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Sustainability spotlight

Food packaging is essential to prevent physical, chemical, and biological deterioration of food products. However, the widespread use of plastic packaging, coupled with inadequate management after consumption, has led to an unprecedented environmental issue affecting both terrestrial and aquatic ecosystems. Polyhydroxyalkanoates (PHAs) are bio-based, biodegradable polymers with good barrier properties and great potential to replace conventional plastics in food packaging applications. These biopolymers can also be used to obtain active materials designed to extend the shelf life of packaged foods, thereby reducing food waste. This review aims to summarise recent advances in PHA-based active food packaging, analysing the influence of active compounds on the material properties, the preservative efficacy, and biodegradability after the end of service life. The development and utilisation of these materials are aligned with several United Nations Sustainable Development Goals (SDGs), including SDG 2 (Zero Hunger), SDG 12 (Responsible Consumption and Production), SDG 13 (Climate Action), SDG 14 (Life Below Water), and SDG 15 (Life on Land).

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Biodegradable and active materials based on PHBV for sustainable food packaging

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Abstract

Polyhydroxyalkanoates (PHAs) are sustainable biopolymers that offer biodegradability, biocompatibility, and a renewable origin, making them a promising alternative to petrochemical plastics in food packaging. Their environmental benefits, combined with their functional versatility, make them ideal candidates to replace conventional materials that contribute to plastic pollution. Among them, poly(3-hydroxybutyrate) (PHB) and its copolymer with hydroxyvalerate, poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV), are the most studied PHAs for food packaging applications. Both have excellent water vapour and oxygen barrier properties, and are biodegradable under aerobic and anaerobic conditions in different ecosystems. However, their high crystallinity and low melt viscosity make them difficult to process for packaging applications, so different strategies have been used to improve their properties. Various studies have analysed the potential of PHBs to obtain active packaging materials to extend the shelf life of food by incorporating different bioactive compounds with antioxidant and/or antimicrobial properties. Some of the bioactive compounds have been extracted from natural sources, such as plants and agri-food waste, thus fulfilling two objectives: waste recovery and the use of natural preservatives. This comprehensive review provides an analysis of recent studies on PHB-based active materials for food packaging systems and the impact of incorporated bioactive compounds on material properties, their ability to extend the shelf life of certain foods, and the biodegradation pattern of the materials. It compiles information on how active compounds provide enhanced functionality to PHB materials, such as antioxidant and/or antimicrobial activity, while other characteristics relevant to food packaging, such as mechanical and barrier performance, may also be affected.

Keywords: PHAs; PHB; Biodegradable polymers; Active packaging; Active compounds; Natural active extracts; Sustainable packaging.

1. Introduction

1.1. Plastics

Since the 1950s, plastics from fossil sources have become a fundamental part of our daily lives. Their versatility, durability, flexibility and low production costs have made plastics indispensable across numerous sectors, with applications in a wide range of products such as packaging, furniture, toys and electronics. Global plastic production has grown exponentially, surpassing 413 million tons in 2023. The synthesis of these products depends on non-renewable resources, particularly compounds derived from fossil fuels, such as crude oil, and is associated with substantial greenhouse gas emissions, which cause an increase in the global temperature of the planet[1]. Despite ongoing efforts to reduce reliance on petrochemical-based materials, projections

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suggest that global plastic consumption could reach around 900 million tons by 2050[2]. The widespread use of plastics poses significant environmental challenges, particularly in waste management. In 2020, 24% of plastics were incinerated, 39% were landfilled, and 22% were recycled, while 15% were managed improperly[3,4], highlighting a critical inefficiency in global waste management systems and underscoring the urgent need for alternative materials and circular economy strategies. The massive manufacture and use of these materials, especially in single-use packaging, coupled with their poor post-consumer management, have led to serious pollution problems in all ecosystems: terrestrial, marine and atmospheric[5,6]. Due to its highly recalcitrant nature, the polymer is predicted to persist in the environment for nearly 400 years before undergoing significant degradation. In addition, they also harm biodiversity and ecosystems and have entered the food chain[7–10]. So much so that microplastics have been found in species that contribute to our food supply and even in humans themselves, which may be linked to health problems[6,11–13].

Numerous efforts have tried to address these problems by offering different alternatives to conventional plastics management or use. Reduction, reuse and recycling of traditional plastics have long been the most widely used strategies[14–16]. Reducing the demand for plastics is the best way to avoid the problem, but in some cases, such as food packaging, plastic packaging is necessary to ensure food quality and safety throughout the supply chain, as well as to extend the shelf life of the packaged food products[17]. Reuse and recycling are also essential for reducing the production of new plastics, but they can also pose risks due to the release of hazardous chemicals during these processes[18]. Furthermore, the recycling of multilayer packaging, typically used in food packaging, poses a significant challenge due to the complex composition of the material. As a result, many of these materials cannot be recycled using traditional mechanical methods and end up being incinerated or landfilled, leading to further pollution[19,20].

1.2. Bioplastics

The development of new materials to counteract these problems has become essential. Bioplastics play a crucial role in offering alternatives to traditional plastics due to their renewable origin and/or their ability to biodegrade in different media[21]. These materials can be bio-based, biodegradable or both. Figure 1 shows the classification of plastics depending on their origin and biodegradable nature. The production of bioplastics is still limited, but the global production capacity is set to increase significantly from around 2.47 million tonnes in 2024 to approximately 5.73 million tonnes predicted for 2029[22]. Due to a strong development of biobased and biodegradable polymers, such as polylactic acid (PLA) and polyhydroxyalkanoates (PHA), biobased polyethylene (PE), as well as a steady growth of biobased polypropylene (PP), the production capacities will continue to increase significantly within the next 5 years[22].

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Figure 1. Classification of plastic materials according to their source of origin and their biodegradation capability, based on data reported by the Institute for Bioplastics and Biocomposites (ifBB)[23] and European Bioplastics (EUBP)[22].

Despite ongoing advances in biopolymer research, significant challenges remain, particularly in adapting mechanical or barrier performance to the requirements of their application. Specifically, for food packaging applications, many biopolymers have poor mechanical properties (such as brittleness or excessive rigidity) or exhibit insufficient barrier performance. For instance, polylactic acid (PLA) and polycaprolactone (PCL) typically demonstrate good water vapour barrier properties but high oxygen permeability. In contrast, starch-based materials exhibit better oxygen barrier performance but are water sensitive and more permeable to water vapour. To address these limitations, different strategies have been used, such as producing multilayer systems (laminates) combining biopolymers with complementary properties or obtaining polymer blends or composites to create monolayer materials with modulated properties of the blend matrix [24–26].

