Appl Biochem Biotechnol (2015) 176:1498–1510
DOI 10.1007/s12010-015-1660-9

Material Properties and Antimicrobial Activity of Polyhydroxybutyrate (PHB) Films Incorporated with Vanillin

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Received: 14 February 2015 / Accepted: 3 May 2015 /
Published online: 23 May 2015

Abstract Polyhydroxybutyrate (PHB) was produced by *Bacillus mycoides* DFC 1, isolated from garden soil. Antimicrobial (AM) films of PHB were prepared by incorporating vanillin (4-hydroxy-3-methoxybenzaldehyde) from 10 to 200 μg/g of PHB. The films were assessed for antimicrobial activity against foodborne pathogens and spoilage bacteria comprising of *Escherichia coli*, *Salmonella typhimurium*, *Shigella flexneri*, and *Staphylococcus aureus* and fungi such as *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus parasiticus*, *Aspergillus ochraceus*, *Penicillium viridicatum*, and *Penicillium clavigerum*. The minimum concentration of vanillin required to exhibit antimicrobial activity was ≥80 μg/g PHB for bacteria and ≥50 μg/g PHB for fungi. The PHB films with and without vanillin were studied for mechanical and thermal properties such as tensile strength, Young's modulus, percentage elongation to break, melting temperature, and heat of fusion. The thermal stability of the films was studied using thermogravimetric analysis. The release kinetics of vanillin into food matrices was also checked using food stimulants. The study is intended to find applications for PHB films containing vanillin to enhance the shelf life of foods in the form of biodegradable wrapper.

Keywords Antimicrobial film · Biodegradable · Polyhydroxybutyrate · Vanillin

Introduction

Polyhydroxyalkanoates (PHAs) are a class of microbially produced polyesters that exhibit thermoplastic and elastomeric properties with potential applications as environment-friendly

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biodegradable polymers. Interest in such alternative materials has increased due to the nonbiodegradable nature of petrochemical-derived plastics. PHAs could be divided into three broad classes according to the size of monomers, i.e., PHAs containing up to C5 monomers are classified as short-chain-length PHAs (scl-PHA), whereas PHAs containing carbon chain length in the range of C6–C14 and C14 monomers are classified as medium-chain-length (mcl-PHA) and long-chain-length (lcl-PHA) PHAs, respectively [1]. The poly-β-hydroxybutyrate (PHB) is the first discovered and most studied PHA with advantages of biodegradability by microorganisms as well as UV light [2]. Bacterial PHB could be produced from renewable resources with ecological advantages as compared to thermoplastics and elastomers produced from fossil carbon sources [3]. PHB has been proposed for short-term food applications such as food packaging. It has the advantage of biodegradability by action of PHA hydrolases and PHA depolymerases forming (R)- and (S)-hydroxybutyrates and non-toxic compounds under aerobic and anaerobic conditions [4, 5]. It was demonstrated that PHB films plasticized with Lapol 108, a high molecular weight polyester plasticizer, are more biodegradable in compost conditions in comparison with polylactic acid (PLA) films [6]. Brasava and Dukalska mentioned that polypropylene films could be replaced by PHB films for packaging fat-rich products such as mayonnaise, margarine, and cream cheese and for storage of sour cream [7]. The development of antimicrobial packaging films is required to extend the shelf life of foods and to reduce the health risks caused by foodborne pathogens. Active packaging materials are incorporated with antimicrobial additives for deliberate release into foods for improving food quality and safety as well as extended shelf life [8, 9]. Biodegradable active packaging films made with PLA and PHB blends containing D-limonene, a natural terpene, were shown to be transparent and flexible with enhanced oxygen barrier and water-resistant properties [10]. The PHB films containing antimicrobial compounds are known to play an essential role in food technology as active food packaging materials. Furthermore, PHB films incorporated with eugenol and pediocin were found to be active against food spoilage microorganisms and used for food packaging applications [11]. The slow release of antimicrobial compounds incorporated into the polymeric matrix covering the surface of food might effectively inhibit bacterial growth over a longer period which is more relevant in comparison to direct incorporation of antimicrobial components into food matrices [12]. Natural compounds such as fatty acids and flavoring agents showing antimicrobial activity are desirable for use as natural preservatives with sustained antimicrobial action throughout the storage of food material. Vanillin, a phenolic aldehyde, is a major component of vanilla bean and is an important flavor compound used worldwide with GRAS status [13]. It is widely used as a flavoring agent and food preservative due to its antioxidant and antimicrobial properties according to Karathanos et al. [14]. Vanillin is known to have antimicrobial activity against bacteria, fungi, and yeasts. Chitosan methyl cellulose films with vanillin for wrapping fresh-cut cantaloupe and pineapple showed inhibitory effect against *E. coli* and *Saccharomyces cerevisiae* [15]. Potassium sorbate- and vanillin-incorporated chitosan films inhibited mold growth and were found suitable for extending the shelf life of butter cake [16]. Chitosan films with vanillin as a cross-linking agent were effective against *E. coli* ATCC 8737 and could be used as flavor release films for food or cosmetic product packaging [17]. The biodegradable PHB films produced by *Bacillus* sp. are free from endotoxins and on incorporation with antimicrobial agents may also find applications in the field of biomedicine [18]. The present study was carried out to impart the antimicrobial activity to the biodegradable PHB film by incorporating synthetic vanillin. The antimicrobial efficacy of the vanillin-incorporated PHB film was investigated against food-contaminating bacterial and fungal pathogens. To assess the

