Poly(hydroxyalkanoates) for Food Packaging: Application and Attempts towards Implementation

Koller, M.

University of Graz, Institute of Chemistry, Division of Physical and Theoretical Chemistry, NAWI GRAZ, Heinrichstrasse 28/III, A-8010 Graz, Austria

Abstract

Plastics are well-established for convenient and safe packaging and distribution of food and feed goods. At present, this special sector of the plastic market displays remarkably increasing quantities of its annual production. Caused by the ongoing limitation and strongly fluctuating prices of fossil feedstocks, classically used for plastic production, there is an evident trend to switch towards so-called "bio-plastics". Especially for bulk applications such as food packaging, a broad implementation of "bio-plastics" constitutes a future-oriented strategy to restrict the dependence of global industry on fossil feedstocks, and to diminish current problematic environmental issues arising from plastic disposal. However, food packaging demands a great deal of the utilized packaging material. This encompasses tailored mechanical properties such as low brittleness and adequate tensile strength, a sufficient barrier for oxygen, CO_2, and aromatic flavors, high UV-resistance, and high water retention-capacity to block the food´s moisture content, or to prevent humidity, respectively. Due to their hydrophobic character and the broad flexibility of their mechanical features, prokaryotic poly(hydroxyalkanoates) (PHAs) are considered as promising materials to compete with petro-plastics on the food-packaging market. Nevertheless, short-comings in particular aspects of their material performance and economics of their biosynthesis and purification constitute stumbling blocks on the long way towards broad implementation of PHAs for food packaging. This article discusses advantages and drawbacks of PHAs as food packaging materials, and demonstrates how desired properties can be improved by the designing of novel composite materials, and also encompassing techniques by applying nanoparticles.

1. Introduction

1.1 *The plastic age*

Plastic industry represents an enormous economic factor as illustrated by the fact that, in 2011, about 1.45 million people were employed in the EU-27 countries by plastic manufacturers or converters with an impressing annual turn-over of about $3 * 10^{11}$ €. As a consequence, our today's world is literally covered with often highly persistent plastics stemming from the chemical conversion of fossil feed stocks; analogous to the prehistoric "*Stone and Metal Ages*", mankind nowadays factually lives in the "Plastic Age". This is valid since the start of the rapid increase of the production of petroleum-

based plastics in the mid of the 20th century. In 1930, the annual world production of plastics barely reached 23,000 t. Only about 20 years later, this value was already increased more than 50-fold, surpassing a quantity of annually 1,3Mt. For the beginning of the 1990s, a global annual plastics output exceeding 100 Mt is reported. At the end of 2010, the overall production volume reached over 250 Mt. At the moment, the global production is estimated with about 280 Mt per year; approximately 40% thereof are allotted to Asia [1].

Contemporary applications of synthetic plastics, also known as "techno-polymers", are immense including packaging materials (37% of the entire plastic market), textile fibers, parts for various electrical equipment, high performance medical & surgical devices, different components in cars and other means of transport, pipes, lacquer components, products for sport and leisure and many more [2]. This massive presence on the market is due to the broad range of beneficial chemical and mechanical properties of plastics, allowing them to replace traditional materials like minerals, wood, natural fibers or diverse metals. These properties encompass the enormous versatility of the material characteristics, low density, imperviousness to humidity, transparency, oxidation resistance, and high recalcitrance towards microbial and chemical degradation [3]. The latter feature causes increasing accumulation of these artificial materials in the aquatic and terrestrial ecosphere; hence, plastic waste provokes severe ecological problems [4].

1-2 *Biopolymers for sustainable replacement of petrol-based techno-polymers*

Low-tech techniques for treatment of plastic waste like thermal conversion or ultimate disposal in landfills for sure have to be avoided in the future due to negative ecological consequences like generation of toxic incineration products or growing piles of plastic waste, respectively. From the sustainability point of view, one can figure out two basic strategies to minimize the problems arising from the tremendous amounts of plastic waste:

The first strategy implements efficient recycling technologies that are sustainable both economically and environmentally in the long term. Recycling technology has been improving at fast rate since its beginnings regarding purification, mono-fractioning of different plastic types, and number of possible recycling cycles. However, the success of this idea does not only depend on the availability of functioning recycling technologies and infrastructure, but also on mankind's willingness to learn and to accept new ways of handling waste [5]. Today, the world's average plastic recycling rate amounts to modest 20%. This approach should go in parallel with the development of high-quality and resistant materials that are functional and useable for a long period; here, we talk about the elongation of the classically short life span of plastic products. In order to meet the requirements of the numerous different types of plastic, high efforts in research and development are still needed.

As the higher sophisticated and more promising approach, bio-based plastic materials can be applied, which, after being subjected towards composting conditions, are naturally mineralized towards CO_2 and water as sole degradation products in the matter of only months. Although widely discussed and ambitiously investigated, bio-based and bio-degradable plastics contemporarily occupy not even 5% of the entire plastic market (here, estimations are strongly fluctuating!). Several plastic-like materials with properties similar to petroleum-based techno-polymers (often frivolously classified as "bio-plastics"), are already on the market. These include thermo-plasticized starch, poly(lactic acid) (PLA), bio-based poly(ethylene) (PE), poly(trimethylene terephthalate) (PTT), poly(butylenes succinate) (PBS), poly(p-phenylene) (PPP), and, as the materials of choice in this review, microbial poly(hydroxyl-alkanoates) (PHAs) [6]. Each of these polymers displays certain mechanical and chemical properties allowing them to be used in special fields. However, only PHAs are characterized by their manifold structural variations, resulting in numerous different properties, and, subsequently, different fields of application. Along with these multi-farious properties, PHAs are the only class of "bio-plastics" with a complete "green" life cycle: renewable resources act as feedstocks of the production (*bio-based*), both synthesis of their monomeric building blocks, and their subsequent polymerization are biocatalyzed by living cells (*bio-synthesized*), they do not cause any adverse effects on the biosphere (*bio-compatibility*) and, finally, they undergo degradation by the action of living organisms (*biodegradability*) [6-8].

Nature equipped various prokaryotic microbial species with the enzymatic prerequisites to accumulate PHAs as storage compounds for carbon and energy in order to

provide them an advantage for survival under famine conditions [9]. They occur as highly organized intracellular granules, amounting in dependence on the microbial species and the environmental conditions, from traces to more than 80% of the cell dry mass [10]. Figure 1 provides a scanning transmission electron microscope (STEM) picture of PHA-granules, well visible as bright spherical inclusions in *Cupriavidus necator* cells cultivated in a multistage bioreactor cascade [11].