1.3. Polyhydroxybutyrate (PHB): a member of the Polyhydroxyalkanoates family

Within the group of bio-based and biodegradable materials, PHAs are of particular interest. These polymers were first discovered in 1926 by the French microbiologist Lemoigne[27]. Later, in 1958, scientists Macrae and Wilkinson identified the role of PHAs as energy storage compounds within microbial cells. However, it was not until the 1980s that the commercial potential of these biopolymers was realised. During this period, Imperial Chemical Industries (ICI) introduced a PHA-based product known as BIOPOL, which consisted specifically of the copolymer poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV). The distribution of BIOPOL was initially managed by Monsanto and subsequently by Metabolix, marking the beginning of industrial interest in PHAs for commercial applications. Nowadays, PHAs are biopolymers synthesised by various microorganisms, including bacteria and archaea, under nutrient-limited conditions: when carbon sources are in excess, while essential nutrients such as nitrogen, phosphorus, or oxygen are scarce. Under these conditions, microorganisms

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accumulate PHAs as intracellular granules, serving as reserves of carbon and energy, from which these can be later extracted. Due to their biocompatibility and non-toxic nature, PHAs are increasingly explored in applications related to food, cosmetics, drug delivery and human health[28–32]. These materials also exhibit resistance to UV radiation and are insoluble in water but are soluble in chloroform and other chlorinated hydrocarbons. A wide diversity of these aliphatic polyesters exists, depending on the microbial strain and the type of carbon source used[33,34]. PHAs are typically classified according to chain length (number of carbon atoms in each monomer unit), as shown in Figure 2. They are generally grouped into short-chain-length (scl: 3–5 carbon atoms), medium-chain-length (mcl: 6–14 carbon atoms), and long-chain-length (lcl: more than 15 carbon atoms) PHAs. The structural diversity of monomers (over 150 types) imparts specific chemical and physical properties, such as crystallinity, melting and degradation temperatures, and viscosity, to the corresponding polymers[35,36]. Scl monomers are the most abundant. These have greater crystallinity and are produced by bacteria such as *Cupriavidus* sp., *Azotobacter* sp., *Halomonas* sp. and *Bacillus* sp. or *Pseudomonas* sp[37–40]. As the monomer chain lengthens, the crystallinity of the polymer decreases, giving rise to less rigid materials. Examples of bacteria that produce mcl-PHAs include *Pseudomonas* sp. and *Burkholderia* sp. Nowadays, work is underway on genetic modifications of PHA-producing bacteria to increase polymer yield and/or modify chain length, thereby modulating their properties[40,41].

PHA production research is currently focused on: 1) the use of inexpensive raw materials, such as agri-food waste, to reduce the cost of biopolymers[42–44], 2) the generation of new biomass from CO_2 and H_2 for PHA biosynthesis by cyanobacteria or wild-type and engineered “Knallgas” bacteria[45,46], 3) the use of robust extremophilic PHA-producing strains[43,44,47,48], 4) the development of novel tools for the rapid *in situ* determination of PHA in photobioreactors, 5) optimisation of bioreactor design and modelling of the dynamics of PHA production; and (6) the development of sustainable and efficient PHA recovery methods from biomass using supercritical water or natural deep eutectic solvents (NADES)[49]. Recent studies[42] are also being conducted on the separation and purification process of PHAs, to mitigate the problems associated with the use of toxic solvents such as chloroform[50].

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R-	n=1 Poly(3-hydroxy-	n=2 Poly(4-hydroxy-	n=3 Poly(5-hydroxy-	n=4 Poly(6-hydroxy-
H	-propionate)	-butirate)	-valerate)	-hexanoate) o PCL
CH_3	-butyrate)	-valerate)	-hexanoate	
CH_2-CH_3	-valerate)	-hexanoate)	-heptanoate)	
$(CH_2)_2$-CH_3	-hexanoate)	-heptanoate)		
$(CH_2)_3$-CH_3	-heptanoate)	-octanoate)		
$(CH_2)_4$-CH_3	-octanate)			
$(CH_2)_5$-CH_3	-nonanoate)			-dodecanoate)
$(CH_2)_6$-CH_3	-decanoate)			
$(CH_2)_7$-CH_3	-undecanoate)			
$(CH_2)_8$-CH_3	-dodecanoate)			
$(CH_2)_9$-CH_3	-dodecanoate)			
$(CH_2)_{10}$-CH_3	-tridecanoate)			

Figure 2. Characteristic molecular structure and names of typical PHAs monomers, depending on the n values and R; m: 100-30000.

Polyhydroxybutyrate (PHB), a scl member of the PHA family, is one of the most studied for packaging uses due to its specific properties. Its biosynthesis yield is strongly influenced by the nature of the carbon substrate used, making the selection of feedstock a key parameter in ensuring the environmental and economic viability of PHB production[51]. In industrial-scale operations, various substrates (including both food-grade crops and alternative biomasses or waste cooking oil) can be used to cultivate PHB-producing microbial strains[52]. For instance, the production of 1 Mt of PHB may require approximately 4.63 Mt of corn or, as much as, 22.00 Mt of sugarcane[23]. However, these figures must also be contextualised by considering the environmental footprint associated with the agricultural and pre-processing stages. In response to sustainability concerns and the cost of the process, recent research has explored the valorisation of food industry by-products as cost-effective and non-competitive raw materials. This approach not only reduces reliance on edible biomass but also promotes waste valorisation, contributing to a circular bioeconomy[53–57].

Among PHAs, only a few are commercially available, although the market trend for these materials is increasing. These include PHB, PHBV and PHBH. Furthermore, PHBs are used industrially in food packaging, but their adoption is limited due to material stiffness and high production costs. Nevertheless, PHBV is actively being developed and validated for industrial packaging use, which would also include food packaging, and the strong industrial and EU support indicates growing commercial adoption [31,58–61].

Although PHB exhibits good gas and water vapour barrier capacity that are advantageous for food packaging, its applicability is hindered by its inherent brittleness and stiffness, which are attributed to its high degree of crystallinity. PHB exhibits tensile strength (TS) values between 1.62-37.6 MPa and elastic modulus (EM) between 3-3.35 GPa, while its elongation at break (E%) ranges from 5 to 10%[62–64]. Moreover, although these materials are impermeable to water vapour, they exhibit significant sensitivity to water, which can promote hydrolytic degradation of their molecular chains. To address these drawbacks, PHB co-polymers have been obtained, resulting in materials with improved flexibility, toughness, and thermal performance. Copolymers are produced by

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cultivating microorganisms in the presence of multiple carbon sources, where the molar ratio of these substrates in the culture medium allows modulation of the copolymer composition[65]. Notable examples include poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBH) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV). The latter is the most commonly studied and incorporates random hydroxyvalerate (HV) units (short-chain monomers) that reduce crystallinity and enhance processability. This copolymer has better thermal processability than PHB and improved mechanical properties (TS: 18-40 MPa; EM: 1.2-3.26 GPa; E%: 1.9-970%)[66–70]. However, the PHBV barrier performance is slightly reduced when the proportion of HV in the chains rose[71]. So, this copolymer represents a more appropriate candidate for use in food packaging systems.

The water vapour permeability (WVP) and oxygen permeability (OP) of PHB and PHBV films are shown in Table 1, in comparison to commonly used polymers in food packaging. Barrier performance is a key property in food packaging systems, as it directly affects the preservation of the packaged products. Some polymers, such as starch or polyvinyl alcohol (PVA or PVOH), exhibit excellent oxygen barrier capacity but high water-vapour permeability. In contrast, other polymers, such as polypropylene (PP) or polyethylene (PE), are highly permeable to oxygen while providing an effective barrier to water vapour. Advantageously, PHBs have good barrier properties against both gases, avoiding the use of multilayer packaging systems (Laminates). Laminates are widely used in food packaging to address the limitations of mono-material films for food applications. These are produced by combining polymers with complementary barrier properties. Some polymers, such as polyethylene terephthalate (PET), ethylene-vinyl alcohol (EVOH), and PHB or PHBV, have relatively low permeability to both oxygen and water vapour, and can be used as single-material packaging for different products, thereby eliminating the need for multilayer structures. The single-material composition facilitates recycling and mitigates the end-of-life challenges associated with multilayer systems, which often require advanced recycling technologies or are ultimately incinerated [19].

Table 1. Water vapour permeability (WVP) and oxygen permeability (OP) of some polymers and biopolymers commonly used in food packaging.