effect of vanillin on material properties of PHB, the mechanical and thermal properties of the film were also studied. The specific migration of vanillin into food stimulants was also investigated for food applications.

Materials and Methods

Materials and Chemicals

The bacterial strains *Escherichia coli* (MTCC 23058), *Salmonella typhimurium* (MTCC 98), *Shigella flexneri* (MTCC 1457), and *Staphylococcus aureus* (MTCC 737) and fungi *Aspergillus flavus* (MTCC 277), *Aspergillus fumigatus* (MTCC 15066), *Aspergillus niger* (MTCC 478), *Aspergillus parasiticus* (MTCC 8189), *Aspergillus ochraceus* (MTCC 1877), *Penicillium clavigerum* (MTCC 9182), and *Penicillium viridicatum* (MTCC 4953) used for antimicrobial activity testing were obtained from Microbial Type Culture Collection (MTCC), Chandigarh, India. The bacterial cultures were grown on nutrient agar slants and fungi were grown on potato dextrose agar slants (HiMedia Laboratories, India) and were maintained at 4 °C. The bacterial and fungal cultures were subcultured once in every month to maintain their viability. Vanillin (99 % purity) was purchased from Acros Chemicals, USA, and PHB standard was purchased from Sigma–Aldrich, India.

PHB Production and Extraction

The PHB-producing bacteria *Bacillus mycoides* DFC 1 (GenBank accession number HM 989923.1) isolated from garden soil was grown in a medium with potato starch as carbon source and tryptone as nitrogen source at pH 7.5 and incubated for 48 h at 37 °C in a rotary shaker at 150 rpm. Cell biomass was separated by centrifugation at 8000 rpm for 15 min, and the same was added with an equal amount of 5 % sodium hypochlorite solution and incubated for 1 h at 37 °C. The biomass was centrifuged to remove sodium hypochlorite and washed twice with distilled water to remove excess sodium hypochlorite. The biomass was treated with hot chloroform and kept in a rotary shaker at 150 rpm for 30 min for extracting PHB granules. Finally, the chloroform-containing PHB was filtered using Whatman No. 1 filter paper. Subsequently, the chloroform from PHB was evaporated to obtain PHB films. PHB produced was subjected to Fourier transform infrared spectroscopy (FTIR) analysis using a Thermo-Nicolet FT-IR spectrophotometer, Model 5700 (Madison, WI), and compared with standard PHB.

PHB–Vanillin Film Preparation

Antimicrobial PHB films were prepared using the solvent casting evaporation method. Vanillin (4-hydroxy-3-methoxybenzaldehyde) was added at varying concentrations (20, 40, 50, 80, 100, or 200 μg) to the film-forming viscous solution of PHB–chloroform (1.0 %, *w*/*v*). The solution was vortexed to attain uniform dispersion and was poured on a 90-mm-diameter glass Petri dish. The mixture was allowed to evaporate at 40 °C for 2 h. The films were peeled off and conditioned at room temperature for 48 h before testing. The films were cut into 6.0 mm for assessing the antimicrobial activity.

Preparation of Inoculum

To obtain an early stationary phase culture, a loopful of bacteria was inoculated in nutrient broth and incubated at 37 °C for 18 h prior to experiment. The overnight-grown cultures were twofold serially diluted in 0.85 % normal saline to obtain a density of about 2×10^5 cells. The fungal inoculum was obtained by scraping the PDA plates containing spores prior to experiment. Spore suspensions of the fungal strains were diluted in Tween 80 to obtain 1×10^6/ml for the antifungal assays.