1.3 *Material features of PHAs*

Material properties of PHAs range from highly brittle and crystalline thermoplasts regarding the most simple case of the homopolyester poly(3-(*R*)-hydroxybutyrate) (PHB), a material with rather restricted applicability, to more elastomeric materials such as copolyesters of 3-hydroxybutyrate (3HB) and 3-hydroxyvalerate (3HV) or 4-hydroxybutyrate (4HB), PHBV and poly(4HB), respectively. 3HB, 3HV and 4HB are the most common representatives of the so-called short chain length PHA (*scl*-PHA) building blocks [12]. Especially among the *scl*-PHA copolyesters, potential candidates for food packaging application can be found due to their high plasticity and accessibility towards melt extrusion, injection molding or thermoforming. In the case of poly(4HB), an enormous elongation to break of up to 1000% is reported, representing a highly elastic material with a bright future in the medical field [13]. Application of poly(4HB) for food packaging is not reported yet. This polyester's composition is determined by the feeding strategy applied during its biosynthetic generation.

Bio-degradable resins, namely medium-chain-length PHAs (*mcl*-PHA), are accumulated by several pseudomonad strains. *mcl*-PHAs are polyesters of hydroxyalkanoates (HAs) with at least 6 carbon atoms. In addition to simple representatives like 3-hydroxyhexanoate (HHx) or 3-hydroxyoctanoate (HO), these HAs frequently exhibit chemical functionalities like double bonds or aromatic rings, and can even contain chemical elements other than the common PHA-constituents (carbon, oxygen and hydrogen), such as sulfur, nitrogen or halogens. In contrast to *scl*-PHA, *mcl*-PHAs often display latex-to resin-like properties with extremely low glass transition temperatures, making them interesting for storage of goods like foods under freezing conditions [14-16]. Figure 2 provides the general chemical structure of PHAs, whereas Table 1 compares the characteristic features of different PHAs.

2- Requirements for Food Packaging Materials in the Context of PHA Characteristics

2.1 *General*

To be used in food packaging application, selected materials have to fulfill several tasks:
a) Enclosing the food;
b) Protecting it from the environment (microbial attack, chemical contaminants, dust, UV-radiation, mechanical damage, humidity, desiccation);
c) Maintaining the sensory food quality;
d) Maintaining a modified atmosphere in the product's headspace (e.g. nitrogen atmosphere for conservation of potato chips);
e) Stability under extreme storage conditions (e.g. low temperature);
f) The environmental conditions leading to biodegradation must be circumvented during the storage of the food product, whereas optimized conditions for biodegradation must arise after discarding;
g) Food grade quality of the packaging material is required regarding the purity.

Table 1: Characteristic thermo-mechanical features of different PHAs [10, 17-20]

	PHB	Poly-(3HB-*co*-3%-3HV)	Poly-(3HB-*co*-11%-3HV)	Poly-(3HB-*co*-20%-3HV)	Poly-(3HB-*co*-3%-4HB)	Poly-(3HB-*co*-16%-4HB)	Poly-(3HB-*co*-64%-4HB)	Poly (4HB)	P(3HB3HV 4HB)[1]	P(3HB3HV 3HHx)[2]	Poly-(3HO-*co*-12%-3HHx)
Melting temperature [°C]	177	170	157	145	166	152	50	60	140	114	61
Glass transition temperature [°C]	4	-	2	-1	-	-8	-	-50	-2	-2	-35
Tensile strength [MPa]	40	38	38	32	28	26	17	104	n.r.	8	9
Elongation at break [%]	6	-	5	50	45	444	591	1000	n.r.	481	380

[1]Poly-(3HB-*co*-21,8-%-3HV-*co*-5,1-%-4HB) [2]Poly-(3HB-*co*-2,4-%-3HV-*co*-13,4-%-3HHx) n.r.: not reported

In order to guarantee these functions, it is important to control the mechanical and barrier properties of the polymeric packaging materials during the experimental storage conditions; in the case of PHA-based materials, these properties highly depend on the structure and monomeric composition of copolymers [12, 17-20]. Chemical or enzymatic post-synthetic modification of the polymeric material, generation of blends and composites with other compatible materials [21], and design of functional multilayer films [22] are strategies to develop high-performance food packaging.

Furthermore, it is crucial to get deep insight into the mechanisms that are responsible for changing of the characteristics (odor, taste, texture, *etc.*) of the packaging material as well as of the food during different time periods of interaction of the packaging material with the food under diverse conditions (temperature, humidity, *etc.*).

Hence, it is of utmost importance to understand not only the physical and mechanical properties of the biomaterials selected for food packaging, but also the compatibility with the food, which has been recognized as a potential source of food quality shortcoming [23].

Until today, only a very restricted number of biopolymers were the subject of investigation as food packaging materials, and reports are mainly based on lab-scale to pilot-scale set-ups [24].

Most prominently, cellulose, PLAs, protein-based films, thermo-processed starch, chitosan, and, to a still evanescent extent, PHAs are reported [24]. This is in strict contrast to the fact that especially PHAs should attract high attention as basic material for food packaging due to several aspects:

- PHAs can be processed to excellent packaging films *via* thermoforming; this can be done using PHAs as the sole material or in combination with other compatible synthetic or bio-based polymers, thus creating composite materials or blends, respectively [7].

- Based on their adaptable degree of crystallinity and elasticity, PHAs can be processed towards differently formed parts such as flexible foils for enwrapping (production of thermo-forming films), or rigid and robust cast components (*via* injection molding) acting as storage boxes and containers [23, 24].

- Thanks to the pronounced hydrophobicity of these water-insoluble materials, PHA films display a high water vapor barrier; this goes in parallel to high barrier properties against CO_2 [20, 22].

- The homopolyesters PHB, poly(4HB) and in dependence on the composition, *scl*-PHA copolyesters (PHBV) exhibit a high oxygen barrier (or low oxygen transmission rate, OTR), which is required to restrict the growth of aerobic microbes and the oxidative spoilage of unsaturated fatty acids. The fine-tuning of polyester composition during the biosynthesis determines the performance of the material in terms of gas barrier properties [13].
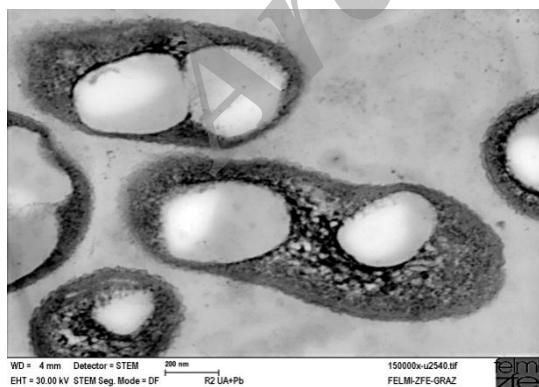