Polymer	WVP ($x10^{12}$) g/m·s·Pa	OP ($x10^{13}$) cm^3/m·s·Pa	Reference
EVOH	17	0.0077	72
PET	2.3	1.35	72
PP	0.726	67.5	72
LDPE	1.2	215	72
PHB	7.88	7.94	73
PHBV (5 % HV)	7.63	6.77	73
PHBV (2 % HV)	4.1	3.6	74
PHBH	29	3.9	75
PCL	85	115	76
PLA	24	150	77
PVA	686	1.1	78
Starch (Cassava)	2777	0.42	79
Starch (Corn)	789	--	80

The special properties of PHBs and their high biodegradability make them good candidates for food packaging. These properties can also be modulated and improved by incorporating other polymers or bioactive compounds into the polymer matrix, thereby

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enhancing their food preservation capacity. Various studies have been conducted to develop active materials based on PHB or mixtures of PHB with other polymers, but this information has not previously been compiled and analysed in order to systematise the advancement of knowledge and make it more widely available. This comprehensive review provides an analysis of recent studies on PHB-based active materials for food packaging systems and the impact of incorporated bioactive compounds on material properties, their ability to extend the shelf life of certain foods, and the biodegradation pattern of the materials. It compiles information on how active compounds provide enhanced functionality to PHB materials, such as antioxidant and/or antimicrobial activity, while other characteristics relevant to food packaging, such as mechanical and barrier performance, may also be affected.

2. Modulating the properties of the PHB

Despite the many advantages associated with PHB or PHBV, several limitations persist, including mechanical brittleness, thermal instability, and elevated production costs. Furthermore, its low viscosity in the molten state results in processing challenges, such as poor heat-sealing properties and a limited thermoforming ability, thereby limiting its application in food packaging systems. To overcome these limitations, different modification strategies of PHBs have been applied, such as biological, chemical, or physical modifications, that modulate the properties of the polymer matrix. Biological modification strategies, as commented on above, encompass the optimisation of microbial cultivation parameters, thereby influencing the incorporation ratio of HV units in the PHBV copolymers. Alfano et al.[71] demonstrated that increasing the 3HV monomer fraction within PHBV molecular chains results in a lower melting point and greater elongation at break of the produced material. Chemical modifications involve structural changes such as carboxylation, halogenation, or hydroxylation. As reported by Lawless et al.[81], the free-radical graft copolymerisation of PHBV with different alkenes resulted in a decrease in melting and crystallisation temperatures and disruption of the crystalline structure, hence improving processability.

As concerns physical modifications, blending of PHB with other polymers or additives has also been studied to modulate the properties[26,82]. These are generally performed with biodegradable components to ensure that the final composite remains fully degradable. Kumar et al. [83] reported a comprehensive review, analysing blends of different PHAs that are more efficient in improving their characteristics, leading to better production and enhanced quality-based uses. PHAs have been blended with natural polymers such as starch, lignin, or cellulose derivatives, which provide lower oxygen permeability, and with biodegradable synthetic polymers such as polycaprolactone (PCL), PLA, polybutylene adipate terephthalate (PBAT) or PVA, where enhanced biodegradability and optimised properties could be found. Nanda et al.[84] demonstrated that blending PHBV with PLA enhances the elongation at break of the resulting material with respect to the neat polymers. Different plasticisers, such as PEGs with various molecular weights, have also been incorporated into PHBV to improve its mechanical properties. This modification reduced the material's brittleness and rigidity but reduced its water vapour barrier performance[85]. Some studies have also explored blending PHAs with non-biodegradable polymers, such as post-consumer PE. The blend showed measurable degradation effects (such as weight loss and visible changes in colour and texture) within 30 to 90 composting days[86].

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The performance of polymer blends is determined by their morphology, resulting from the polymer miscibility and compatibility of the constituent polymers[87]. In blends with total or partial immiscibility of the polymers, heterogeneous systems are formed with phase separation. Interfacial adhesion and fine morphology of the blend are usually promoted by compatibilising agents that facilitate molecular interactions at the interface[88]. PHBV has been extensively combined with various materials to develop blends and composites with superior mechanical and processing characteristics, thereby addressing the intrinsic brittleness and high crystallinity of the polymer. Blending PHBV with more ductile polymers has proven effective in enhancing the extensibility of the resulting materials[89]. PHBV/PLA blends have been compatibilized using specific diisocyanates, leading to a broader thermoforming temperature range and a marked improvement in the blend processability, with a minor effect on the barrier properties of thermoformed trays[90]. Feijoo et al. demonstrated that the incorporation of PHBH into PHBV matrices in varying proportions resulted in improved thermoformability and an extended thermoprocessing window[91].

Food packaging must provide an effective barrier against external contaminants, which requires the packaging to be hermetically sealed. Heat sealing is a fast and widely used method in which polymers are thermally activated on contact, allowing their molecular chains to interdiffuse and form stable bonds after cooling [92]. To enhance the heat sealability of PHBV, blending strategies with other polymers have been explored. One such approach involves incorporating PCL into PHBV matrices. In these blends, PCL acts as a phase that facilitates interfacial bonding, as its polymer chains are capable of diffusing and forming sealing bonds. This effect is achieved at sealing temperatures above the melting point of PCL and lower than the melting point of PHBV, which remains solid during the process[93]. Moll and Chiralt reported a good heat-sealing capacity of PHBV/PCL blends in different proportions, close to 1:1[76].

3. Active films based on PHB

Food spoilage poses a potential risk to human health when spoiled products are consumed, and contributes to increased food waste, economic losses, and inefficient use of resources in food production[94]. Therefore, efficient food packaging is essential for preserving food quality and safety throughout the supply chain. Packaging acts as a physical barrier that protects food from physical, chemical, and biological deterioration, facilitating transport and handling. This fact makes the food industry one of the largest consumers of single-use plastic packaging. However, according to the FAO, despite global population growth, one-third of the food produced worldwide is lost or wasted every year. Active packaging that incorporates substances capable of exerting a preservative action (Figure 3) can extend the shelf life of food, preserving its quality and safety for longer, which is a promising strategy to address food waste. Active packaging systems vary in terms of their functionality and the method of incorporating the active compound, which must be selected based on its function in a specific product[95]. The most common mechanisms of food spoilage are biochemical and oxidative reactions, and microbial spoilage. Therefore, incorporating active compounds with antioxidant and antimicrobial properties into food packaging can control these deterioration processes, extending the shelf life of the packaged products. Simple designs, such as moisture-absorbing pads in food packaging, coexist with more complex systems involving encapsulated substances (such as antioxidant and/or antimicrobial agents) for controlled release[96]. These advanced systems may require costly technological investments that could limit industrial feasibility[97]. Incorporation of the active components into the polymer

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matrix of the package, using an adequate method, is the most usual practice to obtain active films. If the active components are not heat-sensitive or volatile, mixing them by fusion with the polymer during compounding represents an industrially viable alternative for obtaining active films for food packaging.

Figure 3. Typical scheme of an active food packaging system, based on a multilayer film, with an active layer containing releasing or absorbing/scavenging substances.

3.1. Incorporation of active compounds

As concerns food preservatives, consumer demand is shifting towards natural and health-promoting additives instead of synthetic preservatives, while affordable products are still expected[98,99]. In this context, plants and biomass represent a viable and abundant source of active compounds for use in packaging materials. Several factors, including climate, geographical location, and plant genotype, influence their composition[100]. Phytochemicals are particularly valued for their natural origin; many are phenolic in nature and can impart antioxidant and antimicrobial properties to packaging materials. However, these compounds may also alter the structural and functional properties of the packaging polymer matrix, such as its mechanical, optical, thermal, and barrier characteristics[24,101–103]. Therefore, it is essential to assess the changes induced by these active compounds in polymeric matrices to determine whether the resulting materials remain effective for food packaging applications.

