crosslinked fibrillar network protein. Its main sources are porcine, bovine, and ovine with many applications in the food, cosmetics, pharmaceutical, and biomedical industries [34]. The disadvantages of collagen are poor thermal instability, poor mechanical properties, and the possible contamination by pathogenic bacteria and chemical substances [93]. Particularly, collagen has been combined with TiO_2 to improve its physicochemical properties [5]. Preparation of collagen–TiO_2 hybrid composites is usually by dip-coating, followed by freeze-drying for aerogel development. Furthermore, the nanoparticles used are commercially available or synthesized by the Sol–gel method with sizes ranging from 10 to 30 nm in its anatase phase, and in some cases in its rutile phase (Table 6).

Table 6. Effect of TiO_2 incorporation on collagen-based materials.

Application	Method/Presentation	* Composition	TiO_2 Specifications	Relevant Results	Ref.
Biomedical	Dip coating/Composite	Collagen-MWCNTs composite coated Ti incorporated with 20 μg/cm^2 of MWCNTs	Commercial (TiO_2): NI	The high roughness of hybrid materials improved cell proliferation.	[5]
Biomedical	Dip coating/NI	Volume ratio 1:1.5 GPTMS-TiO_2 solutions into a Collagen solution (3 mg·mL^{-1}) to cover the Mg alloys	SM: Sol–gel (TiO_2): NI TiO_2 with an amorphous structure	Protect alloy from corrosion, promote fibroblast proliferation.	[93]
Biomedical	NI/Film	Collagen (0.5 mg·m^{-1})	SM: Electrochemical deposition (TiO_2): NI TiO_2 with a crystalline structure	The hybrid film showed rapid cell adhesion and proliferation.	[94]
Biomedical	NI/Composite	Collagen (NI)	Commercial SM: Anodization (TiO_2): 0.3% *w/w* Size: 67 nm	Hybrid composite facilitated epithelial cell stretching and sheet formation.	[95]
Biomedical	Atomic layer deposition/Membrane	Collagen membrane (25 mm × 15 mm)	Commercial (TiO_2): NI	The hybrid membrane exhibited the proliferation of osteoblast.	[96]
Biomedical	NI/Composite	Mol ratio 1:1 of PdO–TiO_2 incorporated to g-PMMA–Collagen	SM: Sol–gel (TiO_2): NI Size: 8 nm CP: Anatase	TiO_2 incorporation improved thermal stability, mechanical strength, and enhancement of collagen.	[97]
Biomedical	Freeze-drying process/Aerogel	Collagen–PVP–TiO_2 1:20:0.5 mass ratio	SM: Sol–gel (TiO_2): NI Size: 24.4 nm CP: Anatase–Rutile	PVP improves the thermal stability and coercivity of the nanocomposite scaffold.	[34]
Biomedical	Freeze-drying process	Collagen–chitosan–TiO_2 1:1:0.1 mass ratio	SM: Sol–gel (TiO_2): NI Size: 20–30 nm CP: Anatase	TiO_2 improves mechanical properties, resistance to degradation, and antibacterial ability, and wound repair.	[27]
Non-food packing	NI/NI	Collagen (4 g 100 mL^{-1})	SM: Sol–gel (TiO_2): 2% *w/w* Size: 30 nm CP: Anatase	TiO_2 increases the thermal stability of collagen film improves and reduces UV light penetration, and solubility.	[98]

Table 6. *Cont.*

Application	Method/Presentation	* Composition	TiO_2 Specifications	Relevant Results	Ref.
Environmental remediation	Dip coating/NI	Collagen (template)	(TiO_2: Tb^{3+}): 2% *w*/*w* Size: 9.6 nm CP: Anatase	Collagen structure was preserved and photocatalytic performance of TiO_2 increased.	[99]
Electrochemical studies	Chemical reactions/NI	NI	SM: Template (TiO_2): NI Size: 10–20 nm CP: Anatase	Hybrid material showed excellent electrochemical lithium and sodium storage properties.	[100]

* Material composition was based on the best-reported results. NI: No information; MWCNTs: multiwalled carbon nanotubes; g-PMMA: poly(methylmethacrylate); GPTMS-TIP: (3-glycidoxypropyl)trimethoxysilane; PVP: poly(vinyl pyrrolidone); SM: synthesis method; (TiO_2): concentration of titanium dioxide; CP: crystallite phase.