Antimicrobial Activity of Vanillin–PHB Films

Agar disk diffusion assay was carried out to determine the minimal inhibitory concentration (MIC) of the antimicrobial (AM) films. Working cultures of the bacterial strains were prepared by twofold serial dilution of overnight-grown cultures in 0.85 % normal saline. Fungal inoculum was also prepared from spore suspensions. Antimicrobial activity was carried out by spreading 0.1 ml of the bacterial cells or fungal spore suspension over 1.0 % (*w*/*v*) tryptone soy/potato dextrose agar. The AM disks were placed on the surface of the lawn culture, and the plates were incubated at 37 °C for 24 h for bacteria and 28 °C for 3–4 days for fungi. The MIC was determined as the lowest concentration of vanillin-incorporated PHB films showing maximum zone of inhibition against the bacterial and fungal strains.

Analysis of the Thermal Properties of the AM Films

The thermal stability of the polymer was studied using a thermogravimetric analyzer (TGA; Q50, TA Instruments, USA). TGA data reveal change in weight in the material as a function of temperature (or time) under a controlled atmosphere. The sample was placed in a platinum crucible and heated from 50 to 600 °C at a heating rate of 20 °C/min. The mean of percentage of weight loss (T_d) and maximum degradation temperature (T_{max}) was calculated for the samples using the Universal Analysis Software. The melting temperature (T_m) of the neat PHB and antimicrobial PHB films was determined from the position of the endotherm peaks using a differential scanning calorimeter (DSC 2910, TA Instruments, USA). The sample was sealed in an aluminum pan and cooled to −50 °C along with the reference pan under nitrogen atmosphere held isothermally for 1 min and was further heated from −50 to 350 °C at the rate of 20 °C/min.

Analysis of the Mechanical Properties of the AM Films

The films were subjected to measurement of thickness using a digital micrometer and were also analyzed for tensile strength, Young's modulus (Y) (MPa), and percentage elongation to break using a universal testing machine (Lyold Instruments, Model: LRX Plus) with a gauge length of 25 mm and a cross-head speed of 100 mm/min. Samples were prepared by making rectangular strips with dimensions of 5 cm×1 cm, and five strips were tested for each sample.

Migration of Vanillin into Food Stimulants

The migration kinetics of vanillin from the PHB films was studied by total immersion migration method where 1.0 g PHB film strip (1×10 cm) was immersed in the respective

stimulant in amber vials, sealed tightly, and used for experiments. Even if these materials are designed for antimicrobial packaging for foods stored at room temperature, the release experiments were carried out at 37 °C to accelerate mass transfer. Food stimulants were selected as recommended by European food packaging regulations (EC, 1997) [19]. As the migration of vanillin is dependent on the food matrix, the specific migration was investigated in various food stimulants such as distilled water for water-based products, 3.0 % (*v*/*v*) acetic acid for acidic products, 50 % (*v*/*v*) ethanol for fat-rich products, and *n*-hexane for dairy foods.

Statistical Analysis

All experiments were conducted in triplicate and results were expressed as mean±standard deviation.

Results and Discussions

PHB Production and Antimicrobial PHB Film Preparation

The PHB-producing strain of *B. mycoides* DFC 1 (GenBank accession number HM 989923.1) was isolated from garden soil. The PHB production was carried out in media containing potato starch and tryptone. The extracted biomass-containing PHB granules were treated with chloroform to obtain PHB films. The FTIR spectroscopy analysis of PHB produced from *B. mycoides* showed a similar peak when compared with standard PHB; an intense band at 1720 cm^{-1} corresponding to the ester carbonyl (C=O) stretching vibration was observed indicating short-chain-length monomers of PHB (Fig. 1). The PHAs with short-chain-length and medium-chain-length monomers show a strong characteristic ester carbonyl band vibration (C=O stretching vibration) between 1720 and 1740 cm^{-1}. The variation in the peak position also indicates complex chemical environment surrounding the PHAs [20]. Antimicrobial PHB films were prepared by incorporating vanillin at various concentrations from 10 to 200 μg/g of PHB. The solution of PHB–vanillin blend was viscous at a higher concentration of vanillin, and the cast film was pale yellow and opaque when the films were prepared and stabilized for 48 h before further experiments.