When obtained from agri-food by-products, the active extracts may support waste valorisation and reduce the cost of active packaging while promoting a circular economy[104]. The incorporation of these extracts into biodegradable polymers, such as PHB, can result in active, biodegradable materials for extending the food shelf life without the environmental problems of conventional plastics. The applications of these compounds go beyond food packaging and extend to sectors such as pharmaceuticals, cosmetics, and other industries that require extended shelf life for high-value perishable products[105,106]. Table 2 summarises recent studies on PHB materials incorporating different active compounds, and the main effects produced on the functional properties of materials.

Table 2. Recent studies on the active materials based on PHB: active components, incorporation method and main effects on the material. The percentage by weight (wt.) of active compound relative to the polymer has been specified.

Polymer	Active Compound (AC)	Wt. % Active Compound	Active Incorporation	Effect on PHB's properties	Reference

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PHBV	Oregano essential oil (OEO), rosemary extract (RE), and green tea extract (GTE)	10	Electrospun fibres	Antimicrobial (*E. coli*/*S. aureus*) and antioxidant (DPPH) activities; OEO was the most effective, producing more opaque films.	107
PHB/TPS (65:35)	Eugenol	3	Melt blending	Antifungal (*Botrytis cinerea*) and antioxidant (DPPH) capacity. More fragile films.	108
PHBV/ PEG1000	Essential oils of oregano and clove (OR or CLO) or their major compounds: eugenol or carvacrol (EU or CA)	17.6 or 33.3	Spraying between PHBV layers	Antibacterial activity (against *E. coli* and *L. innocua*) in the inoculated culture medium but reduced in the inoculated food matrices. More fragile films, with higher WVP and UV blocking effect.	109–111
PHBV	Ferulic (FA) or p-coumaric acid (PCA	3.1, 6.4, or 9.9	Melt blending	Lower WVP and OP. More extensible films (except for PCA at 9 %), with UV blocking effect.	70
PLA/PHBV (75:25)	Ferulic, p-coumaric or protocatechuic acid	2	Meld blending	More rigid and fracture-resistant films. Lower WVP and OP. Higher thermal degradation, except for protocatechuic acid.	112
PHBV	Ferulic acid (FA), vanillin (V) or catechin (C)	5.25	Melt blending	Anti-plasticising effect of FA and C, but plasticising effect of V. Lower OP and crystallinity. Vanillin increased WVP.	113
PHB	Vanillin	(10-200) $x10^{-4}$	Solvent casting	More extensible films. Antibacterial and antifungal capacity.	114
PHBV	Quercetin or gallic acid	0.5 -15	Extrusion	More fragile and rigid films. Gallic acid promoted chain scission of PHBV, decreasing thermal stability.	115
PHBV	Resveratrol	1	Electrospun fibres	Better antioxidant activity (30 % more), of films with resveratrol solubilised in DES than those with non-solubilised resveratrol.	116
PHB	Quercetin or curcumin	1 or 7	Electrospun fibres	Antioxidant capacity of the fibres (DPPH assays).	117
PHB	Polydatin or (+)-catechin	1	Extrusion	Higher resistance of the films to oxidation and to UV light degradation.	118

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PHB	Gallates (ethyl, propyl, octyl, and lauryl)	1	Extrusion	Higher resistance of the films to oxidation and UV light degradation. Slight decrease in the tensile strength and an increase in elongation at break.	119
PHB	Tannic acid	5, 10 or 15	Solvent casting	Enhanced thermal stability and processing window of the polymer (Crosslinking effect).	120
PHBV	Aloe emodin	(135 -540) $x10^{-4}$	Solvent casting	Photodynamic antimicrobial action (*E. coli*) in papaya and pork products.	121
PHBV	Chestnut tannins	1, 5 or 10	Solvent casting	UV-blocking and antioxidant (DPPH) capacity. Colour changes in response to NH_3 (smart films for food applications).	122
PHB/PLA	Herb extracts (*Hypericum* L., *Urtica* L. and *Chelidonium* L. (E)), ZnO and a combination of both (EZnO)	1	Extrusion	PHA-EZnO exhibited the highest tensile strength and the lowest OTR and WVTR values, as well as the highest transparency. Higher inhibition (*S. aureus*, *E. coli* and *C. albican*s) of films with EZnO than films containing only E or ZnO.	123
PHBV	Rice straw extracts (RSE)	6.4	Melt blending	Reduced crystallinity, stretchability, fracture resistance and OP, but enhanced WVP, LP and UV blocking effect.	74
PHBV	Grape leaf extract from *Vitis vinifera* var. *L. País* leaf	1	Encapsulating microparticles	Preservation of the extract antioxidant activity in the microparticles as a function of the encapsulation method.	124
PHB/ PHA (50:50)	Grape seeds lignin from Green Veltliner and Sauvignon Blanc	1, 5 or 10	Solvent casting	Increased crystallinity and stiffness. Decreased O_2 and CO_2 permeability; Antioxidant capacity of the films (ABTS assay)	125
PHBH	Lignin from poplar	1- 9	Solvent casting	Decreased WVP and OP. Increased extensibility of the films and UV-light barrier.	75

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PHBH	Modified lignin from poplar	5	Solvent casting	Lignin-PHBH biocomposites modified with maleic anhydride and KH550 had better thermal stability, mechanical and barrier properties than non-modified biocomposites.	126
PHBV	Horchata solid residue from tiger nut	11.1-66.7	Melt blending	Increased WVP. Decreased tensile properties. Antioxidant activity of films (TPC and DPPH assay).	127
PHBV	Grape stalks, grape stalk extracts.	11.1	Melt blending	Increased barrier capacity against O_2 and UV-light.	128
PHBV	Wheat straw fibres	10, 20 or 30	Extrusion	Larger fibre size decreased tensile properties and increased WVP.	129

Different plant-derived compounds with proven antioxidant or antimicrobial properties have been added to the PBH matrices to obtain active materials by using different processes. Essential oils or their main components, such as oregano essential oil[107] or eugenol[108], were incorporated into PHB matrices, providing the films with antimicrobial and antioxidant capacity. Incorporation of essential oils into the polymer solutions to obtain cast films or electrospun fibres requires the solubilization or emulsification of the compounds, depending on their relative solubility. In the case of emulsification, the stability of the emulsion highly affected the compound retention in the matrix and the microstructure of the final material[78]. During thermoplastic processing, a part of the active compound was lost during thermal treatment of the material due to its high volatility. To mitigate this problem, Requena et al.[110,111] incorporated carvacrol (CA) or eugenol (EU) by spraying them between two layers of PEG-plasticised PHBV that were thermo-adhered, and a retention percentage with respect to the amount initially incorporated of nearly 90 % was achieved. At equilibrium, an almost total release of both CA and EU occurred in food simulants with 50 % ethanol in water, while about 20 and 50 % of CA and EU, respectively, were delivered in the more aqueous food simulants and 65–70 % in fatty systems. Compound migration and antibacterial effect of these films were also studied in contact with different food matrices (fresh cheese, chicken breast, fresh-cut pumpkin and melon). The antibacterial activity in inoculated food matrices was less remarkable than in inoculated culture medium. The most significant antibacterial effects against *Escherichia coli* were observed in cheese and pumpkin, whereas the highest migration of both CA and EU took place in melon. This suggests that many compositional factors of the food matrix affected the availability of active compounds to exert their antibacterial action[109]. However, the biggest problem with essential oil compounds for use in food preservation is their significant sensory impact, which can negatively affect the product's evaluation.

Plant extracts, such as rosemary and green tea extracts[107], herb extracts[123], rice straw extracts[74], cider by-products extract[130] or grape leaf extracts[124] have been incorporated into PHB matrices to provide them with antioxidant/antimicrobial activity. Likewise, numerous phenolic compounds, such as phenolic acids (ferulic, p-coumaric, and

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protocatechuic[112,131], vanillin[114], catechin[113,118], quercetin, gallic acid[115,117], gallates[119], resveratrol and its glycosides[116] and polyphenols such as tannic acid[120], tannins[122] or lignin[125] have also been used to formulate PHB-based active materials with antioxidant or antimicrobial properties.