5.3.1. Biomedical Applications of Collagen–TiO_2 Hybrid Composite

Table 6 lists, works on collagen-based materials functionalized with TiO_2 for biomedical applications. Park et al. [5] evaluated the effect of collagen-multi-walled carbon nanotubes (MWCNTs) composite coating deposited on titanium, using a dip-coating method on osteoblast growth. Cell proliferation studies confirmed a strong dependence of the extent of cell proliferation on the amount of MWCNTs incorporated in the composite in a dose-dependent response. Collagen–MWCNT–Ti showed higher cell proliferation than the collagen–MWCNT composite, where TiO_2 was responsible for cell proliferation. Truc et al. [94] studied the interaction between fibroblast and collagen modified on titanium (Ti) surface by electrochemical deposition (ECD), to reduce dental implant failure. They found that the Ti/Collagen hybrid composite showed rapid cell adhesion and proliferation.

Nojiri et al. [95] evaluated the establishment of perpendicularly oriented collagen attachments on TiO_2 nanotubes (TNT), which exhibited significant binding resistance, and the chemically linked collagen–TiO_2 facilitated epithelial cell stretching and sheet formation. Similarly, Bishal et al. [96] informed that collagen–TiO_2 promotes human osteoblast growth and proliferation in a dose-dependent manner with no inflammatory response detected, which was associated with the ability of TiO_2 to interact with calcium and phosphate elements, suggesting that this material could be used for applications in bone tissue engineering. On the other hand, Vedhanayagam et al. [97] informed that the poly(methyl methacrylate)–collagen–PdO–TiO_2 hybrid scaffolds did not show toxic effects on MG 63 cells (human osteosarcoma), and enhanced the alkaline phosphatase activity during in vitro osteogenic differentiation by the secretion of the osteogenic protein, leading to bone formation. Moreover, the hybrid scaffold exhibited higher thermal stability (83.45 °C), and mechanical strength (Young’s modulus 105.57 MPa) than the pure collagen scaffold (71.64 °C, 11.67 MPa, respectively), due to the chemical and physical interaction between collagen and Palladium oxide (PdO)–TiO_2.

Additionally, collagen–silane–TiO_2 has also been used as a functional agent of Mg alloys. The hybrid composite promotes the formation of a stable $Mg(OH)_2/MgCO_3/CaCO_3$ structure that effectively protects its corrosion. Moreover, the collagen–silane–TiO_2 improved osteoblasts and fibroblasts proliferation compared to bare and silane–TiO_2-coated alloys. In the long term, collagen–silane–TiO_2 is a viable strategy to prevent Mg alloy degradation due to the formation of a complex structure [93]. On the other hand, Li et al. [34] made 3D nanocomposite scaffolds composed of collagen, polyvinyl pyrrolidone (PVP), and TiO_2 nanoparticles, with good degradation resistance in PVP dose-dependent response for potential tissue engineering applications. Likewise, collagen–chitosan–TiO_2 scaffolds exhibited antimicrobial activity against *S. aureus* and improved permeability, stability to degradation, and cell aggregation to stop bleeding, which are suitable for the development of wound-healing materials [27].

Significant evidence shows that collagen functionalization with TiO_2 nanoparticles improved its biological properties for dental implants and bone and dermal regeneration.

5.3.2. Other Applications of Collagen–TiO_2 Hybrid Composite

Other researched applications of the collagen–TiO_2 hybrid composite include the development of packaging materials, catalysts, and electronics (Table 6). Erciyes et al. [98] proposed the use of leather solid wastes as a source of collagen hydrolyzed to make composites functionalized with TiO_2. The hybrid film exhibited improved water vapor permeability, water-solubility, elongation at break, and tensile strength. The authors highlighted the potential reuse of collagen-waste to develop packaging materials.

Additionally, Luo et al. [99] informed that collagen–TiO_2: Tb^{3+}-doped hybrid material exhibited excellent photocatalytic performance against methyl orange (93.87%) dye after 6 h of exposure in UV-light irradiation (150 W).

Furthermore, Cheng et al. [100] proposed a facile synthetic strategy to engineer a one-dimensional (1D) hierarchically ordered mesoporous TiO_2 nanofiber bundles (TBs) by using low-cost natural collagen fibers as a bio-template. In general, the hybrid structure can offer shortened ion diffusion paths, ensuring an efficient electrolyte penetration for ion access without affecting its structural integrity. They conclude that the hybrid materials had excellent electrochemical lithium and sodium storage properties.

