



UNIVERSITÀ DI PARMA

UNIVERSITA' DEGLI STUDI DI PARMA

**DOTTORATO DI RICERCA IN
Ingegneria Industriale**

CICLO XXXVII

***Design, Fabrication and Characterization of Biodegradable
Biocomposite Materials Reinforced with Natural Based Fillers for
Sustainable Packaging***

*Progettazione, Realizzazione e Caratterizzazione di Materiali Biocompositi Biodegradabili
Rinforzati con Filler di Origine Naturale per il Packaging Sostenibile*

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Anni Accademici 2021/2022 – 2023/2024

A chi ha creduto in me
A chi ancora ci crede
A chi ci crederà

Abstract

In accordance with the transition to a circular economy, this research project examines the design, fabrication and characterization of biocomposite materials based on biopolymer matrices of proven scientific and industrial interest, such as poly(butylene adipate-co-terephthalate) (PBAT), poly(butylene succinate) (PBS) and poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBH). The goal is to improve the mechanical properties of these materials while ensuring their biodegradability and compatibility with potential conventional processing techniques.

Therefore, the study focused on the production and characterization of biocomposite materials based on PBAT blended with different concentrations of natural lignocellulosic fibers derived from agricultural processing wastes, such as hemp flour (HF), and PBS and PHBH blended with different concentrations of a lignocellulosic filler derived from agricultural processing wastes, particularly brewers' spent grain (BSG). In addition, considering BSG as a promising economic biofiller to these PBS- and PHBH-based biocomposite materials, the use of a naturally derived bioplasticizer called trillevulinate glycerol (GT) was integrated.

The influence of the fillers and bioplasticizer on the rheological, structural, and morphological properties of the composites was evaluated using melt-mass flow rate (MFR) analysis, gel permeation chromatography (GPC), scanning electron microscopy (SEM), and Fourier transform infrared spectroscopy (FT-IR). To evaluate the influence of the incorporation of filler particles on the mechanical and thermal properties of the polymers, uniaxial tensile testing (UTT), differential scanning calorimetry (DSC) and dynamic-mechanical analysis (DMA) were performed on the generated formulations. This made it possible to evaluate the effectiveness of the reinforcement and bioplasticizer, going on to identify problems associated with the use of new materials.

Finally, considering the expected expansion of the wood-plastic composites (WPC) market, coupled with rising prices and decreasing wood availability, the goal of this research activity is the development of environmentally sustainable composite materials, using HF and BSG as economical biofillers, alternatives to wood flour in the production of WPC.

Keywords: biopolymers, PBAT, PBS, PHBH, biocomposites, biofillers, bioplasticizer, WPC, circular economy

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Chapter 1 - Introduction

1.1 Sustainable development and management of plastics.

Plastics are indispensable in several fields of the world economy, as they are utilized in a wide range of applications and offer a multitude of properties and benefits [1,2]. The advantageous of plastic properties include mechanical strength, which provides remarkable resistance to impact and mechanical stress [3]; low density, which makes them suitable for applications requiring weight reduction [4]; effective barrier properties that hinder moisture and gases, making them ideal for the preservation of food and sensitive products [5,6]; and versatility, as they are easily molded, colored, and modified for various applications [2]. Furthermore, plastics can provide excellent thermal, electrical, and chemical insulation, making them suitable for use in industrial settings [7,8]. Additionally, the production and processing of plastics is relatively cost-effective when compared to alternative materials. These attributes make plastics an attractive option for a different industrial field, including packaging, healthcare, cosmetics, agriculture, fashion, automotive, aerospace, construction, electronics, and technology [9].

The large-scale production of plastics has been perfected since their invention over a century ago, and in recent years an impressive rate of over 460 million tonnes has been achieved [10]. Predictions indicate that the production will continue to expand in response to the growth of the world's population and needs, as well as the increasingly industrialized emerging markets [11]. More over 57 million tons were produced in Europe alone in 2022, making up almost 14% of the world's total plastics output [12]. The use of conventional polymers derived from fossil fuels, such as polypropylene (PP), low-density polyethylene (LDPE), high-density polyethylene (HDPE), and polyethylene terephthalate (PET), to produce films, bags, bottles, food containers, and protective packaging, dominates the packaging industry in Europe, accounting for around 40 % of total demand [13,14].

Although conventional plastics perform many essential functions in various industries, the problems of overproduction and uncontrolled use underline the need for strategies that address their environmental consequences with more sustainable methods [15]. The interplay of these factors, along with reliance on fossil fuels and insufficient recycling infrastructure, has led to ineffective plastic disposal, resulting in pervasive and escalating pollution that poses

significant threats to both the environment and human health [15–17]. Plastics play a substantial role in greenhouse gas emissions, including CO₂, presenting a significant challenge to achieving climate objectives and limiting the increase in global temperatures to 1.5°C [18]. Currently, when recycling proves ineffective, the alternatives frequently narrow down to energy recovery or disposal in landfills. In 2015, packaging constituted approximately 63% of post-consumer plastic waste, resulting in a notable environmental impact [16]. This situation illustrates the increasing strain stemming from ineffective plastic waste management, which compounds the difficulties linked to the unregulated production and use of plastic products [19,20].