Antimicrobial Activity of Vanillin–PHB Films

The inhibitory effect of PHB films incorporated with vanillin was tested against bacterial and fungal food contaminants using agar diffusion assay. The minimum inhibitory concentration against bacteria was ≥80 μg/g PHB, whereas for fungi, it was ≥50 μg/g PHB. The difference in the zone of inhibition indicates the sensitivity of the bacterial and fungal strains to the PHB–vanillin film (Fig. 2 and Table 1). The data are in accordance with the earlier reports by Matamoros et al. [21] that vanillin is more effective in inhibiting bacteria, yeasts, and molds. The mode of action of phenolic compounds on living organisms is concentration dependent which at low concentrations inhibits energy production, while at higher concentrations, it was shown to cause protein denaturation [22]. The antimicrobial activity of vanillin against bacteria and fungi has been reported earlier

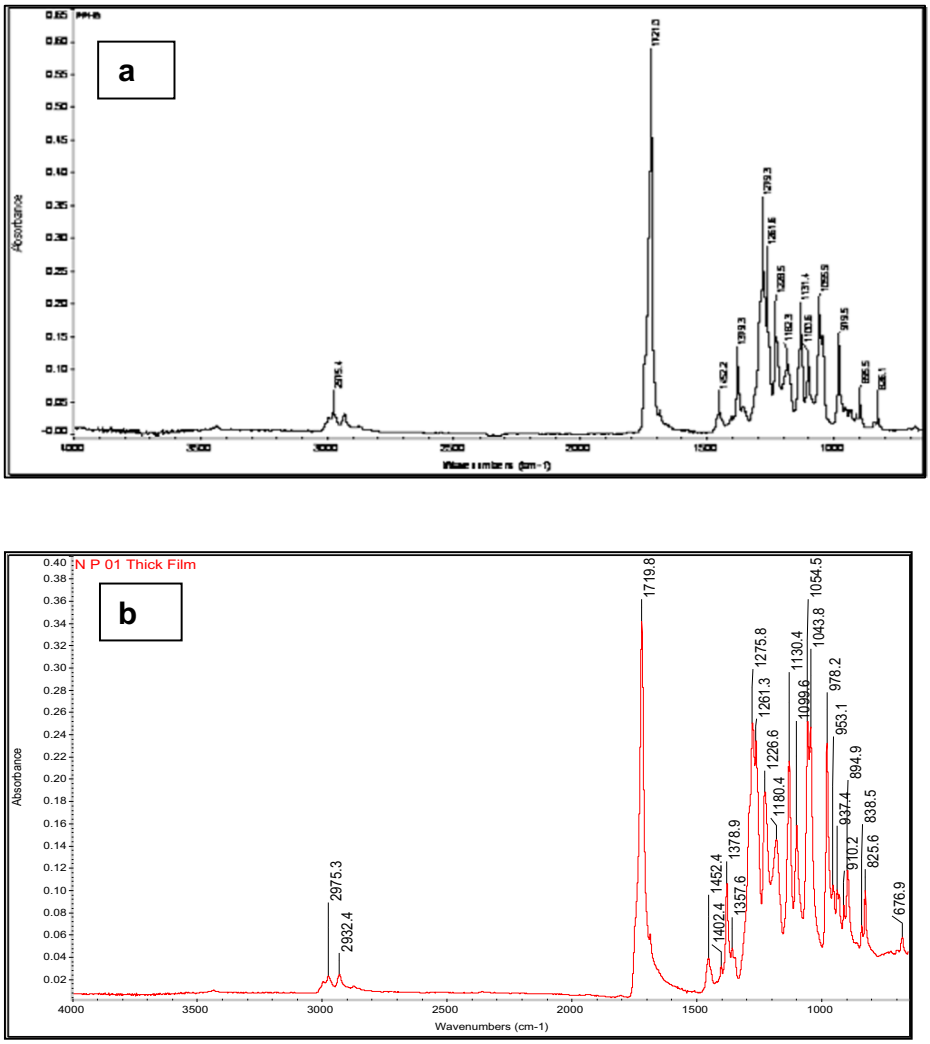

Fig. 1 FTIR spectra of PHB (standard) (**a**) and PHB (*B. mycoides*) (**b**)