In general, the collagen–TiO_2 hybrid material exhibited potential applications such as food and non-food packaging, environmental remediation, and electrochemical studies.

5.4. Soy Protein–TiO_2 Hybrid Composite

Soy protein isolate (SPI) is a by-product attained from the manufacture of soybean oil with a complex mixture of proteins (β-conglycinin and glycinin) with a minimum protein content of 90% on a moisture-free basis [101,102]. It is readily available, biodegradable, and biocompatible for edible coatings [19] with potential usage on food packaging [72,103]. However, the main disadvantages of SPI-based films include weak mechanical properties and high sensitivity to humidity [102,104]. In that sense, SPI films have been functionalized with TiO_2 to enhance their physical properties, where the most common method for its preparation is evaporative casting. Furthermore, the nanoparticles used are commercially available in its anatase phase, with concentrations ranging from 0.5% to 2% in weight of total solid content (Table 7).

Table 7. Effect of TiO_2 incorporation on soy protein isolate-based materials.

Application	Method/Presentation	* Composition	TiO_2 Specifications	Relevant Results	Ref.
Food packaging	Evaporative casting/Films	Soy protein isolate ($5\ g\ 100\ mL^{-1}$) glycerol (0.4 g)	Commercial (TiO_2): 1.5% *w*/*v* CP: Anatase	TiO_2 improved the physicochemical and antimicrobial properties of the soy protein isolate film.	[101]
Food packaging	Evaporative casting/Films	Soy protein isolate ($5\ g\ 100\ mL^{-1}$), sorbitol (20%), glycerol (10%)	Commercial (TiO_2): 2% *w*/*w*	The hybrid film exhibited improved mechanical properties.	[102]
Food packaging	Evaporative casting/ Films	Soy protein isolate (5%), glycerol (2%),	Commercial (TiO_2): 0.5% *w*/*w* Size: 15–30 nm CP: Anatase	TiO_2 improved the physicochemical and antimicrobial properties of the soy protein isolate film.	[19]
Food packaging	Evaporative casting/Films	Soy protein isolate ($4.5\ g\ 150\ mL^{-1}$), glycerol ($3.75\ g\ 150\ mL^{-1}$)	(TiO_2): 1.33% *w*/*w*	Hybrid composite effectively extended the shelf life of strawberries and antimicrobial activity.	[105]
Food packaging	Evaporative casting/ Films	Soy protein isolate (NI)	(TiO_2): NI	Grapes treated with hybrid films showed higher quality parameters than uncoated fruits.	[106]
Food and non-food packaging	Evaporative casting/Films	Soy protein isolate ($4.5\ g\ 150\ mL^{-1}$), glycerol (2%)	Commercial (TiO_2): 1.33% *w*/*w* TiO_2 with crystalline structure	The hybrid composite showed antimicrobial activity.	[23]

* Material composition was based on the best-reported results. NI: No information; SM: synthesis method; (TiO_2): concentration of titanium dioxide; CP: crystallite phase.

5.4.1. Food and Non-Food Packaging Applications of Collagen–TiO_2 Hybrid Composite

Table 7 lists the work on soy protein isolate–TiO_2 hybrid material for food and non-food packaging development with enhanced properties. Malathi et al. [102] informed that TiO_2 incorporation into an SPI film promotes an increase in thickness, opacity, tensile strength, and elongation at break of the cast film, which was associated with the hydrogen bonding or O–Ti–O bonding. Moreover, a strong charge and polar interaction between side chains of soy protein molecules restrict segment rotation and molecular mobility, leading to an increase in the elongation of the hybrid film. Furthermore, Lu et al. [101] reported that the functionalization of an SPI film with TiO_2 promoted a decrease in water vapor (from 5.43 to $4.62\ g{\cdot}mm{\cdot}m^{-2}{\cdot}day^{-1}{\cdot}kPa^{-1}$) and oxygen (from 0.470 to $0.110\ g{\cdot}cm^{-2}{\cdot}day^{-1}$) permeability, as well as an increase in tensile strength (from 6.6683 to 14.5642 MPa) in a TiO_2 concentration-dependent response. They argue that the presence of TiO_2 in protein structure significantly changes the hydrophilic nature of the film, due to the stable covalent (Si–O–C, Ti–O–C, and Si–O–Ti) and non-covalent (hydrogen bonds and Van der Waals forces) interactions between TiO_2 and SPI. Moreover, the hybrid film exhibited antimicrobial effects against *E. coli* (inhibition zone by agar test diffusion assay of 27.34 mm). Wang et al. [23] demonstrated the bactericidal efficiency of an SPI–TiO_2 hybrid film under UV-light (at 365 nm during two hours) against *E. coli* (reduction of 71.01% of viable cells) and *S. aureus* (reduction of 88.94% of viable cells), which was associated with the synergistic antimicrobial effect between TiO_2 and β-conglycinin and glycinin peptides present in the SPI [107].