The potentially active compounds produced different effects on the properties of the materials, depending on their ratio in the formulation. Some phenols, such as quercetin and catechin, interact with the polymer chains, producing crosslinking effects through hydrogen bonds with the polyester carbonyl. This increased the glass transition temperature of the material and enhanced its thermal stability, opening the processing window[113,115]. In contrast, vanillin had a plasticising effect on the PHBV matrix, reducing Tg and tensile strength of the material. Gallic acid produced chain scission when its concentration rose, decreasing the thermal stability of the polymer[115]. Similar effects were observed for ferulic and p-coumaric acids when used above 6 % in the blend[70].

Lignin obtained from poplar biomass was well integrated in the PHBH matrix, with good interfacial adhesion[75]. The tensile strength and Young's modulus of biocomposites with 3 - 5 wt. % lignin increased by 46.1 % and 130.4 %, respectively, and the degradation temperature (maximum rate) increased by 50 °C. Likewise, a 30 % and 52 % reduction in the permeability to water vapour and oxygen was, respectively, found. Biocomposites exhibited excellent UV resistance and good DPPH radical scavenging capacity. Lignin-PHBH biocomposites modified with maleic anhydride and KH550 had better thermal stability, and mechanical and barrier properties than non-modified biocomposites[126]. Lignin from grape seeds was also incorporated into PHB, blended with amorphous PHAs, and positively influenced the gas barrier properties of the films, their antioxidant activity and biodegradability[125].

Basar et al.[116] analysed the effect of the resveratrol solubilisation in deep eutectic solvents (DES) for the development of continuous electrospun PHBV films with antioxidant properties, compared to the non-solubilised resveratrol. The electrospun and annealed films containing DES-solubilised resveratrol exhibited a 30% improvement in antioxidant activity with respect to those containing non-DES-solubilised resveratrol. Mechanical and barrier testing demonstrated that DES-resveratrol not only confers additional functional attributes to PHBV but also maintains mechanical and barrier performance.

Chestnut (*Castanea* spp.) tannins were also incorporated into PHBV films at different ratios by solvent casting, using formic acid to reach a homogeneous distribution of tannins in the polymer matrix. Tannins at 5 % provide the films with good transparency and tensile strength, comparable with those of the common packaging polymers. Tannins provided the PHBV matrix with antioxidant capacity, while the PHBV/tannin films could detect ammonia vapours through colour change, which makes this material potentially applicable as a smart indicator for food spoilage[122].

Different lignocellulosic residues, dried and powdered with different particle sizes, have also been incorporated into PHBV matrices. These residues contained structural components such as cellulose, hemicellulose and lignin, as well as a high phenolic content. The release of active components into the polymer matrix, the potential reinforcing effect of the fibres, and the low cost of the filler represent potential advantages of these by-products in developing food packaging materials. Blend films of PHBV and the tiger nut horchata solid residue (HSR) at different ratios were obtained by melt blending and compression moulding[127]. The composite films showed lower water vapour barrier capacity and reduced tensile strength (43–81 %) and elongation at break (46–77

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%), while the rigidity increased or remained unchanged when using up to 20 % of HSR. However, the HSR provided the films with remarkable antioxidant capacity and an effective UV light-blocking effect. Similar results were obtained by Maté et al.[132] for PHBV biocomposites containing powdered grape stalk lignocellulosic fractions at 10 %. Berthet et al.[129] analysed the impact of size, morphology and wheat straw lignocellulosic fibres (WSF), and their content on the processability and functional properties of PHBV-based composites. They observed that increasing fibre size and/or content led to a significant worsening of tensile properties and water vapour barrier capacity of the composites.

As commented on above, PHBV exhibits good barrier properties against both water vapour and oxygen. However, the incorporation of some active compounds can modify this barrier capacity. Antioxidant phenolics usually promote the oxygen barrier capacity of the material due to their capacity to scavenge oxygen. Likewise, their molecular interactions with the polymer chains may affect the overall chemical affinity of the active material and the molecular diffusion of the permeant molecules[70,79,112,130,133]. For extracts with complex composition, the overall interactions of their components with the polymer chains can affect the barrier and mechanical properties of the resulting materials. Moll et al.[74] reported very few changes in barrier properties of PHBV containing 6 % wt. of active extracts from rice straw (23 % lignin, 10 % protein, 5-8 % total phenols and about 50 % carbohydrates), while films tended to be brittle and less resistant to fracture.

In general, the addition of the biomass fractions (lignocellulosic fibres or extracts) or plant phenolic compounds to PHB matrices resulted in materials with antioxidant and/or antibacterial properties due to the action of the incorporated active compounds. Likewise, many of the biomass fractions are coloured and provide the films with their typical colour and UV-light blocking effect due to the light absorption of the phenols. This may protect packaged foods from photooxidative reactions[123,128].

The active components from agri-food waste fractionated by sustainable processes are promising ingredients for developing active packaging materials due to their low cost and availability, contributing to the waste valorisation and circular economy, while reducing their environmental impact. Likewise, the melt extrusion with the polymer is the most scalable process for incorporating active fractions into the polymer matrices, always considering the thermal stability of the active compound and its potential impact on the material's functional properties as a packaging material.

3.2. Release of active compounds

The key factor controlling the activity of active materials when applied in food packaging is the release kinetics and the equilibrium partition of the active compounds between the packaging and food. In order for an active compound present in the packaging material to exert its antimicrobial effect, it must be released from the polymer matrix into the packaged food, in enough amount to inhibit the microbial growth[24]. Antioxidants may also act on the packaging itself due to their oxygen scavenging capacity. The release of active compounds occurs through their diffusion across the polymeric film, which requires sufficient molecular mobility and solubility in the polymer matrix to allow their migration into the food. As commented on above, PHBs exhibit a highly crystalline structure, which significantly limits the segmental mobility of their polymer chains. This restricted molecular mobility can, in certain cases, impede the effective release of active agents from the polymer matrix to the food or its environment. The composition of the product in contact with the package can also influence the diffusion of active compounds. For instance, swelling of the polymer matrix induced by food contact can lead to structural changes in the polymer, potentially enhancing molecular mobility and diffusion[131]. The

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relative chemical affinity of the active compound for the polymer and the product in contact determines the partition coefficient (the ratio of the mass of compound released into the medium to that retained in the polymer) at long times, once equilibrium has been reached[131,134]. Figure 4 summarises the variables that can impact the migration of active compounds from the polymeric matrix into the food system, as well as how the composition of the food (or of the food simulant) can affect the structure of the polymer, as reported by Ordóñez et al. [24].

Figure 4. Mass transfer between an active packaging system and the packaged food, including the factors that may influence the mass transfer process.

To study the release kinetics and equilibrium concentration of active compounds in food, different food simulants have been standardised to avoid the problems associated with the complex composition and variability of food. These food simulants are commonly used as standardised test media[135]. These simulants are designed to emulate the chemical characteristics of specific food categories, allowing for a controlled assessment of release behaviour. However, although these studies provide valuable preliminary information, testing with real foods remains essential, given the unique and complex composition of each food product.