and is dependent on the time of exposure, concentration, and target organism. The compound is also known to suppress fungal and total microbial growth in yoghurt significantly when added at 2000 mg/l [23]. The MIC of vanillin was reported in the range of 6 to 18 mM against bacterial pathogens such as *E. coli*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, and *Salmonella enterica* and fungi such as *Candida albicans*, *Penicillium expansum*, and *Saccharomyces cerevisiae* [24]. Chitosan methylcellulose film wrapping containing vanillin 0.45 % (*w*/*v*) was found to be more efficient in reducing the number of *S. cerevisiae* in fresh-cut pineapple [15]. The vanillin-incorporated imino-chitosan biodynameric films showed antifungal activity against *Candida albicans* as reported by Marin et al. [25]. Vanillin used at a concentration of 250 ppm in combination with 210 ppm of licorice extract was found to inhibit growth of *E. coli O157:H7* in liquid coconut endosperm processed under mild heating conditions [26].

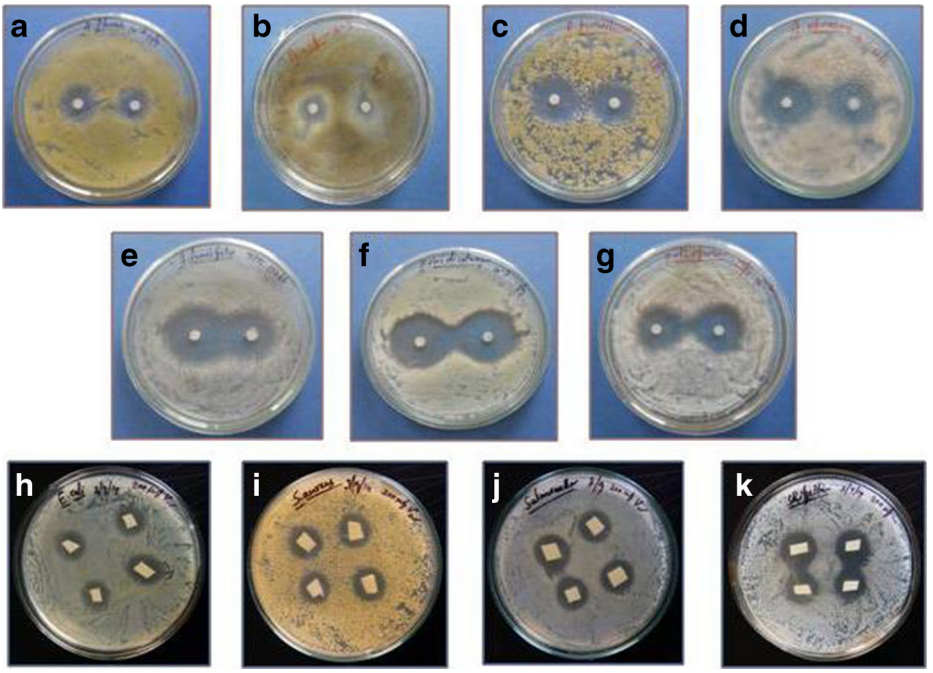

Fig. 2 Minimum inhibitory concentration of PHB–vanillin antimicrobial (AM) films showing antifungal and antibacterial activity against **a** *Aspergillus flavus*, **b** *A. niger*, **c** *A. parasiticus*, **d** *A. ochraceus*, **e** *A. fumigatus*, **f** *P. viridicatum*, **g** *P. clavigerum*, **h** *Escherichia coli*, **i** *Staphylococcus aureus*, **j** *Salmonella typhimurium*, and **k** *Shigella flexneri*

Thermal Properties of the AM Films

The thermal stability of the polymer was studied using TGA (Fig. 3). The initial weight loss for antimicrobial PHB films incorporated with vanillin was observed at 50.0 °C, while for neat

Table 1 MIC of vanillin-incorporated PHB films by agar diffusion assay

Microorganism	MIC[a]	Zone size (mm)
Staphylococcus aureus MTCC 737	80	14±1.0
Salmonella typhimurium MTCC 98	80	14±1.0
Escherichia coli MTCC 23058	100	12±0.5
Shigella flexneri MTCC 1457	80	16±0.5
Aspergillus flavus MTCC 277	50	16±1.0
A. fumigatus MTCC 15066	50	32±1.0
A. niger MTCC 478	50	16±1.0
A. parasiticus MTCC 8189	50	19±0.5
A. ochraceus MTCC 1877	50	20±0.5
Penicillium viridicatum MTCC 4973	50	24±0.5
P. clavigerum MTCC 9182	50	21±0.8