Additionally, Wang et al. [19] informed that TiO_2 incorporation in an SPI film positively influences its tensile strength (90.79% higher than control). On the other hand, the addition of nano-TiO_2 reduced the flexibility (70.21% less than control), and water vapor (65.67% less than control), and oxygen (46.50% less than control) permeability in comparison with control groups. This was due to the strong hydrogen bonds formed between the two main components, which could prevent water and oxygen from diffusing through the films. The reduction in flexibility values could be associated with a collapse of the crystalline structure of the hybrid material by the formation of aggregates by an excess of TiO_2.

The reported application of SPI–TiO_2 hybrid film includes fruit preservation and water-dye degradation. Zhang et al. [105] reported that SPI–TiO_2 hybrid film was effective to extend the shelf life of strawberries stored at 4 °C up to 8 days without significant weight losses (<17.3%) and color changes with stable microbial quality in comparison with the uncoated fruits. Similar trends were reported in grapes coated with an SPI–TiO_2 hybrid film by Hoseiniyan et al. [95], who reported that coated grapes exhibited good performance during cold storage (31 days at 4 °C) without significant effects in the total soluble solids, titratable acidity, and weight losses. The hybrid film prevents the fungal infection of the fruits, and the coated fruits also had a good appearance and marketability compared with the uncoated fruits.

In summary, the incorporation of TiO_2 into SPI significantly improved its physicochemical properties and exhibited good fruit preservation performance.

5.4.2. Other Applications of Soy Protein Isolate–TiO_2 Hybrid Composite

Calza et al. [108] fabricated a system composed of soybean peroxidase and TiO_2 nanoparticles for environmental remediation purposes (Table 7). They informed that the hybrid material effectively remove orange II dye (100%) and carbamazepine (100%) drug from aqueous solutions after 60 min of exposure compared with the soybean peroxidase structure (<80% and <10%, respectively, after 120 min of exposure), which was associated with the synergistic properties of peroxidase and TiO_2. Further studies are needed to understand the removal and degradation mechanism of soybean peroxidase–TiO_2, which could be used as an alternative for wastewater treatment.

5.5. Other Proteins Functionalized with TiO_2

Table 8 lists various non-conventional proteins functionalized with TiO_2, such as zein, keratin, sodium caseinate, lactoferrin, and sesame, to enhance their physicochemical properties, where the most common method for their preparation is evaporative casting for films and freeze-drying for hydrogels and scaffolds. Furthermore, the nanoparticles used are commercially available with sizes ranging from 10 to 200 nm in its anatase phase, and in some cases in its rutile phase, using concentrations ranging from 0.5% to 10% in weight of total solid content.

Table 8. Effect of TiO_2 incorporation on non-conventional protein-based materials.

Application	Method/Presentation	* Composition	TiO_2 Specifications	Relevant Results	Ref.
Food and non-food packaging	Evaporative casting/Film	Zein (13.5% *w*/*v*), glycerol:PEG 600 (3.3% *w*/*w*)	Commercial SM: Hydrothermal (TiO_2:SiO_2): 1.5% *w*/*v* Size: 100–180 nm	TiO_2 improved the mechanical, thermal, and water-related properties of zein film.	[24]
Food packaging	Evaporative casting/Nanofibers	Zein (3 g 10 mL^{-1} of 70% aqueous ethanol)	Commercial (TiO_2:SiO_2): 5% *w*/*w* Size: <25 nm CP: Anatase Purity: 99.7%	Coated fruits extend their shelf life.	[33]
Food and non-food packaging	Evaporative casting/Film	Zein: sodium alginate (90:10), betanin (1%)	Commercial (TiO_2): 0.5% *w*/*w* Size: 10–25 nm	The hybrid film exhibited antimicrobial activity.	[109]
Food and non-food packaging	Evaporative casting/Film	Sodium caseinate (8 g 100 mL^{-1}), guar gum (0.3% *w*/*w*), CEO (2% *w*/*w*)	Commercial (TiO_2): 1% *w*/*w* Size: 10–25 nm CP: Anatase Purity: >99%	The hybrid film exhibited antimicrobial activity.	[11]
Food and non-food packaging	Evaporative casting/Film	Sodium caseinate (2.5% *w*/*w*), glycerol (2% *w*/*w*)	Commercial (P25) (TiO_2): 0.5% *w*/*w*	TiO_2 improved the mechanical, thermal, and water-related properties of the film.	[30]