Figure 1: Cells of *Cupriavidus necator* harboring PHA granules as refractive intracellular inclusions.
The mass fraction *m* of PHAs in total dry biomass amounts to 0.48 g.g^{-1}, magnification 1/150000. Picture has been provided by the courtesy of Dr. Elisabeth Ingolić, FELMI-ZFE Graz.
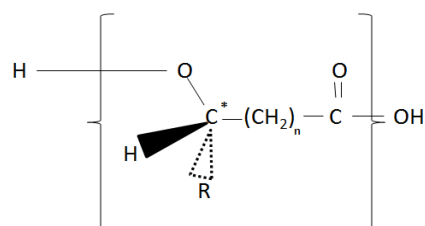

Figure 2: General structure of PHAs

R = CH_3, n = 1: 3-Hydroxybutyrate   (3HB)
R = C_2H_5, n = 1: 3-Hydroxyvalerate   (3HV)
R = H, n = 2: 4-Hydroxybutyrate   (4HB)
R = C_3H_7, n = 1: 3-Hydroxyhexanoate   (3HHx)
R = C_5H_9, n = 1: 3-Hydroxyoctanoate   (3HO)

: indicates chiral carbon atom in the case of R ≠ H

• These barrier properties for oxygen, water and CO_2 make PHAs interesting as the basic materials for producing bottles for liquid foods, and also for CO_2-containing liquids [23]. Barrier performance can further be enhanced by designing composite materials, as will be shown later [24].

• Their high UV-barrier is desirable to protect especially unsaturated lipid components in food from formation of radicals, thus accelerating their spoilage [23].

• PHA-based packaging spoiled with food residues (blood, tissue liquor, fruit juices, *etc.*) can conveniently be discarded by composting [21].

• Classical biotechnological PHA production is based on the feed stocks such as cane sucrose, sugar beet sucrose, or corn starch–based glucose [7]. A considerable amount of PHA is also produced using the plant oils such as soybean oil, palm oil, and corn oil. Hence, the manufacture of PHA is closely connected with food industry. Waste lipids from gastronomy or animal processing industry and milk whey are alternative raw materials with excellent performance on the laboratory scale, and are expected to be commercialized soon [7]. Life cycle assessments (LCAs) and economic appraisal for PHA production, if compared to petrochemical plastics, were carried out for the cases of the raw materials, cane sugar [25], whey lactose [26], and waste lipids from the animal processing industry [27]; all these calculations demonstrate an environmental benefit of replacing common plastics by PHA produced by different feed stocks.

2.2 *Purity and sensory quality*

For superior sensory properties, a high degree of purification is required from a biopolymer to be used for food packaging. In the case of PHAs, lipid residues often remain attached to the biopolymer after extraction. This causes a typical rancid odor and smell of the material that can easily and negatively affect the quality of the packaged food. Efficient and convenient methods to highly purify microbial PHAs in order to meet the requirements of norms and standards for application in the pharmaceutical or food sector are being investigated and developed in various laboratories globally [28]. Here, simple chemical methods [29], combinations of organic PHA solvents and anti-solvents [30], or high-pressure extraction with classical anti-solvents like acetone [31] can be applied in order to remove the remaining lipids and pyrolytic

lipopolysaccharides (endotoxins) that are frequently spotted attached to PHAs from Gram negative production strains [32]. Alternatively, supercritical solvent extraction is regarded as a viable, sound strategy to quantitatively remove such impurities in an environmentally safe way [33-35].

2.3 *Oxygen barrier*

The oxygen barrier performance of a food packaging vessel for fresh products (e.g. meat and follow-up products, fruits, salad, various convenience foods, *etc.*) displays a decisive role for its conservation. Oxidative degradation can affect the color, flavor and microbial stability of a variety of foods. Maintaining product quality relies on the packaging film's ability to act as a barrier, overcoming the driving force of the difference in oxygen partial pressures inside the package (0-2%) *vs.* outside the package (21%). The oxygen barrier is quantified by the OTR, defined as the steady state rate at which oxygen can permeate through a polymeric test film; it indicates the amount of oxygen that permeates a packaging materials *via* diffusion per unit of area and time ($cm^3.m^{-2}d^{-1}bar^{-1}$, $nmol.m^{-1}s^{-1}GPa^{-1}$, *etc.*) at standardized conditions for temperature (23°C) and humidity (often 50% relative humidity RH) [23]. Oxygen permeability is determined according to the standardized norm DIN 53380 [36]. In this test, a permeation cell is separated by the polymer film to be tested, and the test gas (here: oxygen) is introduced into one half of the cell. The permeation of the gas into the second half of the cell is determined by an adequate sensor. So, when a polymer film packaging has a low oxygen permeability coefficient, the oxygen pressure inside the container drops to the point where the oxidation is retarded, extending the shelf-life of the product [24, 36].

As a common rule, a polymeric film is regarded to act as barrier if it features a transmission rate below 2 $nmol\ ms^{-1}GPa^{-1}$; such materials are often termed as "barrier polymers" [37]. Generally, biodegradable polymers present a value one or more orders of magnitude below the synthetic polymers used in the same field like poly(ethylene terephthalate) (PET) [24]. Several authors have reported values for oxygen permeability coefficients of commercialized biodegradable polymers like PLAs and also different types of PHAs [20, 23, 24]. It was shown that the composition of PHAs on the monomeric level does not only affect the properties like crystallinity, melting temperature, *etc.*, but also the permeability for oxygen and

water. The higher the molar share of 3HV in PHBV copolyesters, the higher the reported permeability to water and oxygen and higher moisture sensitivity in investigated PHA samples [38].

2.4 *Water barrier*

From chemical point of view, PHA polyesters present the advantage of considerable hydrophobicity when compared to the other biopolymers of natural origin such as starch. The water vapor barrier properties for the packaged products, whose physical or chemical deterioration is related to their equilibrium moisture content, are of major importance for maintaining or extending the products' shelf-life. The water vapor barrier is quantified by the water vapor permeability coefficient (WVPC) or the water vapor transfer rate (WVTR), which indicates the amount of water vapor that permeates per unit of area and time in or out of a packaging material. Requirements for high or low water vapor barriers are dependent on the type of the packaged food; for fresh food products, it is important to avoid desiccation while for example, for the bakery products that are easily subjected to fungal infections by molds, it is essential to avoid water inflow. For different biodegradable polymers, WVPC is reported in the literature with 172 g m^{-2} d^{-1} in the case of PLA, while for PHAs, significantly lower values typically below 20 g m^{-2} d^{-1} can be found [39]. Generally, the value for PHB is very similar to that of the petrochemical opponents (PET) and poly(vinyl chloride) (PVC) [38]. Regarding PHBV copolyesters, the water barrier function generally decreases in comparison to the PHB homopolyester [38-40]. Hence, excellent water barrier performance of PHAs can be concluded by comparison of different related polymers. In addition to PHAs, PLAs present a medium water and oxygen permeability level comparable to poly(styrene) (PS), another common petrochemical, non-biodegradable plastic used for packaging purposes, or the petroleum-based biodegradable polyester; poly(ε-caprolactone) (PCL) [40].