[a] Minimum inhibitory concentration of vanillin-incorporated PHB (in micrograms per gram of PHB)

PHB films, the initial weight loss was noticed at 150.0 °C. The thermal decomposition (T_d) of PHB–vanillin combination films showed a gradual weight loss from 119.0 °C T_d (5 %) to 225.0 °C T_d (80 %), and the maximum thermal decomposition (T_{max}) occurred at 200.0 °C. The thermal decomposition (T_d) for neat PHB films showed a gradual weight loss from 221.2 °C T_d (5 %) to 270.0 °C T_d (80 %) with maximum thermal decomposition (T_{max}) at 275.0 °C. Weight loss occurred in three stages for PHB–vanillin films. The initial weight loss was noticed below 100.0 °C which is due to water losses; the second weight loss was between 100.0 and 175.0 °C due to evaporation of vanillin, and the major weight loss occurred at 225.0 °C due to complete degradation of PHB–vanillin films. The antimicrobial PHB films tested showed reduced thermal stability from 275.0 to 200.0 °C in comparison to neat PHB films (Fig. 3 and Table 2). The observations in our study are similar to studies on PHB–PLA blends by Arrieta et al. [10], which showed a decreased maximum thermal decomposition temperature (T_{max}) from 286.0 to 278.0 °C of the films in combination with D-limonene due to interfacial interactions between the polymer and additive. The thermal stability of PHB–PLA blends improved on addition of acetyl tributyl citrate (ATBC) and polyethylene glycol (PEG) [27]. Furthermore, improved thermal stability of PVA–vanillin films was reported due to encapsulation of vanillin using cyclodextrin in PVA nanowebs [28]. In another study, PHB blended with PEG and citrate esters, such as ATBC, as plasticizers improved the properties desirable for large-scale film production [29].

The AM films were also studied using differential scanning calorimetry (DSC) for thermal properties. The melting temperatures (T_m) for neat PHB and vanillin were found to be 173.0 and 63.8 °C, with melting enthalpy values of neat PHB and vanillin of 28.0 and 49.5 J/g, respectively. These are similar to data reported by Matamoros et al. [20] where the T_m of neat PHB was shown in the range of 173.0 to 180.0 °C, while the T_m of vanillin was 80.9 °C. Neat

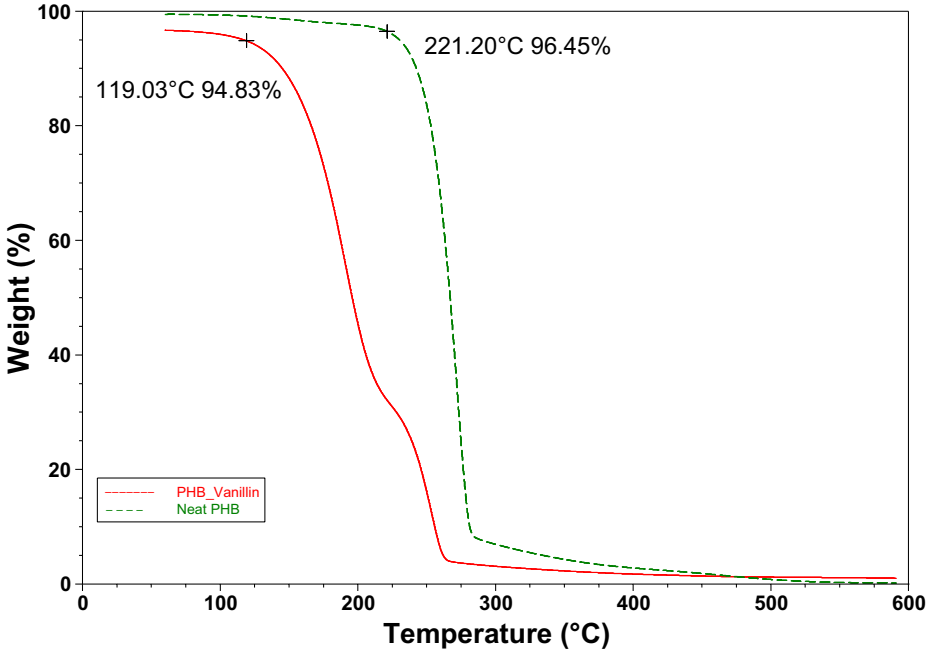

Fig. 3 TGA thermogram of neat PHB and PHB–vanillin films

Table 2 Comparison of properties of vanillin-incorporated PHB films and neat PHB films