Table 8. *Cont.*

Application	Method/Presentation	* Composition	TiO_2 Specifications	Relevant Results	Ref.
Food and non-food Packaging	Evaporative casting/Films	Feather keratin (1.2 g), PVA (13.33 g)	Commercial (P25) (TiO_2): 3% *w*/*w* Size: 60 nm CP: Anatase Purity: 99.8%	The hybrid material exhibited improved physicochemical properties.	[110]
Food and non-food Packaging	Catalyst curing/Composite	Raw wool keratin (350 g/m^2), BTCA (12.6%)	Commercial (P25) (TiO_2): 0.6 $g{\cdot}L^{-1}$ Size: 21 nm CP: Anatase-Rutile Crystalline structure	The hybrid material showed an improved UV-protective effect.	[22]
Environmental remediation	Evaporative casting/Film	Sesame protein (3 g 100 mL^{-1}), glycerol (30% in total solid content)	Commercial (P25) (TiO_2): 3% *w*/*w* Size: 21 nm CP: Anatase-Rutile Crystalline structure	The hybrid film exhibited photocatalytic activity against methylene blue.	[16]
Environmental remediation	Hydrogel synthesis/Hydrogel	Keratin (1% *w*/*v*)	Commercial (P25) (TiO_2): 10 *w*/*w* CP: Anatase-Rutile	Hybrid hydrogel effectively removes trimethoprim from wastewater.	[111]
Environmental remediation	Electrospinning/ Nanofibers	Keratin–PLA–TiO_2 mass ratio of 33:33:33	Commercial (P25) CP: Anatase	The hybrid nanofibers effectively remove methylene blue dye from the aqueous solution.	[112]
Environmental remediation	Biometic/Microspheres	NI	Anatase	The hybrid composite showed good photocatalytic properties again or dye yellow and blue acid dyes.	[113]
Biomedical	Freeze dried/Scaffolds	Silk fibroin (2% *w*/*v*), F (2% *v*/*v*)	Commercial (P25) (TiO_2): 15 *w*/*w*	SF–TiO_2:F exhibited biocompatibility and improved mechanical properties.	[114]
Biomedical	Freeze-dried/Scaffolds	Silk fibroin (2.5% *w*/*v*), chitin (2.5% *w*/*v*), glutaraldehyde (0.25% *v*/*v*)	Commercial (P25) (TiO_2): 1.5% *w*/*w* Size: 10–15 nm CP: Anatase Purity: >99%	Hybrid material exhibited antimicrobial activity, also it is biocompatible and biodegradable.	[115]
Biomedical	Dip-coating/Coating	Lactoferrin (0.2 $mg{\cdot}mL^{-1}$), collagen (0.2 $mg{\cdot}mL^{-1}$)	SM: Sol-gel (TiO_2): NI Size: 200 nm CP: Anatase Crystalline structure	The hybrid coating showed enhanced biocompatibility with MG-6e cells.	[116]

* Material composition was based on the best-reported results. NI: No information; CEO: cumin essential oil; PVA: polyvinyl alcohol; PEG: polyethylene glycol; BTCA: 1,2,3,4-butane tetracarboxylic acid; PLA: poly(Lactic acid); SM: synthesis method; (TiO_2): concentration of titanium dioxide; CP: crystallite phase.