2.5 *CO_2 barrier*

Similar to oxygen and water vapor barrier properties, the CO_2 barrier property is also of particular importance for food packaging application. The CO_2 barrier is quantified by the CO_2 permeability coefficients (CO$_2$PC), which indicate the amount of CO_2 that permeates a packaging material per area unit and time. Alternatively to the permeability coefficients, the CO_2 transmission rate (CO$_2$TR), expressed in cc m^{-2} s^{-1} (or g m^{-2} day^{-1}), is frequently reported in the literature [23].

PHB generally shows a CO_2 permeability value very close to that of PET and non-plasticized PVC, which are usually considered as materials only modestly permeable to CO_2 [24]. At 4 *atm* (the pressure relevant to carbonized bottled drinks), PHB even outperforms PET in the capture of CO_2. Accordingly, PHB can be considered as a good barrier material against CO_2 permeation; in the case of PHBV copolyesters, inferior barrier performance against CO_2 permeation is reported in the literature [23, 24, 38].

2.6 *Flavoring substances*

High barrier for flavoring compounds is important in order to preserve the flavor of the packed food. Due to its barrier properties for the substances responsible for the sensory quality, 1-methyl-4-(1-methylethenyl)-cyclohexene (limonene) is a frequently used test substance to quantify the properties of polymeric materials. In the case of PHB, lower values for limonene permeability are reported if compared to PET (0.088 * 10^{-13} *vs.* 1.17 * 10^{-13} kg m s^{-1} m^{-2} Pa^{-1}); whereas this value significantly rises in the case of PHBV copolyesters [40].

2.7 *Chemical resistance*

Products that are packaged in food containers may have weak or strong acidity that might cause hydrolytic reactions to the packaging material. As a well-known feature, PHAs easily undergoes acid-catalyzed hydrolytic degradation. Therefore, it is necessary to assess the performance and the suitability of biopolymers stored with common food packaging solution as a function of time. The interaction and absorption between the chemical compounds and the polymers can affect the final mechanical properties of a polymer. Normally, the chemical resistance is tested by measuring the tensile stress, elongation at break, and modulus of elasticity of the polymer specimens submerged in weak and strong acid solutions as a function of time, and simulating real conditions at different temperatures. For test purposes, a weak acetic acid solution is used to mimic slightly acidic conditions, while strong acid conditions are imitated by the solutions of hydrochloric acids [36].

Regarding the organic vapors, Miguel and Iruin tested PHB films at 100% vapor saturation at 30°C with the organic solvents of highly varying polarity. PHB displayed high barrier

properties against solvents of low (n-hexane, isopropyl ether) or very high (methanol, similar performance to water) polarity, whereas the solvents exhibiting polarities similar to PHB (acetone, butyl acetate and toluene) easily permeated PHB [20].

2.8 *Cost performance*

As a major drawback in production of PHA biopolyesters on a large scale, e.g. for bulk applications such as food packaging, a satisfying cost-performance scenario is still not sufficiently achieved, mainly based on the high expenses of raw materials [7, 21]. Production of petrochemical polymers constitutes an old routine technology, well-established since decades, especially in the case of "simple" materials such as PE, poly(propylene) (PP), PET, PS or PVC. According to the literature, the production prices of such petrochemical polymers are reported to be in a magnitude of 1 US-$, whereas, depending on the polyester composition and the final product purity, roughly the 5-10 fold price has to be calculated for PHA [6, 7, 9, 21, 41].

In the case of biopolymers, PLA used to be already be produced at low price due to the high yields of the lactic acid obtained by anaerobic fermentation of simple, inexpensive carbon sources and the simple chemical requirements to polymerize lactic acid towards PLA *via* ring-opening polymerization [42]. Here, it has to be emphasized that the application of PLA, which is highly restricted due to the rather brittle character of this material in its pure form, is not accessible towards thermoforming for production of enwrapping foils. Hence, its application in the food packaging sector is rather restricted to rigid cups and containers [21].

In contrast, PHAs are more flexible based on a myriad of possible compositions on the monomeric level. Special building blocks different from 3HB require the supply of the producing strain with structurally related carbon sources during PHA-biosynthesis. 3HV, for example, is incorporated into growing PHA chains by numerous microbes if odd-numbered fatty acids like propanoic acid or pentanoic acid are provided. These substances constitute a considerable cost factor in the entire PHA production process. It has to be stressed that, other than anaerobic lactic acid production that converts more than 90% of a supplied carbon source into lactic acid, PHA biosynthesis occurs under aerobic conditions. This results in a considerable loss of carbon sources of about 50%

by intracellular conversion towards CO_2 and minor side-products [41].

Applying inexpensive carbon sources for production of catalytically active biomass and, subsequently, PHA is a central task of global research to render PHA production economically efficient [21, 43]. Beside waste streams from food industry as mentioned in 2.1, crude glycerol from biodiesel production [44, 45], hydrolysis products of (ligno) cellulosic materials [46], or even toxic C1-compounds like methanol [47, 48] can be used for heterotrophic PHA production; autotrophically, CO_2 can act as the main carbon source by the specialized microbes like cyanobacteria [49-51], namely "Knallgasbacteria" like *Cupriavidus necator* [52], or novel strains like *Ideonella* sp. 01 [53].

Apart from the raw material side, the process design [11] and method for recovery of PHA from biomass [29] significantly determine the cost efficiency of PHA-production [reviewed by 9, 21].

3- Examples of Biopolymers as Food Packaging Materials

3.1 *Basic performance of PHA for food packaging*

Miguel and Iruin [20] carried out basic studies on water, CO_2 and organic solvent transport through the films of PHB and PHBV with the 3HV molar ratios of 0, 8, 14, and 24 mol/mol. The films of 25 to 50 μm thickness were obtained by simple casting of chloroform solutions. The authors reported similar values for the barrier performance, regarding the water, CO_2 and solvents, known from the common plastics (PET and PVC), especially for the food-relevant compounds (water and CO_2). On the other hand, they found a low barrier against organic compounds in the case of solvents with similar polarity like the tested PHAs [20].