Properties	Neat PHB films	Antimicrobial PHB films
Melting point, T_m (°C)	173.0	217.3
Thermal decomposition, T_{max} (°C)	275.0	200.0
Tensile strength (MPa)	9.4±0.81	1.89±0.32
Young's modulus (MPa)	25,186±3218	1863.3±252
Elongation at break (%)	0.91±0.085	2.09±0.075

PHB showed two endotherm melting peaks, and the peak at higher temperature at 270.7 °C could be attributed to complete degradation of PHB and the lower temperature peak at 173.0 °C is due to melting of imperfect PHB crystals formed during sample preparation. Double melting peaks for neat PHB were reported by Sindhu et al. [30] while studying the thermal properties of PHB produced from *Bacillus sphaericus* using DSC. The PHB–vanillin films also showed three endothermic melting peaks at 63.8, 217.3, and 256.7 °C which indicate the formation of crystalline structures with different perfection and thermodynamic stabilities between neat PHB and vanillin. The results showed a decreased T_m for vanillin at 63.8 °C, while T_m for PHB increased to 217.3 °C with changes in melting behavior. The melting peak observed at 256.7 °C for PHB–vanillin films could be due to partial or complete degradation of vanillin at higher temperatures (Fig. 4 and Table 2). Similar results were obtained by Bartczak et al. [4] while studying the thermal properties of poly(lactide) and amorphous poly([*R*,*S*]-3-hydroxybutyrate). Thermal oxidation of vanillin to vanillic acid at temperatures ranging from 258.0 to 278.0 °C using DSC curves and GC-MS was reported

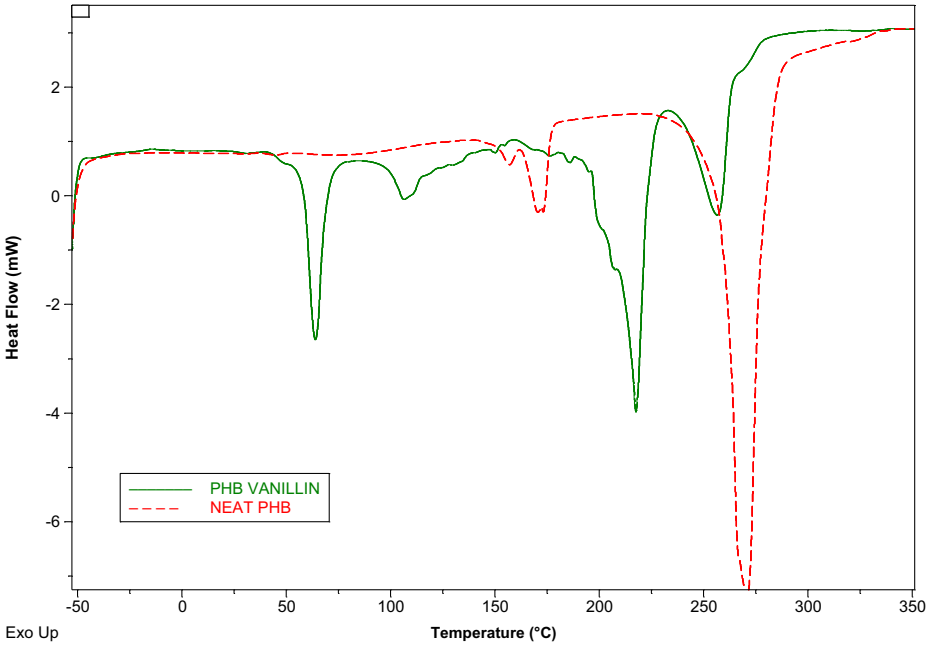

Fig. 4 DSC thermogram of neat PHB and PHB–vanillin films

[31]. The T_m of neat PHB showed reduction from 167.7 to 164.9 °C on addition of PLA and limonene [10].

Mechanical Properties of the AM Films

The thickness of neat PHB films was determined as 51.40 μm, while the thickness of PHB–vanillin films was found as 93.40 μm. The addition of vanillin probably contributed to the increase in thickness of the films. The films became soft due to the addition of vanillin as shown by reduction in tensile strength and Young's modulus data (Table 2). The percentage elongation at break for PHB films with vanillin improved to 2.09 %, while for the neat PHB films, it was only 0.91 %. Earlier reports showed reduction in tensile strength and Young's modulus of PHB films with the incorporation of eugenol while other material properties such as percent elongation to break and seal strength of the polymer improved [11]. The thickness and elasticity of chitosan methylcellulose films incorporated with vanillin increased while tensile strength was reduced [12]. Our results were similar to the data reported by Arrieta et al. [10] where the addition of D-limonene to PHB and PLA blends contributed to a significant reduction in tensile strength and Young's modulus while the elasticity of the films was found to increase.