5.5.1. Packaging Applications of Non-Conventional Proteins Functionalized with TiO_2

Table 8 lists reports on the use of non-conventional protein materials functionalized with TiO_2 for food and non-food packaging development. Kadam et al. [24] evaluated the effect of TiO_2:SiO_2 nanoparticles incorporation on the thermal and mechanical properties of a cast zein film. They reported that mechanical properties (tensile strength) of the hybrid film were enhanced; however, its flexibility was reduced two-fold compared with zein film, possibly associated with the formation of TiO_2 aggregates. Furthermore, the water contact angle, water vapor permeability, and thermal properties of the hybrid film were improved by the addition of inorganic nanoparticles, associated with the interaction between zein and TiO_2:SiO_2, which promotes a stable and strong hydrogen bonds formation. Similarly, Amjadi et al. [109] made zein–sodium alginate (90:10) film functionalized with TiO_2–betanin (0.5%:1%) nanoparticles and informed that the hybrid film exhibited antioxidant properties (by the presence of bioactive compounds in betanin) and high antimicrobial effects (by agar test diffusion assay) against *E. coli* (15.4 mm of inhibition zone) and *S. aureus* (16.9 mm of inhibition zone), which was attributed to the antimicrobial properties of TiO_2. Moreover, Böhmer-Maas et al. [33] developed a zein–TiO_2 nanofiber as an ethylene absorber for cherry tomatoes preservation (25 °C). They reported that coated fruits with the hybrid film exhibited less ethylene concentration (9.38 $\mu g \cdot L^{-1} \cdot g^{-1} \cdot h^{-1}$) than those coated with a zein film (10.27 $\mu g \cdot L^{-1} \cdot g^{-1} \cdot h^{-1}$), which permits extended the shelf life of cherry tomatoes up to 22 days. According to the authors, the ethylene degradation occurs by the oxidation of ethylene into CO_2 and water by the OH radicals and reactive oxygen species generated by the photocatalytic ability of TiO_2.

Montes-de-Oca-Ávalos et al. [30] investigated the effect of TiO_2 incorporation on the physicochemical properties of a sodium caseinate film. They informed that mechanical, thermal, water vapor permeability characteristics of the caseinate film were improved in a TiO_2 concentration-dependent way, associated with good dispersion of TiO_2 through the film polymeric matrix. According to the authors, the presence of TiO_2 avoids protein agglomeration due to the stable hydrogen bond formation. Additionally, Alizadeh-Sani et al. [11] informed that a sodium caseinate–guar gum film functionalized with TiO_2 (1% *w*/*w*) and cumin essential oil (2% *w*/*w*) showed remarkable antimicrobial activity against *L. monocytogenes* (16 mm of inhibition zone), *S. aureus* (15 mm of inhibition zone), *E. coli* O157:H7 (14 mm of inhibition zone), *S. enteritidis* (12 mm of inhibition zone) in a strain-dependent manner. These results were associated with the cell wall differences between bacteria (outer membrane) and the synergistic antimicrobial effect among TiO_2 and cumin essential oil. Moreover, the water vapor permeability, tensile strength, and flexibility of the combined film were improved by a synergistic effect of TiO_2 and cumin essential oil.

Additionally, Montazer et al. [22] informed that the incorporation of TiO_2 in a wool keratin film stabilized by butane tetracarboxylic acid (BTCA) exhibited excellent UV-barrier properties related to the C–N and N–H bonds promoted for TiO_2 and BTCA interactions, with an optimum concentration of 0.6 $g \cdot L^{-1}$ and 12.94% *w*/*v*. Similarly, Wu et al. [110], who informed that thermal stability, mechanical resistance, and water vapor permeability of the keratin–tris film were improved by its functionalization with TiO_2 that may act as a physical cross-linker agent.

According to evidence, functionalization of non-conventional proteins like zein, keratin, and sodium caseinate with TiO_2 nanoparticles exhibited interesting properties for food and non-food packaging development.

5.5.2. Environmental Applications of Non-Conventional Proteins Functionalized with TiO_2

Usage of zein, keratin, and sesame proteins as a supporting material of TiO_2 for the removal and degradation of water pollutants have been explored (Table 8). Babitha and Korrapati [113] made mesoporous microspheres formed by zein and TiO_2 as an alternative for acid yellow (AY110) and acid blue (AB113) dyes decolorization under UV-light irradiation. They reported that the hybrid microspheres (1 $mg \cdot mL^{-1}$) showed a dye removal efficiency of 96% and 89% in AY110 and AB113, respectively, at lower dye concentration (10 $mg \cdot L^{-1}$) but decreased at higher concentrations (100 $mg \cdot L^{-1}$), which was associated with the saturation of active sites into the hybrid matrix.