PHB packaging for the storage of food products was investigated by Bucci et al. [54]. They produced PHB-packaging containers by injection molding. The packaging was evaluated by means of dimensional (dimensions, volumetric capacity, mass and thickness), and mechanical (dynamic compression and impact resistance) tests, and compared it with the classically used poly(propylene) (PP) packaging of the same dimensions. In addition, the potential for organoleptic contamination of some food types (cream cheese, margarine, and mayonnaise) was studied. The authors reasoned that, compared to PP, PHB had a significantly lower resistance to dynamic compression. This is revealed by a

deformation value of about 50% lower than that reported for PP; hence, PHB constitutes a more rigid and less flexible material, if compared to PP. Under normal freezing and refrigeration conditions, the performance of PHB turned out to be inferior to that of PP, whereas at higher temperatures, PHB performed even superior to PP. The dimensional characterization assay results underlined the necessity of designing special mold mixtures for the injection of PHB or for the optimization of injection conditions and mold temperature. The results of the sensory tests accomplished with selected foods revealed no significant difference to PP. Based on the results, the authors reasoned the feasibility of designing packaging made from bio-based materials such as PHB [54].

Later, the same authors carried out additional studies to investigate the potential of PHB as packaging material for food products. The polymer, through the injection process, was used for the manufacture of 500 mL packaging set consisting of vessel and cup. The performance of the packaging was evaluated through physical tests (visual analysis, and UV-light transmission), and compared with PP packaging. In addition, migration tests of packaging components and biodegradation assays were carried out. The problems identified during the production of the PHB packaging were overcome by adjusting the manufacturing process in terms of triggering the mold and the applied PHB structure. It turned out that the packaging acted as an efficient barrier to UV-light transmission in the spectral range of $\lambda = 250–350$ nm. Biodegradation experiments were successfully performed under different environmental conditions [55].

The results demonstrated the great potential of PHB as food packaging material. The PHB packaging underwent fast degradation in different environments, including a desired property regarding the enormous contemporary consumption of processed food, which requires huge quantities of packaging for conservation. This is in contrast to the PP and PET packaging that is recalcitrant towards biodegradation, causing the accumulation of solid waste material in the environment. The investigated PHB was revealed to be promising to be applied in food packaging, since it acts as a good barrier to light incidence in the UV range. In relation to the migration of components, pigmented PHB packaging can be also recommended for different foods under conservation conditions in both the long- and near- term [55].

3.2 *PHA-coated paper*

An interesting strategy might be the development of sheets consisting of paper coated with a PHA layer for enwrapping of food goods. As a matter of fact, paper-based materials represent an alternative to the developing compostable and biodegradable packaging. Coating of paper with (bio)polymers provides a way to decrease the high hydrophilicity of the paper. Dagnon et al. described the first attempts to produce *scl*-PHA-Kraft paper–composites. For this purpose, *scl*-PHA sheets were first produced by compression molding between the Teflon sheets. The obtained *scl*-PHA sheets were further compressed on Kraft paper sheets. The biodegradation of poly(4HB)-coated Kraft-paper has already been demonstrated successfully [22]. Based on the low melting points and the high sticky character of *mcl*-PHA, a thin layer of these bio-resins attached on simple paper sheets could be an innovative alternative to contemporary paper-plastic two-layer latex composite sheets as used in retail. As a prerequisite, the breakthrough of *mcl*-PHA production on a large scale has finally to be launched.

3.3 *Enhanced barrier properties by developing composite materials*

The development of PHA-based composites has attracted increasing attention during the last two or three decades. Generally, such composite materials can exhibit a further range of changed material properties such as decreased density compared to basic PHAs. In the case of using inexpensive filler- and composite materials, e.g. straw, bagasse or saw dust, one can achieve a better price performance of the product. Regarding the food packaging sector, barrier properties for the water vapor, oxygen, and flavorful compounds of PHB and PHBV copolyesters can be enhanced by manufacturing composite materials with various compatible materials like other polyesters (PLA, PCL and PVA), proteins (zein, keratin and whey proteins), or carbohydrates (cellulose, lignocellulose, starch). As a novel field of development, different compatible materials can be incorporated into the PHA matrix as nanoparticles. Nanoparticles have the potential to improve special material features such as gas permeability and certain thermo-mechanical properties, thus providing a strategy to further fine-tune the properties of the final composite material [56].

The possibility to design high-performance composite materials of different biopolymers (in this case, PLA and PHB) was recently

demonstrated by Guinault *et al.* [57]. PHBV was allied with PLA in order to improve its gas barrier and mechanical properties. Two processes, classical 3 layer co-extrusion and original polymer blending extrusion, have been used in this study to combine PLA and PHBV to obtain films with different PLA-PHBV structures. Thermo-formability of the different films was compared and the final structure in relationship with their gas barrier properties was investigated. The effect of the thermoforming step has been studied by separating heating and stretching effects. It was demonstrated that the blend morphology results in superior mechanical and barrier properties than single PLA or 3-layers PLA/PHBV films [57]; these experiments open the door to design flexible thermoformed films to be used for enwrapping of food.

In recent years, multilayer polymeric films have attracted increasing interest for many applications, also in food industry. Therefore, multilayer structures based on PHBV with a molar fraction of 12% 3HV, containing a high barrier interlayer of electrospun nanofibers of zein, a storage protein in corn kernels, were developed by Fabra et al. [58]. The authors observed that the method used for the preparation of the outer PHBV layers (casting *vs.* compression-molding) affected their functional properties; the mechanical resistance was increased, and the water vapor permeability and transparency decreased in the multilayer containing outer layers prepared by compression-molding to a higher extent as compared which the samples prepared by casting. The addition of zein interlayer produced only marginal changes in the mechanical and optical film properties while the incorporation of zein nanofiber interlayers significantly improved the oxygen barrier properties of the multilayer films prepared by both processing technologies. The effect of the interlayer on permeability for water vapor, and the flavorful model compound 1-methyl-4-(1-methylethenyl)-cyclohexene limonene highly depended on the zein fraction. Thus this approach provides an innovative way to develop fully renewable microbial biopolyester-based multilayer structures with enhanced barrier performance of significant interest in food packaging applications [58].

Similar attempts were recently described by Pardo-Ibanez *et al*. [59]. Sustainable biocomposite materials based on the combination of PHA with a keratin additive derived from poultry feathers were successfully developed *via* melt compounding. Suitable dispersions for low loadings of the additive in the PHA matrix were achieved by a melt-mixing technique. Satisfying physical interaction between the polymeric matrix and the additive was observed by scanning electron microscope (SEM). Reductions in water, limonene, and oxygen permeability of the pure polymer to less than a half of their initial values for the composite containing 1 wt% of keratin additive were achieved. This composition was also found to exhibit the optimum mechanical performance. As a result, these materials offer significant potential in fully renewable PHA-based packaging applications with enhanced barrier performance [59].

Apart from the carbohydrate fraction, surplus whey from dairy industry contains a fraction of proteins, namely "whey retentate", which predominately consists of the milk albumins [60]. Recently, the WHEYLAYER project developed a biopolymer-coating based on whey protein for plastic films able to replace the currently used expensive synthetic oxygen barrier reaching an OTR of 1 cm^3 $m^{-2}d^{-1}$ bar at standard conditions and a WVTR of 2 g m^{-2} d^{-1}. The multi-layer film materials can easily be recycled, and show significant reduction in the environmental impact of the resulting packaging; preliminary evaluation showed that they fulfilled food safety regulations. At the moment, the new materials are tested and evaluated as packaging solution for food and other goods [61]. A combination of these protein-based materials with PHA layers might open the door for new food packaging strategies.