Next to the desired migration of the active compounds into the food, undesired migration of some components of the polymeric material, such as manufacturing additives or non-intentionally added substances (NIAS), resulting from reaction and degradation processes or impurities, can also occur[136]. Regulatory frameworks governing migration testing specify the conditions under which the overall migration of packaging materials must be evaluated[135]. Although these protocols are designed for assessing the total mass transfer of inert components, they can be adapted for quantifying the migration of active substances. It is important to note that active compounds are intentionally added substances (IAS) and are not considered part of the overall migration, as defined by Regulation[137]. Active compounds must exhibit high functional performance while maintaining safety for the end user. To prevent potential adverse health outcomes, it is essential to regulate their release into the food, ensuring that total intake remains within the established acceptable daily intake (ADI)[138]. Europe has one of the strictest regulatory frameworks worldwide and food contact materials require the authorisation of

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the use of the substances as active compounds by the European Food Safety Authority, according to Regulation (EC) No 1935/2004(EFSA, 2004). These difficulties have limited the presence of active packaging in the European market, whereas in North America dominate the global market with 36.38 % share[140].

3.3. Effectiveness of active PHBs films

Validation of active packaging materials in real foods is necessary to ensure their effectiveness for quality and safety preservation in a target product. Table 3 summarises recent studies on the effect of PHB materials containing different active compounds that have been tested on several food products to evaluate their preservation capacity. Fresh-cut apples, peaches and cheese or meat products have been used to test the active materials, as shown in Table 3.

Table 3. Studies on the effect of PHA-based active materials incorporating active compounds (phenolic acids/plant extracts) on food preservation and shelf life.

Polymer	Active Compound	Active Incorporation	Food product	Packaging	Main Results on Food Shelf Life	Reference
PHBV	Bis-O-dihydroferuloyl -1,4-butanediol	Solvent casting	Fresh-cut apple	Sample wrapping with the films	Decrease of polyphenol oxidase activity, weight loss and ascorbic acid degradation in fresh apple.	141
PHB-mcl PHA (90:10)	Phloretin	Solvent casting	Fresh-cut apple	Sample wrapping with the films	Antimicrobial (L. monocytogenes) and antioxidant activity (DPPH, TEAC, FRAP and chelating assays). Stabilisation of weight and colour of fresh-cut apple samples.	142
PLA/PHB/ BXA (76:19:5)	α-tocopherol	Meld blending	Peaches	Heat-sealed bags	Reduction in rancidity and water loss, prolonging the firmness and preservation of packaged peaches.	143
PHBV	Lauroyl Arginate Ethyl (LAE®)	PHBV films coated with PHBV/LAE	Spreadable cheese	Active pads in PP trays with PP/PA sealed films	PHBV films coated with LAE® were effective pads for preventing the growth of inoculated fungi, at the same level as MAP.	144

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PHBV/ Sepiolite	Oregano essential oil (OEO)	Meld blending	Mozzarella cheese slices	Interleaves	Inhibition of the growth of *S. aureus* and *E. coli* inoculated in cheese slices.	145
PHBV	Rice straw extracts and ferulic acid	Meld blending	Pork meat	Heat-sealed bags	Reduction of microbial growth and oxidation state in pork meat.	146
PHBV/PCL (1:1)	Rice straw extract and ferulic acid	Meld blending	Pork meat	Heat-sealed bags	Reduction of microbial growth and oxidation state in pork meat.	147
PLA/PHBV (75:25)-Starch	Ferulic, *p*-coumaric or protocatechuic acid	Meld blending	Pork loin steaks	Heat-sealed bags	Inhibition of bacterial growth and oxidation of meat lipids. Extension of the shelf life of pork loin.	148
PHBV	Nanoemulsion with dill essential oil and nisin	Coated films by electrospinning of an active solution	Minced chicken meat	Active pads in sealed PET trays	Inhibition of microbial growth. Extension of the shelf life of minced chicken meat stored under refrigeration.	94
PHB/PLA (75:25)	Rice bran extracts	Solvent casting	Pork loin steaks	Interleaves in PE vacuum bags	The interleaves were not effective at controlling meat oxidation or microbial growth.	149

Jiang et al.[143] used heat-sealed bags of PHBV/PLA blend with and without α-tocopherol (4 %) to analyse the shelf extension of packaged peaches, stored at 1±0.5 °C and 90±5 % relative humidity for 1 month, under a light storage environment. The active packaging with α-tocopherol could effectively reduce cell membrane damage by lipid oxidation, and better maintain the fruit firmness and weight, showing a great potential in fruit preservation as biodegradable food packaging. PHBV films with bis-O-dihydroferuloyl-1,4-butanediol (BDF, a ferulic acid derivative)[141] and PHB with phloretin (a dihydrochalcone from apple tree leaves)[142] were tested as preservation films in fresh-cut apples. In both cases, apple samples were wrapped with the films and different quality parameters were evaluated as a function of storage time. The presence of BDF in the films significantly reduced the activity of polyphenol oxidase, the weight loss and ascorbic acid degradation of apple samples stored at 4 °C and 60 % of relative humidity, for 10 days in dark conditions. Phloretin also reduced the weight loss and colour changes of apple samples for 72 h, at room temperature. Therefore, the presence of antioxidants in the PHB films highly contributes to preserving packaged fruits during storage.

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Different studies reported the effect of PHB films with active compounds to preserve cheese products. Cerruti da Costa et al.[145] designed PHBV formulations to obtain active interleaves for preserving mozzarella cheese slices. Films based on PHBV/sepiolite with oregano essential oil were effective at preventing the bacterial growth of inoculated samples with *S. aureus* and *E. coli*. Bruni et al. obtained PHBV films with a potent antimicrobial agent, LAE (Lauroyl Arginate Ethyl), to produce active pads for preserving spreadable cheese from fungal growth[144]. Inoculated cheese samples packaged in PP trays sealed with PP/PA films containing active pads remained as stable as those packaged with modified atmosphere packaging (MAP). The incorporation strategy of the active compound within the packaging system plays a key role in modulating its release into the food product. Bruni et al. produced active pads incorporating LAE, either directly into the polymer matrix via melt extrusion or as a surface coating[144]. The study showed that the coated active pad was much more effective than the melt-blended material at preventing the growth of inoculated fungi on cheese samples. The amount of LAE released from the coated pad was sufficient to reach the minimum inhibitory concentration (MIC) of the fungi (typical cheese contaminants) while remaining below the acceptable daily intake (ADI), thus ensuring food safety.

Encapsulation can also be an effective strategy to control the release kinetics of the active compound into the packaged food, as well as to protect the active agent (especially volatile or heat-sensitive compounds) during the packaging processing, or to mitigate the impact of certain compounds, such as essential oils, on the organoleptic properties of the food[138]. Different microencapsulation techniques, categorised as physical (solvent evaporation, spray drying, freeze-drying), chemical (interfacial polymerisation, molecular inclusion complex formation), and physicochemical (coacervation, liposome formation, ionic gelation), exhibit differing efficiencies in terms of active compound retention, stability, and functional performance[150]. Fiorentini et al. encapsulated citrus peel extracts with β-cyclodextrins by spray-drying for their incorporation into PHB/PLA blends by extrusion. These films inhibited the growth of *Escherichia coli* and *Staphylococcus aureus* in bacterial challenge tests[151].

Fresh pork meat has been used in several studies as a food model to analyse the preservation capacity of active PHB films. PHBV and PHBV/PCL (1:1) bags containing ferulic acid or rice straw extracts (RSE) at 6 % wt. were used to analyse the shelf-life extension of cold-stored meat (5 ˚C), in comparison with the active-free bags[146,147]. The changes in pH, weight, colour, oxidation and microbial counts were evaluated. Active compounds were highly effective at reducing meat oxidation and microbial growth, extending the shelf life of the meat. In PHBV bags, ferulic acid and RSE prolong the meat shelf life by 8 and 4 days, respectively, compared with the active-free bags, while in PHBV/PCL bags, the extension was by 5 days with both active components. A similar preservation effect was observed in pork loin steaks packaged in PHBV/PLA (25:25) bags with ferulic, p-coumaric or protocatechuic acids[148]. In contrast, Cabeza de Vaca et al.[149] did not observe a protective effect of active interleaves of PHBV/PLA films with rice bran extracts against meat oxidation or bacterial growth. PHBV active pads with electrospun nano-emulsions of dill essential oil and nisin were used to improve the preservation of minced chicken meat, packaged in PET trays. These pads contribute to controlling the microbial growth, extending the meat shelf life by 2 days with respect to the control[1].