Additionally, Villanueva et al. [111] fabricated a hydrogel combining keratin (from cow's horn) and TiO_2 to remove trimethoprim from wastewater. They reported that the hybrid material exhibited good degradation efficiency (>95%) against antibiotic removal from aqueous solution in a TiO_2 dose-dependent response, with an optimum TiO_2 concentration of 10% *w*/*w* with performance up to four consecutive cycles (90%). It was associated with the swelling and adsorptive abilities of the hybrid film and to the presence of active sites on the catalyst surface due to the strong attachment between keratin and TiO_2 through covalent and non-covalent interactions. Moreover, Siriorn and Jatuphorn [112] reported that a chicken feather keratin–poly(lactic acid)–TiO_2 nanofibers (0.05 g) effectively remove methylene blue (90%) dye from aqueous solution (5×10^{-6} M) under visible light due to the improved adsorptive properties of the hybrid nanofibers.

Fathi et al. [16] made a sesame protein isolate film functionalized with TiO_2 for water-dye removal purposes. They reported that the hybrid film (64 cm^2) effectively degraded 76% of methylene blue dye

(10 mg·mL^{-1}) under UV-light irradiation after 120 min of exposure. Moreover, the hybrid material exhibited enhanced water vapor permeability, water resistance, water contact angle, and mechanical strength in a TiO_2 dose-dependent response with an optimum TiO_2 concentration of 3% *w*/*w* associated with the interaction chemical and physical interactions between sesame protein and TiO_2. On the other hand, the morphological studies through scanning electron microscopy revealed that a high concentration of TiO_2 exhibited an inhomogeneous dispersion, causing aggregations in the protein matrix that negatively affects its functionality.

To summarize, non-conventional proteins like zein, keratin, and sesame functionalized with TiO_2 nanoparticles could be a viable, low-cost, and efficient alternative for environmental applications as photocatalysts for wastewater treatment.

5.5.3. Other Applications of Non-Conventional Proteins Functionalized with TiO_2

Other potential uses of non-conventional proteins functionalized with TiO_2 include bone regeneration, antimicrobial activity, and textiles (Table 8). Johari et al. [114] made a fluorated silk fibroin–TiO_2 hybrid scaffold for bone tissue engineering with non-toxic effects in human osteoblast cells (SaOS-2) and suitable cell attachment and spreading on the hybrid material, which was associated with the fluoridation of TiO_2 nanoparticles (TiO_2–F). Moreover, the hybrid scaffold exhibited good porosity (200 to 500 μm), mechanical resistance (tensile strength of 1.7 MPa), and adequate biodegradation rate (from 1% to 5% of weight loss in 30 days) in a TiO_2 dose-dependent response due to the formation of Ti–O–C bonds and the partial substitution of OH groups present in the TiO_2 surface by fluorine anions, that significantly increase the functional properties of TiO_2. On the other hand, with high amounts of TiO_2 (>15%), some agglomerates could appear that negatively affect the technological properties of the hybrid scaffold.

Mehrabani et al. [115] informed that a chitin–fibroin–TiO_2 hybrid composite did not show cytotoxic effects on a human Caucasian fetal foreskin fibroblast cell line at low TiO_2 concentrations (<1.5% *w*/*w*). Nonetheless, the hybrid material exhibited a porosity of 94%, a density of 3118 mg·mL^{-1}, and water resistance with a swelling degree of 93% after 24 h. In addition, it showed antimicrobial properties against *E. coli*, *S. aureus*, and *C. albicans*, which are suitable for the development of wound-healing materials. According to Feng et al. [117], incorporation of TiO_2 into fibroin (mostly α-helix) matrix promotes structural changes that permit a strong interaction with the β-sheets changing from typical silk I to Silk II structure in a TiO_2-dependent manner, attributed to the presence of hydroxyl groups on the TiO_2. The enhanced properties of fibroin could be related to the conformational structure. On the other hand, the authors reported that a high concentration of TiO_2 might negatively affect the mechanical properties of the hybrid material associated with the damage of its microscopic structure mainly by the formation of TiO_2 agglomerates, and possibly to the extra water used for the preparation of the hybrid material.

Kazek-Kesik et al. [116] coated a lactoferrin–collagen composite on titanium alloys for bone replacement. It was found that the presence of lactoferrin and TiO_2 enhanced osteoblast-like effect on MG-63 cells after seven days of evaluation in comparison with collagen-treated cells, mainly by the ability of both components to promote cell adhesion.

According to the evidence, the functionalization of non-conventional proteins with TiO_2 nanoparticles exhibited interesting properties and applications. However, further studies are needed to validate their potential uses.