Another example to improve biopolymer properties by designing nanostructured composites is the application of nano-whiskers of bacterial cellulose. These nano-whiskers were incorporated into PHA by solution casting technique by Martínez-Sanz et al. [62]. It was demonstrated that the barrier properties strongly depend on the relative humidity, and can be improved at low cellulose nano-filler loadings. This increase of barrier capacity can easily be understood by considering the fact that the incorporated cellulose nano-particles display roadblocks for the materials to be transported; similar effects have been reported for inorganic nanoparticles, e.g. clay [63].

3.4 *Functionalized packaging materials*

Attempts to develop food packaging materials with immanent antimicrobial activity as a form of "active packaging" to elongate the shelf-life of food, and to avoid the contamination with microbial pathogens like *Listeria monocytogenes*,

Salmonella typhimurium, pathogenic *Escherichia coli*, or molds were already reviewed comprehensively in 2002 by Appendini and Hotchkiss [64]. Incorporation of bioactive compounds for food packaging was also commercially applied; silver-substituted zeolithes are most frequently used for this purpose. Antimicrobial activity was supplied to the polymers, e.g. by covalently linking the polymers to bacteriocins or lysozyme. As carrier materials, polymers like PS, PVC and nylon were investigated; the use of PHA for this purpose was not reported [64]. As a well-known drawback, many antibiotics are sensitive against the temperature need for polymer processing; as an outdoor, they can be coated on the polymeric films after processing or added on cast films. Reported examples encompass nisin-methylcellulose coatings on PE-films, or nisin-zein coatings [65]. A more simple strategy encompasses the enclosure of small bags to the interior of a food package. These bags can function as oxygen absorbers, moisture absorbers or even ethanol vapor generators [64]. Another remarkable approach is the generation of packaging surfaces consisting of polymers with inherent antimicrobial character. As an example, positive surface charge enjoying, chitosan causes adverse effects on the microbial cells [66].

Also PHA, can be functionalized and equipped with antimicrobial activity, thus supporting their performance in food packaging. Loading of *mcl*-PHA with functionalities against bio-fouling (zosteric acid) was already successfully demonstrated by Hany et al. [67]. Recently, synthesis and structural investigations of 3HB-oligomer conjugates with sorbic acid and benzoic acid, explicitly designed for active food packaging systems, was reported by a Polish research group. The preservative–oligomer conjugates were obtained *via* the anionic ring-opening oligomerization of racemic β-butyrolactone initiated by sodium sorbate or sodium benzoate, respectively. The structures of the resulting conjugates have been elucidated at the molecular level by electrospray ionization multi-stage mass spectrometry supported by ^{1}H-NMR. Fragmentation of selected ions of the resulting conjugates confirmed that molecules of preservatives (sorbate and benzoate, respectively) are covalently bound to the 3HB-oligomers with unchanged structure [68]. These results might pure the way for functional, biological food packaging.

4- Conclusion

The presented work demonstrated that PHA biopolyesters meet many required material features needed for developing useful, biodegradable food packaging. Advanced, inexpensive and simple purification methods have already been developed, competitive production prices can be reached by applying inexpensive raw materials like (agro) industrial waste or CO_2, upgraded to function as carbon sources, for large-scale PHA biosynthesis.

Shortcomings regarding the high crystallinity of "simple" PHA homopolyesters (PHBs) can be overcome by switching into high-quality copolyesters. Such copolyesters display excellent characteristics as low melting points and high flexibility, although their barrier properties for O_2, CO_2, flavors and water significantly decrease as compared to simple PHBs. This drawback can be overcome by creating novel composite materials with additional compatible matters of natural origin, such as various surplus proteins. Furthermore, the combination of multilayer materials with functionalization of the PHA moieties may open the door for high-tech, antimicrobial packaging devices with tailored target application in the food sector. Future work should especially focus on new antimicrobial compounds with wide spectrum of activity and low toxicity bound to the biopolymers like PHA.

Declaration of interest

The author states that there exists no conflict of any interest.

References

1. Electronic resource: http://www.plasticseurope.org/documents/document/20121120170458-final_plasticsthefacts_nov2012_en_web_resolution.pdf downloads 30122013
2. Electronic resource: http://www.worldpackaging.org/i4a/doclibrary/getfile.cfm%3Fdoc_id%3D7+&cd=2&hl=de&ct=clnk&gl=at downloads 30122013
3. Keshavarz T, Roy I. Polyhydroxyalkanoates: Bioplastics with a green agenda. Curr Opin Microbiol. 2010; 13: 321-326.
4. Shah A, Hasan F, Hameed A, Ahmed S. Biological degradation of plastics: A

comprehensive review. Biotechnol Adv. 2008; 26: 246-265.

5. Braunegg G, Bona, Koller M. Sustainable polymer production. Polym-Plast Technol. 2004; 43: 1779-1793.

6. Chen GQ. Introduction of bacterial plastics PHA, PLA, PBS, PE, PTT, and PPP, Plastics from bacteria: Natural functions and applications. 2010; 14: 1-16.

7. Koller M, Salerno A, Muhr A, Reiterer A, Braunegg G. Polyhydroxyalkanoates: Biodegrad-able polymers and plastics from renewable resources. Mater Tehnol. 2013; 47: 5-12.

8. Verlinden RA, Hill DJ, Kenward MA, Williams CD, Radecka I. Bacterial synthesis of biodegrade-able polyhydroxyalkanoates. J Appl Microbiol. 2007; 102: 1437-1449.

9. Koller M, Gasser I, Schmid F, Berg G. Linking ecology with economy: Insights into polyhydroxy-alkanoate producing microorganisms. Eng Life Sci. 2011; 11: 222-237.

10. Khanna S, Srivastava AK. Recent advances in microbial polyhydroxyalkanoates. Process Biochem. 2005; 40:607-619.

11. Atlić A, Koller M, Scherzer D, Kutschera C, Grillo-Fernandes E, Horvat P, *et al.* Continuous production of poly ([*R*]-3-hydroxybutyrate) by *Cupriavidus necator* in a multistage bioreactor cascade. Appl Microbiol Biot. 2011; 91: 295-304.

12. Kunioka M, Tamaki A, Doi Y. Crystalline and thermal properties of bacterial copolyesters: Poly (3-hydroxybutyrate-co-3-hydroxyvalerate) and poly (3-hydroxybutyrate-co-4-hydroxybutyrate). Macromolecules. 1989; 22: 694-697.