In practice, compounds intended for use in food packaging must be approved by the relevant regulatory agencies before they can enter the market. The main authorities, the Food and Drug Administration (FDA) in the United States and the European Food Safety

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Authority (EFSA) in Europe, are responsible for conducting the necessary assessments to determine whether a compound is safe and thus authorised for food contact. A wide range of regulations exists in this area, which are increasingly strict, aiming to prevent the misuse of additives that could pose health risks[95,106,152].

4. Effect of bio-based compounds on the biodegradation behaviour of PHBs

4.1. Biodegradation of PHBs

Biodegradable polymers are defined by the International Union of Pure and Applied Chemistry (IUPAC) as materials that can undergo degradation via biological activity, leading to a reduction in their molar mass. This degradation process involves the cleavage of polymer chains, causing chemical modifications and the subsequent loss or deterioration of the material's functional properties. In biodegradable polymers, microbial activity is typically the primary driver of this degradation, resulting in the conversion of the material into environmentally benign substances (such as water, carbon dioxide, methane, and mineral residues). In some cases, this transformation can produce beneficial outcomes, including the generation of renewable biomass within relatively short timeframes[138].

Several standardised methods have been developed to monitor the decomposition of biodegradable materials. The principal organisations responsible for establishing these standards at the international level are the International Organisation for Standardisation (ISO) and ASTM International (formerly the American Society for Testing and Materials, ASTM). For example, ISO 14855 defines the procedure for assessing the final aerobic biodegradability of plastic materials under controlled composting conditions. UNE-EN ISO 20200, meanwhile, establishes the testing method for analysing the degree of disintegration of plastics in simulated composting systems through laboratory testing. Additional standards have been designed to assess biodegradation in other environments, including marine ecosystems. Examples include ISO 23977-1:2022, which examines the aerobic biodegradation of plastics in seawater, and ASTM D7991-15, which evaluates degradation in sandy marine sediments under controlled laboratory conditions. PHBs can also be degraded under anaerobic conditions, producing methane and carbon dioxide as products of the process[153,154].

Although the implementation of responsible and well-regulated post-consumer waste management strategies is essential for all packaging materials, it is equally important to recognise the varying biodegradability profiles of different biodegradable plastics. For instance, PHBV has been shown to biodegrade under various environmental conditions, including domestic composting (28 °C), soil, seawater, and wastewater sludge[89,155]. Conversely, PLA require specific environmental conditions to achieve full biodegradation[138].

The biodegradability of PHAs is influenced by their chemical structure, including the type of monomers present in the polymer chains and the nature of their interconnecting bonds[156]. Although these polymers are capable of decomposing in various environments and under a range of conditions, their structural parameters may also influence the kinetics of their degradation. Furthermore, the incorporation of various natural compounds (either as reinforcing within the polymer matrix or as bioactive agents) has been shown to impact the degradation rate of PHAs[157].

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Table 2 summarises recent studies about the biodegradation of different PHAs incorporating various naturally derived materials into the matrix for different purposes, highlighting the biodegradation conditions and the primary effects of these additives on the PHA biodegradation performance. In general, hygroscopic fillers such as cellulosic fractions[158], or some lignocellulosic fractions of plant waste, such as vine shoots[159] or olive pomace[160], accelerated biodegradation by promoting water diffusion through the polymeric matrix, and bulk hydrolysis of the polymer. However, the lignin content of wood flour reduced the degradation rate of PHBV, which was attributed to its interactions with the biofilm microorganisms[161]. Some phenolic compounds with antimicrobial activity, such as ferulic acid or vanillin, favoured the polymer hydrolysis during the degradation process, contributing to its acceleration. This effect was also observed for L-limonene[162] and eugenol[163]. In contrast, catechin, which promotes the polymer crosslinking, delays the PHBV degradation[21,164]. Other phenols, such as gallic acid and quercetin, also delayed the degradation of PHBV[165], as did inorganic fillers such as halloysite clay nanoparticles[166].

Table 4. Recent studies on the biodegradation behaviour of PHA in different environments, as affected by different bio-based additives.

Polymer	Natural Compound	Activity	Active Incorporation	Degradation medium	Standard	Main Results	Reference
PHBV with electrospun internal layer PLA/PHB (75:25)	Catechin	Antioxidant	Electrospun fibres	Solid synthetic waste (58ºC), aerobic conditions	ISO 20200:2015	Decrease in the disintegration rate	164
PLA-PHB (75:25)	Cellulose nanocrystals (CNC)	Reinforcement	Extrusion	Solid synthetic waste (58ºC), aerobic conditions	ISO 20200:2006	Acceleration of the disintegration rate	158
PHBV or PHBV/PCL (1:1)	Ferulic acid (FA) or rice straw extract (RSE)	Antioxidant/ antimicrobial	Melt blending	Solid synthetic waste (58ºC), aerobic conditions	ISO 20200:2023	RSE accelerated biodegradation, and FA promoted the process in PHBV/PCL, but not in PHBV. PCL retarded the disintegration of active films.	167
PLA/PHBV (75:25)	Ferulic, p-coumaric or protocatechuic acid	Antioxidant/ antimicrobial	Melt blending	Compost or solid synthetic waste (58ºC), aerobic conditions	ISO 20200:2004 and ISO 14855:1-2012	No effect of phenolic acids on the film disintegration pattern.	168
PLA-PHB (75:25)	L-limonene	Plastizer	Melt blending	Solid synthetic waste (58ºC), aerobic conditions	ISO 20200:2006	L-limonene promoted the biodegradation rate	162
PHBV	Pure α cellulose (C) and pine woodflour (WF)	Reinforcement	Extrusion	Compost or solid synthetic waste (58ºC), aerobic conditions	ISO 14855-1 and ISO 20200:2015	C did not affect the biodegradation rate, and WF reduced it due to the lignin effect.	161

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PHB/PHA (1:1)	Grape seeds lignin	Antioxidant	Solvent casting	Solid synthetic waste (58ºC), aerobic conditions	ISO 20200:2023	Concentration-dependent effect on the degradability degree.	125
PHBV	Acetyl tributyl citrate (ATBC) and lignin-coated cellulose nanocrystals (L-CNC)	Plasticiser/ reinforcing	Extrusion	Compost (58ºC), Vermicomposting (21ºC) Freshwater biotope (27ºC)	ISO 14855-1:2012 No standard No standard	ATBC accelerated biodegradation, and L-CNC partially inhibited this effect in every case.	157
PHBV	Quercetin and gallic acid	Antimicrobial	Extrusion	Compost (28ºC), aerobic conditions	ISO 14855-1:2012	Both decreased the biodegradation rate.	165
PHBV	Olive pomace (OP)	Fillers	Melt blending	Soil (28 °C), aerobic conditions	ASTM D5988-96	OP accelerated the biodegradation of PHBV.	160
PHBV	Halloysite clay nanoparticles and oregano essential oil (OEO)	Reinforcing/ Antimicrobial	Melt blending	Soil (23 °C), aerobic conditions	ASTM G 160–03	OEO accelerated biodegradation, with and without Halloysite clays.	166
PHBV	Vine shoots	Fillers	Melt blending	Soil (28 °C), aerobic conditions	No standard	Acceleration of the biodegradation rate.	159
PHB	Eugenol	Antioxidant/ antimicrobial	Solvent casting	Agricultural (18-37ºC), sandy (19-34ºC) and landfill soil (18-41). Aerobic conditions.	No standard	The films in the agricultural soil presented a faster degradation rate	163
PHBV	Human hair waste (HHW), sawdust (SD) and chitin from shrimp shells	Reinforcement	Extrusion	Soil (21.5 °C), aerobic conditions	No standard	All residues accelerated PHBV biodegradation. HHW showed the highest acceleration effect.	169
PHBV	Catechin, ferulic acid and vanillin	Antioxidant/ antimicrobial	Melt blending	Marine medium (25ºC)	ISO 23977-1:2022	Ferulic acid and vanillin accelerated the biodegradation, and catechin slowed it down.	21
PHBV	Purified cellulose (TC), wood flour (WF), and almond shell (AS)	Fillers	Extrusion	Marine medium (a port and the open sea)	No standard	PHBV/AS showed the highest disintegration degree. The port environment promoted the biodegradation.	170