6. Disadvantages of Protein–TiO_2 Hybrid Composites and Perspectives

Despite the observation that protein–TiO_2 hybrid composites exhibited excellent technological and functional properties with great potential to be used in several applications, it is necessary to evaluate the safe use and implementation of this kind of hybrid composites, mainly due to the presence of TiO_2 in their composition.

In this context, it has been reported that pure TiO_2 exhibited toxicological and adverse effects in cell lines (HeLa and HaCaT), proteins (microtubule and bovine serum albumin), and animal models (Sprague–Dawley rats, Wistar rats, and mussel *Mytilus coruscus*) in a concentration-dependent response, typically at doses ranging from 0.4 to 100 $mg \cdot mL^{-1}$ with direct application [118–123]. Nonetheless, the tested concentrations of TiO_2 in these works were higher than the recommended safe usage (<1% by weight) by international regulations in the use of TiO_2 as a food additive [124].

However, the amount of TiO_2 used as a functional agent to develop protein–TiO_2 hybrid composites ranges from 0.003 to 1 $mg \cdot mL^{-1}$, depending on its application. For example: in food packaging materials manufacturing, the amount average of TiO_2 employed is 0.28 $mg \cdot mL^{-1}$, while for packaging materials with non-food purposes it is 0.85 $mg \cdot mL^{-1}$. Moreover, for the development of scaffolds, dental implants, and wound-healing materials, the average amount of TiO_2 is 0.23 and 0.9 $mg \cdot mL^{-1}$ for making hybrid materials for environmental remediation.

According to Xu et al. (2017) [123], the interaction between protein structure and TiO_2 plays a critical role in the safe use of these materials, which usually depends on the new properties of each hybrid composite and the used concentration of TiO_2 [125]. In this sense, there are a few reports on the toxicity status of protein–TiO_2 hybrid composites, which reported no toxicological or adverse effects on their use, associated with the low concentration of TiO_2 used for the functionalization of protein-based materials. However, most of the published reports cited in this document focused on in vitro evaluations. Therefore, further studies are needed to evaluate the possible human health and environmental risks on the usage of these hybrid composites.

7. Concluding Remarks

Significant evidence indicates that functionalization of protein-based materials by adding TiO_2 nanoparticles is a feasible approach to improve their thermal, mechanical, optical, water-resistance, gas-barrier, and adsorptive properties. The evaporative casting method is one of the most common procedures for the preparation of protein–TiO_2 hybrid films and coating and freeze-drying for hydrogels and scaffolds, using commercial TiO_2 with a particle size ranging from 10 to 200 nm (the most frequently used is 10–25 nm in size) in its anatase phase with a crystalline structure.

Protein–TiO_2 hybrid composites are an active research area for developing eco-friendly and active food and non-food packaging materials with antimicrobial and UV-protective effects. Furthermore, they are attractive and biocompatible materials to fabricate wound-healing patches, tissue engineering scaffolds, or biosensors for biomedical applications.

On the other hand, although the functionalization of protein-based materials with TiO_2 offers significant advantages, some limitations have been reported, especially those associated with the concentration of TiO_2. Higher concentrations of TiO_2 could promote an inhomogeneous dispersion through the polymeric matrix, forming agglomerates that negatively affected the technological and functional properties of the hybrid material, particularly in flexibility and transparency. Likewise, the preparation method could negatively influence the properties of the hybrid material, associated with the physical and chemical interactions between components. For example, if there was no proper mixing ratio between protein and TiO_2, a saturation of the available functional groups in the polymeric matrix can affect the physicochemical properties of the film. Additionally, other possible limitations of the protein–TiO_2 hybrid composites could be related to the type and source of protein and its possible structural changes by the presence of TiO_2 and its stability for diverse applications.

There are some challenges to be achieved for industrial applications; one of the most important is to obtain the correct amounts of protein and TiO_2 nanoparticles because different uses require different formulations with desirable properties. For example, the shelf life of climacteric fruits depends on the correct exchange of oxygen, carbon dioxide, and water vapor permeability. Meanwhile, products with high amounts of lipids require UV-protective effects to prevent their oxidation. On the other hand, wound-healing materials should exhibit high water and mechanical resistance but correct gas exchange, high adherence, and antimicrobial properties. Moreover, standardized protocols for their preparation are needed for industrial-scale implementation. It is also necessary to carry out in vivo tests to evaluate the possible human health and environmental risks on the usage and safe implementation of these hybrid composites in diverse applications. Therefore, further research efforts should be dedicated to solving these challenges.

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