13. Martin DP, Williams SF. Medical applications of poly-4-hydroxybutyrate: A strong flexible absorbable biomaterial. Biochem Eng J. 2003; 16: 97-105.

14. Muhr A, Rechberger EM, Salerno A, Reiterer A, Schiller M, Kwiecień M, *et al.* Biodegradable latexes from animal-derived waste: Biosynthesis and characterization of *mcl*-PHA accumulated by *Ps. citronellolis*. React Funct Polym. 2013; 73: 1391–1398.

15. Muhr A, Rechberger EM, Salerno A, Reiterer A, Malli K, Strohmeier K, *et al.* Novel description of *mcl*-PHA biosynthesis by *Pseudomonas chlororaphis* from animal-derived waste. J Biotechnol. 2013; 165: 45–51.

16. Zinn M. Biosynthesis of Medium-Chain-Length Poly [(*R*)-3-hydroxyalkanoates]. Plastics from bacteria: Natural function and

17. Zhao W, Chen GQ. Production and characterization of terpolyester poly (3-hydroxybutyrate-*co*-3-hydroxyvalerate-*co*-3-hydroxyhexanoate) by recombinant *Aeromonas hydrophila* 4AK4 harboring genes *phaAB*. Process Biochem. 2007; 42: 1342-1347.

18. Koller M, Hesse P, Bona R, Kutschera C, Atlić A, Braunegg G. Biosynthesis of high quality polyhydroxyalkanoate co- and terpolyesters for potential medical application by the archaeon *Haloferax mediterranei*. Macromol Symp. 2007; 1: 33-39.

19. Williams SF, Martin DP. Applications of PHAs in Medicine and Pharmacy. Biopolymers Online. 2005.

20. Miguel O, Iruin JJ. Evaluation of the transport properties of poly (3-hydroxybutyrate) and its 3-hydroxyvalerate copolymers for packaging applications. Macromol Symp. 1999; 144: 427-438.

21. Koller M, Salerno A, Dias M, Reiterer A, Braunegg G. Modern biotechnological polymer synthesis: A review. Food Technol Biotech. 2010; 48: 255-269.

22. Dagnon KL, Thellen C, Ratto JA, D'Souza, NA. Physical and thermal analysis of the degradation of poly (3-hydroxybutyrate-co-4-hydroxybutyrate) coated paper in a constructed soil medium. J Polym Environ. 2010; 18: 510-522.

23. Siracusa V, Rocculi P, Romani S, Rosa MD. Biodegradable polymers for food packaging: A review. Trends Food Sci Tech. 2008; 19: 634-643.

24. Mensitieri G, Di Maio E, Buonocore GG, Nedi I, Oliviero M, Sansone L, *et al.* Processing and shelf life issues of selected food packaging materials. Trends Food Sci Tech. 2011; 22: 72-80.

25. Harding KG, Dennis JS, Von Blottnitz H, Harrison STL. Environmental analysis of plastic production processes: Comparing petroleum based polypropylene and polyethylene with biologically based poly-β-hydroxybutyric acid using life cycle analysis. J Biotechnol. 2007; 130: 57-66.

26. Koller M, Sandholzer D, Salerno A, Braunegg G, Narodoslawsky M. Biopolymer from industrial residues: Life cycle assessment of poly (hydroxyalkanoates) from whey. Resour Conserv Recy. 2013; 73: 64-71.

27. Shahzad K, Kettl K-H, Koller M, Titz M, Schnitzer H, Narodoslawsky M. Comparison

of ecological footprint for bio based PHA production from animal residues utilizing different energy resources. Clean Technol Environ Pol. 2013; 15: 525-536

28. Koller M, Niebelschütz H, Braunegg G. Strategies for recovery and purification of poly [(*R*)-3-hydroxyalkanoates] (PHA) biopolyesters from surrounding biomass. Eng Life Sci. 2013; 13: 549-562.

29. Choi JI, Lee SY. Efficient and economical recovery of poly (3-hydroxybutyrate) from recombinant *Escherichia coli* by simple digestion with chemicals. Biotechnol Bioeng. 1999; 62: 546-553.

30. Shishatskaya EI, Volova TG. A comparative investigation of biodegradable polyhydroxyalkanoate films as matrices for *in vitro* cell cultures. J Mater Sci: Materials in Medicine. 2004; 15: 915-923.

31. Koller M, Bona R, Chiellini E, Braunegg G. Extraction of short-chain-length poly-[(*R*)-hydroxyalkanoates] (*scl*-PHA) by the "anti-solvent" acetone under elevated temperature and pressure. Biotechnol Lett. 2013; 35: 1023-1028.

32. Furrer P, Panke S, Zinn M. Efficient recovery of low endotoxin medium-chain-length poly ([*R*-3-hydroxyalkanoate) from bacterial biomass. J Microbiol Meth. 2007; 69: 206-213.

33. Khosravi-Darani K, Vasheghani-Farahani E. Application of supercritical fluid extraction in biotechnology. Critic Rev Biotechnol. 2005; 25: 1-12.

34. Khosravi-Darani K, Vasheghani-Farahani, E, Shojaosadati SA, Yamini Y. Effect of process variables on supercritical fluid disruption of *Ralstonia eutropha* cells for poly (*R*-hydroxybutyrate) recovery. Biotechnol Prog. 2004; 20: 1757-1765.

35. Khosravi-Darani K, Vasheghani-Farahani E, Yamini Y, Bahramifar N. Solubility of poly (β-hydroxybutyrate) in supercritical carbon dioxide. J Chem Eng Data. 2003; 48: 860-863.

36. Gas permeability (plastic films manometric). Electronic resource:
http://www.mecadi.com/en/permeation_leakage/permeation_measurement/gas_permeability/din_53380-2_din_53380-m

37. Packaging Technology. Electronic resource:
http://packagingtech.net/31-barrier-polymers.html

38. Shogren R. Water vapor permeability of biodegradable polymers. J Environ Polym Deg. 1997; 5: 91-95.

39. Auras RA, Singh SP., Singh JJ. Evaluation of oriented poly (lactide) polymers vs. existing PET and oriented PS for fresh food service containers. Packag Technol Sci. 2005; 18: 207-216.

40. Sanchez-Garcia MD, Gimenez E., Lagaron JM. Novel PET nanocomposites of interest in food packaging applications and comparative barrier performance with biopolyester nanocomposites. J Plas Film Sheet. 2007; 23: 133-148.

41. Koller M, Bona R, Braunegg G, Hermann C, Horvat P, Kroutil M, *et al.* Production of polyhydroxyalkanoates from agricultural waste and surplus materials. Biomacromolecules. 2005; 6: 561-565.

42. Narayanan N, Roychoudhury PK, Srivastava A. L(+) lactic acid fermentation and its product polymerization. Electron J Biotechnol. 2004; 7: 167–178.