Consequently, there has been an increase in global awareness about these issues in recent years, leading to the emergence of new studies focused on comprehending and addressing these challenges. These interventions play a key role in addressing existing harms and averting additional negative impacts associated with plastics and microplastics [21–23].

The EU has traditionally approached policies concerning climate change, biodiversity, and the circular economy as distinct domains, lacking adequate emphasis on the interrelations among these fields. The European Green Deal was introduced to address this fragmentation, serving as a comprehensive strategy aimed at achieving climate neutrality by 2050 [24]. It seeks to transform the EU's linear economic model towards a circular economy and an environmental sustainability paradigm across several key sectors, including energy, transport, agriculture, and industry [25]. Recently, the XXIX Conference of the Parties to the United Nations Framework Convention on Climate Change (UNFCCC), known as COP29, took place in Baku, Azerbaijan [26]. The event brought together prominent political figures, scientific authorities, emerging activists, and various stakeholders to discuss the pressing challenges of climate change mitigation and adaptation.

In this context, the utilization of plastics is the focus of various regulatory and policy initiatives, aimed at reducing the environmental consequences of its use and encouraging sustainable life cycle management of this material. The revision of the Packaging and Packaging Waste Regulation stands out as a significant measure, targeting the goal of making all packaging recyclable or reusable by 2030. This initiative encourages the shift towards sustainable packaging solutions and aims to decrease reliance on single-use packaging (European Commission, 2024) [27]. Progress in plastics management includes addressing the issue of

microplastics reduction. Recent initiatives encompass a prohibition on the intentional incorporation of microplastics in various products, aiming to decrease the environmental release of microplastics by 30% by the year 2030 (European Commission, 2023) [28]. The Packaging and Packaging Waste Regulation (PPWR) will replace the current Packaging and Packaging Waste Directive 94/62/EEC (PPWD). The aim is to promote higher harmonization and at the same time introduce important changes in the legal framework for packaging waste in the EU, as in the case of the prohibition of PFAS [29].

1.2 The advent of biopolymers: what they are and classifications

Polymers are macromolecules composed of smaller units, known as monomers, interconnected by repeating covalent bonds. Polymers can be classified based on the chemical synthesis method, differentiating between addition and condensation polymerization [30].

A further differentiation can be established about their thermal and structural characteristics [31,32]. From a structural standpoint, polymer chains may manifest in linear, branching, or cross-linked configurations, which directly affect the material's physical and chemical characteristics. About their thermal characteristics, polymers can be:

- a) Thermoplastics, that can be molded using heat. Upon cooling, the form acquired in the mold is preserved; however, additional heating permits reshaping and reworking, so making the material reusable.
- b) Thermosets, that achieve permanent hardening via a cross-linking reaction. These materials are non-recyclable, unlike thermoplastics, and deteriorate when exposed to temperatures beyond their thermal stability limit.

In the context of biopolymers, it is crucial to evaluate the feedstock nature (renewable or nonrenewable) and the degree of biodegradability [33]. Consequently, all polymeric materials may be divided into four primary classifications:

- a) Biodegradable polymers from renewable materials, including cellulose [34], chitin [35], starch [36], polylactic acid (PLA) [37], bio-polycaprolactone (bio-PCL) [38], polyhydroxyalkanoates (PHAs) [39], and bio-poly(butylene succinate) (bio-PBS) [40].

- b) Non-degradable polymers from renewable sources, such as bio-poly(ethylene) (bio-PE), bio-poly(propylene) (bio-PP), and bio-poly(ethylene terephthalate) (bio-PET) [13,41–43].
- c) Biodegradable polymers from nonrenewable sources, including poly(butylene adipate-co-terephthalate) (PBAT) [44], poly(butylene succinate) (PBS) [45], and poly- ϵ -caprolactone (PCL).
- d) Traditional polymers, namely nondegradable from nonrenewable materials, including polypropylene (PP) [46], low-density polyethylene (LDPE) [47], high-density polyethylene (HDPE) [48], and polyethylene terephthalate (PET) [49].

Biopolymers provide a sustainable alternative to traditional petroleum-based polymers, which contribute substantially to environmental pollution due to their longevity and carbon emissions [50]. Consistent with the principles of the circular economy, biopolymers have the benefit of minimizing plastic waste, owing to their ability to decompose swiftly in natural or regulated settings, such as industrial composting facilities [51,52].