Determination of the Migration of Vanillin from PHB Films

Samples of solution were removed from food stimulants after 24 h, and vanillin released into food stimulants was quantified using a UV–vis spectrophotometer at 231 nm from a previously obtained standard curve. We conducted our studies under an extended storage temperature of 37 °C for 24 h to study the impact of vanillin in controlling the food pathogens at early stages of food storage. Table 3 shows that the specific migration of vanillin depended on the type of food stimulant used. In the present study, vanillin migrated maximum at 37 °C into the stimulant 50 % ethanol (71.736 μg/ml) followed by distilled water (65.54 μg/ml) which could be due to increase in temperature and faster migration of vanillin into 50 % ethanol than distilled water. Moreover, increased migration is due to the better solubility of vanillin in ethanol than other hydrophobic solvents. Previous studies on migration of vanillin reported equilibrium of vanillin was reached in 20 min at 24 °C for distilled water due to the higher affinity of the polar stimulant towards the nonpolar vanillin [32]. A steady state was attained after 17.4 min in water at 35 °C, and increase in temperature enhanced the quantity of vanillin migration into the stimulant. Antimicrobial polypropylene films were studied for migration of catechins and quercetin into 50 % ethanol, and fast release of antioxidants was reported at 40 °C [33]. Catechin release from PLA films was rapid into 50 % ethanol on addition of PHB with PLA due to the higher affinity of the less polar catechin towards the polar stimulant [10]. Fast release of an antimicrobial agent could be an advantage in order to restrict the microbial growth at early stages of food storage. Faster diffusion of vanillin in an aqueous environment

Table 3 Migration of vanillin from PHB films into food stimulants

Food stimulant	Specific migration (μg/ml) at 37 °C
3 % acetic acid	6.16±0.3
n-Hexane	15.25±0.5
Distilled water	64.54±0.6
50 % ethanol	71.73±0.3

was reported in monolayer films in comparison to multilayered films containing polyvinyl alcohol and bacterial cellulose [33]. Multilayer zein-based films were used for controlled release of thymol [34]. The release of vanillin in food stimulants such as 3 % acetic acid and *n*-hexane water was observed as 6.16 and 15.25 μg/ml, respectively. Our results are similar with those of Narayanan et al. [11] where increased migration was reported for eugenol when tested at 37 °C, which is structurally similar to vanillin in food stimulants such as acetic acid, *n*-hexane, and ethanol. In the present study, vanillin was found to be active at a very low concentration against these food spoilage organisms (50 μg/g for bacteria and 80 μg/g for fungi) which is well below the reported oral LD_{50} values in animals, i.e., 3.0 g/kg for rabbits, 1.5–2.8 g/kg for rats, and 1.4 g/kg for guinea pigs, which indicate low oral toxicity [35]. The migration of the compound from PHB films is very negligible in the case of acetic acid and hexane, which shows that the migration of vanillin from PHB is dependent on the food matrix, which is in contact with the film. The PHB–vanillin film surface was uneven after 48 h of contact with the food stimulant. The migration of vanillin into a hydrophobic milieu such as fats is faster than that into a hydrophilic environment as in ionized acetic acid; the study explains that the PHB–vanillin antimicrobial films are more relevant for applications in foods containing fat as one of the ingredients.

Conclusions

Vanillin was found more effective against fungi than bacteria that commonly occur in preserved foods. Release of vanillin into food stimulants for fatty food resulted in the rapid release of vanillin for eliciting its antimicrobial action. The investigations on thermal properties of antimicrobial films showed a reduction in maximum thermal decomposition and the tensile strength of the films. Although incorporation of vanillin into PHB films has reduced the mechanical strength and melting point, the PHB films containing vanillin might be used as secondary films over the primary wrapping polymer layers. Further studies are required to support encapsulation of vanillin for controlled release, and application of antimicrobial films in food products will be investigated as vanillin has antimicrobial and antioxidant properties. As PHB is a bio-based packaging material, such films could be promising alternatives to synthetic materials. With functional properties, vanillin-incorporated antimicrobial films are promising and have potential food applications.

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