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PHBV	Miscanthus fibres (Misc) and distillers' dried grains with solubles (DDGS)	Reinforcement	Extrusion	Marine medium (25ºC)	ASTM D7991-15	Filler accelerated degradation. GGDS was the most effective.	89

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Some authors also studied the influence of other biodegradable polymers mixed with PHBV on the disintegration of the resulting polymer matrix. Van der Zeed et al.[171] found that mixing PHBV with 20 % or 40 % PCL or PBSA increased the disintegration rate in synthetic compost at 25 ºC and 90 % relative humidity, compared to pure PHBV. However, Moll & Chiralt[167] observed that PCL slightly delayed the disintegration rate of PHBV/PCL (1:1) in other composts with different compositions, at 58 ˚C and 55 % relative humidity. This indicates that the degradation environment and the proportion of polymer added to the mixture affect the degradation process.

4.2. Biodegradation mechanisms

The degradation rate of a given material is significantly influenced by the characteristics of the biofilm formed on its surface during the biodegradation process[172]. A biofilm is defined as a layer formed at the interface between a material and its environment, composed of a dynamic, structured colony of microorganisms (such as bacteria, fungi, or algae) adhered to a given surface. These microorganism communities secrete an extracellular matrix composed of a range of polymeric substances, including enzymes, proteins, lipids, polysaccharides and DNA. Some biofilm components, mainly enzymes, contribute to the degradation of the material. These compounds protect the microbial communities constituting the biofilm against environmental stresses (desiccation, toxic compounds, UV-light…), while also facilitating intercellular interactions[173,174]. The diversity of microbial species involved in the biofilm may be, in turn, modulated by the specific composition of the material to be degraded[172].

The formation of the biofilm is primarily responsible for the biodegradation of polymeric materials occurring predominantly at the surface. This process leads to surface erosion that progresses from the exterior toward the interior of the material, resulting in a decrease in polymer molecular weight. The secreted extracellular enzymes by microorganisms initiate the depolymerisation of the polymer chains, leading to a total degradation process with the formation of CO_2 (or methane in anaerobic conditions) and water[21,161,172].

Recent studies on the biodegradation of polymeric films have increasingly aimed to characterise the microbial communities constituting the biofilm, to elucidate their functional roles in the degradation process[175]. This is typically achieved through the DNA sequencing methodologies that have been extensively employed in other biological systems, such as human colonic or oral microbiome research[176,177]. These studies involve the extraction of microbial DNA from the biofilm matrix, followed by sequencing using Illumina platforms, typically targeting the V3–V4 regions of the 16S rRNA gene through amplification with specific primers. The resulting data are subjected to bioinformatic analysis to determine the relative abundance of each microbial taxon present within the biofilm. The population that forms the biofilm also depends on the environment in which the material degradation occurs[178]. Savenkova et al.[179] found that the dominant microorganisms involved in PHB biodegradation in soils were bacteria (*Pseudomonas*, *Azotobacter*, *Bacillus*, and *Streptomyces*) and fungi (*Penicillium*, *Cephalosporium*, *Paecilomyces*, and *Trichoderma*). However, biodegradation studies conducted in marine soils and marine waters identified different bacterial strains

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(*Enterobacter* sp., *Bacillus* sp., *Pseudomonas* sp., *Aquibacter* sp., *Gracilibacillus* sp. and others), fungi, multicellular algae, and yeasts responsible for the degradation of both PHB and PHBV[175,179,180].

Different studies analysed the ecotoxicity of compost resulting from the degradation of PHBVs according to ISO 18763, which determines the toxic effects of contaminants on the germination and early growth of higher plants. Based on this standard, three plant species (one monocotyledonous (*Sorghum saccharatum*) and two dicotyledonous (*Lepidium sativum* and *Sinapis alba*)) are sown in different composts, and seed germination and seedling growth under controlled environmental conditions are assessed by measuring root and shoot length. Vostrejs et al.[125] determined the germination and root growth of white mustard (*Sinapis alba* L.), confirming the non-toxicity of compost containing degraded PHBV.

It should be noted that materials that are fully biodegradable within a short period of time and under various environmental conditions may present challenges when used as packaging designed for long-term applications, where durability is a key requirement. Therefore, it is essential to find the right balance between the functional lifespan of food packaging and its biodegradation at the end of its shelf life. In addition, the characteristics of the food product to be packaged must be taken into account. These biodegradable materials may be more suitable for perishable products that are not intended to be stored for long periods between packaging and consumption[181].

Final remarks

The need to package food to protect it throughout the food supply chain has contributed significantly to environmental pollution due to the widespread use of single-use plastic materials. The use of biodegradable packaging materials can help mitigate this problem because, at the end of their life cycle, they break down into environmentally harmless compounds such as carbon dioxide, water and biomass, which can be safely returned to nature.

PHBs are particularly suitable for food packaging, both from a functional and environmental point of view. This suitability is due to their excellent gas barrier properties (related to their high crystallinity) and their acceptable mechanical strength. In addition, they can biodegrade in different environmental conditions, such as soil, compost and marine environments. They are also produced biosynthetically, are biocompatible and non-toxic, and some types have already been approved by the FDA. However, there are still significant challenges that limit their widespread use. Among these, their high production cost, fragility and sensitivity to moisture limit their large-scale manufacture and application. Likewise, its compatibility with other polymers is also limited.

PHBV copolymers with reduced crystallinity with respect to PHB, and modulated barrier and mechanical performance, depending on the molar ratio of valerate units, represent a better alternative for food packaging. Likewise, blends with other biodegradable polymers and/or natural additives offer alternative solutions for developing more sustainable packaging materials without compromising the biodegradability of the final product. Specifically, active materials represent an innovative approach to reducing the significant amount of food waste currently generated. These materials have been produced by incorporating bioactive compounds into the packaging material using different techniques, but industrially scalable methods, such as extrusion blending, are the most adequate to ensure the final application of the material. The active compounds

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can be released into the packaged food, slowing down its deterioration and helping to extend its shelf life, ensuring its quality and safety for a longer period. Obtaining bioactive agents from natural resources or agri-food waste is a growing trend, which allows such waste to be valorised within the framework of the circular economy.

There is still a long way to go in the development of active biodegradable packaging that can simultaneously mitigate plastic pollution and food waste. Greater investment is needed to optimise the production of materials, with lower manufacturing costs, and appropriate policies to guide market trends. It is also essential to validate the performance of these packaging materials in real food systems to ensure their effectiveness in extending shelf life and preventing post-consumption contamination.

Autor Contribution

Eva Moll: Investigation, Conceptualisation, Methodology, writing – original draft. **Amparo Chiralt:** Conceptualisation, Methodology, Writing – review & editing, Supervision, Project administration.

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Data Availability Statement

No new data were generated or analysed for this review.

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