43. Khosravi-Darani K, Mokhtari ZB, Amai T, Tanaka K. Microbial production of poly (hydroxybutyrate) from C1 carbon sources. Appl Microbiol Biotechnol. 2013; 97: 1407-1424.

44. Hermann-Krauss C, Koller M, Muhr A, Fasl H, Stelzer F, Braunegg G. Archaeal production of polyhydroxyalkanoate (PHA) co-and terpolyesters from biodiesel industry-derived by-products. Archaea. 2013; 2013: 1-10.

45. Cavalheiro JM, Raposo RS, de Almeida M, Teresa Cesario M, Sevrin C, Grandfils C, *et al.* Effect of cultivation parameters on the production of poly (3-hydroxybutyrate-co-4-hydroxybutyrate) and poly (3-hydroxybutyrate-4-hydroxybutyrate-3-hydroxyvalerate) by *Cupriavidus necator* using waste glycerol. Bioresour Technol. 2012; 111: 391-397.

46. Silva L, Taciro M, Ramos MM, Carter J, Pradella J, Gomez J. Poly-3-hydroxybutyrate (P3HB) production by bacteria from xylose, glucose and sugarcane bagasse hydrolysate. J Ind Microbiol Biotechnol. 2004; 31: 245-254.

47. Braunegg G, Genser K, Bona R, Haage G, Schellauf F, Winkler E. Production of PHAs from agricultural waste material. Macromolecular Symposia. 1999; 144: 375-383.

48. Mokhtari-Hosseini ZB, Vasheghani-Farahani E, Heidarzadeh-Vazifekhoran A, Shojaosadati SA, Karimzadeh R, Darani KK. Statistical media optimization for growth and PHB production from methanol by a methylotrophic bacterium. Bioresour Technol. 2009; 100: 2436-2443.

49. Stal LJ. Poly (hydroxyalkanoate) in cyanobacteria: An overview. FEMS Microbiol Lett. 1992; 103: 169-180.

50. Asada Y, Miyake M, Miyake J, Kurane R, Tokiwa Y. Photosynthetic accumulation of poly-(hydroxybutyrate) by cyanobacteria—the metabolism and potential for CO_2 recycling. Int J Biol Macromol. 1999; 25: 37-42.

51. Wang B, Pugh S, Nielsen DR, Zhang W, Meldrum DR. Engineering cyanobacteria for photosynthetic production of 3-hydroxybutyrate directly from CO_2. Metab Eng. 2013; 16: 68-77.

52. Pohlmann A, Fricke WF, Reinecke F, Kusian B, Liesegang H, Cramm R, *et al.* Genome sequence of the bioplastic-producing "Knallgas" bacterium *Ralstonia eutropha* H16. Nat Biotechnol. 2006; 24: 1257-1262.

53. Tanaka K, Miyawaki K, Yamaguchi A, Khosravi-Darani K, Matsusaki H. Cell growth and P (3HB) accumulation from CO_2 of a carbon monoxide-tolerant hydrogen-oxidizing bacterium, *Ideonella* sp. O-1. Appl Microbiol Biotechnol. 2011; 92: 1161-1169.

54. Bucci DZ, Tavares L, Sell I. PHB packaging for the storage of food products. Polym Test. 2005; 24: 564-571.

55. Bucci DZ, Tavares L, Sell I. Biodegradation and physical evaluation of PHB packaging. Polym Test. 2007; 26: 908-915.

56. Grillo Fernandes E, Pietrini M, Chiellini E. Thermo-mechanical and morphological characterization of plasticized poly(*R*-3-hydroxybutyric acid). Macromol Symp. 2004; 218:157–164.

57. Guinault A, Grandmontagne A, Sollogoub C, Miquelard-Garnier G, Jouannet D, Nguyen AS. The effect of thermoforming of PLA-PHBV films on the morphology and gas barrier properties. Key Eng Mat. 2012; 504: 1135-1138.

58. Fabra MJ, Lopez-Rubio A, Lagaron JM. High barrier polyhydroxyalcanoate food packaging film by means of nanostructured electrospun interlayers of zein. Food Hydrocolloid. 2013; 32: 106-114.

59. Pardo-Ibáñez P, Lopez-Rubio A, Martínez-Sanz M, Cabedo L, Lagaron JM. Keratin–polyhydroxyalkanoate melt-compounded composites with improved barrier properties of interest in food packaging applications. J Appl Polym Sci. 2014; 131(4). DOI: 10.1002/app.39947.

60. Koller M, Hesse P, Bona R, Kutschera C, Atlić A, Braunegg G. Potential of various archae- and eubacterial strains as industrial polyhydroxy-alkanoate producers from whey. Macromol Biosci. 2007; 7: 218-226.

61. Schmid M, Dallmann K, Bugnicourt E, Cordoni D, Wild F, Lazzeri A, *et al.* Properties of whey-protein-coated films and laminates as novel recyclable food packaging materials with excellent barrier properties. Int J Polym Sci. 2012; 2012:1-7.

62. Martínez-Sanz M, Villano M, Oliveira C, Albuquerque MG, Majone M, Reis M, *et al.* Characterization of polyhydroxyalkanoates synthesized from microbial mixed cultures and of their nanobiocomposites with bacterial cellulose nanowhiskers. N Biotechnol. 2014; 31: 364-376.

63. Casariego A, Souza BWS, Cerqueira, MA, Teixeira JA, Cru L, Díaz R, Vicente AA. Chitosan/clay films' properties as affected by biopolymer and clay micro/nanoparticles' concentrations. Food Hydrocolloids 2009; 23: 1895-1902.

64. Appendini P, Hotchkiss JH. Review of antimicrobial food packaging. Innov Food Sci Emerg Technol. 2002; 3: 113-126.

65. Cooksey K. Utilization of antimicrobial packaging films for inhibition of selected microorganisms. In S. Risch, ACS Symposium Series: Food Packaging: Testing Methods and Applications. 2000; 753: 17-25.

66. Goldberg S, Doyle R, Rosenberg M. Mechanism of enhancement of microbial cell hydrophobicity by cationic polymers. J Bacteriol. 1990; 172: 5650-5654.

67. Hany R, Böhlen C, Geiger T, Schmid M, Zinn M. Toward non-toxic antifouling: Synthesis of hydroxy-, cinnamic acid-, sulfate-, and zosteric acid-labeled poly [3-hydroxyalkanoates]. Biomacromolecules. 2004; 5: 1452-1456.

68. Kwiecień I, Adamus G, Bartkowia, A, Kowalczuk M. Synthesis and structural characterization at the molecular level of oligo (3-hydroxybutyrate) conjugates with antimicrobial agents designed for food packaging materials. Des Monomers Polym. 2014; 17: 311-321.