It is essential to distinguish between "biopolymers" and "bioplastics" [53,54]. (Bio)polymers are macromolecules consisting of repeating structural components, or monomers. (Bio)plastics are industrial products that include both (bio)polymers and other additives. The latter play a crucial role in improving material performance and may consist of reinforcements to enhance mechanical strength, plasticizers to increase flexibility, stabilizers to boost durability, flame retardants, and other agents to customize material properties for specific applications and to optimize economic efficiency [55,56]. To ensure the sustainability of bioplastics, biodegradable and bio-based additives are being employed [57,58]. This research aims to create biocomposite materials that improve the mechanical characteristics of polymers such as PBAT, PBS, and PHBH, integrating various natural fillers and/or plasticizers.

1.3 Biodegradability and Compostability

The degradation rate of polymers is a crucial factor in assessing their environmental effect, especially with biodegradable and/or compostable polymers [59,60]. Biodegradable polymers can decompose in the environment without significant ecological harm. This process happens through interactions with biotic variables, such as microorganisms in natural ecosystems, and

abiotic ones, including physical agents, moisture, temperature, acidity, and ultraviolet light [61,62]. In the biodegradation process, polymers are entirely converted from organic compounds into simple inorganic molecules, such as water, carbon dioxide, and occasionally, methane. Composting is a method of recycling organic waste that expedites the decomposition of natural components, resulting in a stabilized, nutrient-dense compost beneficial for soil and crops [59].

The European standard for packaging materials, EN 13432 [63], delineates standards for biodegradability and compostability. Specifically, biodegradable polymers must decompose to a minimum of 90% within six months. Compostable polymers must degrade within three months, leaving no visible residue.

In both cases, the necessary requirements for deteriorated materials are as follows:

- a) the breakdown process must not have any detrimental impacts.
- b) the materials must have a restricted concentration of heavy metals.
- c) the parameters of pH, salt content, volatile solids concentration, and levels of phosphorus, magnesium, nitrogen, and potassium must adhere to the limitations established by European legislation.

It is imperative that the final compost must be safe for the soil and not hinder plant development. The impact of the compost on germination and plant growth (ecotoxicity) must be evaluated to assure this [64,65].

The rate of polymer degradation varies significantly based on several parameters, including chemical structure, environmental conditions, molecular weight, and degree of crystallinity [66]. The polymer chemical composition significantly affects its degradation capability. Polymers with ester linkages, including PLA, PBS, PHA, and PBAT, exhibit accelerated degradation rates owing to the hydrolytic vulnerability of these bonds. Conversely, conventional polymers, exemplified by polyolefins, exhibit substantially stronger carbon-carbon (C-C) covalent bonds, leading to markedly slower breakdown. Molecular weight is also a crucial aspect. Polymers with elevated molecular weight exhibit slower breakdown rates due to the reduced accessibility of their elongated chains to enzymatic or chemical degradation mechanisms. Conversely, low molecular weight polymers are more prone to fragmentation, hence promoting breakdown.

The level of crystallinity influences the material's degradation resistance [67]. Crystalline portions of the material exhibit more organization and density compared to amorphous parts. Consequently, they are less accessible to microbes and chemicals, hence impeding the disintegration process. Conversely, amorphous patches, characterized by their lack of structure, promote infiltration of water and enzymes, therefore expediting breakdown. Furthermore, the environmental factors that predominantly affect the deterioration rate encompass temperature, oxygen availability, acidity, and humidity [68,69]. These elements are crucial in regulating the biological and chemical processes necessary for polymer degradation via mechanisms such as chain scission and cross-linking through Norrish I and II reactions [70,71].

These strategies promote the development of a more sustainable production chain in alignment with the principles of the circular economy. Biopolymers, being predominantly biodegradable and sourced from renewable elements, match seamlessly with this paradigm, wherein products are engineered for recycling or reintegration into nature without leaving detrimental residues [72–75]. To move toward a sustainable economy, the use of biodegradable and compostable biopolymers should be emphasized, in contrast with conventional polymers in terms of their source and their capacity for degradation in natural or regulated settings. Biopolymers like PBS and PBAT constitute a crucial category of materials due to their biodegradability and mechanical qualities for industrial applications. Nonetheless, they are mostly sourced from nonrenewable resources. Simultaneously, PHAs, such as poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBH), produced by bacteria fermentation, serve as a completely renewable and biodegradable substitute, thereby demonstrating the potential of biological resources within the framework of the circular economy.

1.4 Biopolymers: PBAT, PBS and PHBH

1.4.1 Poly(butylene adipate-co-terephthalate)

Poly(butylene adipate-co-terephthalate) (PBAT) is an aliphatic-aromatic copolyester derived from fossil resources, consisting of two different co-monomer segments [33]. The first section, consisting of the butylene adipate (BA) oligomer, is formed from 1,4-butanediol (BDO) and adipic acid (AA), imparting significant flexibility to the polymer. The second section,

conversely, consists of the oligomer butylene terephthalate (BT), synthesized from BDO and terephthalic acid (TPA). This imparts stiffness to the structure. This configuration endows PBAT with a blend of toughness and flexibility, making it a viable option for various industrial applications.

Despite the prevailing dependence on fossil feedstocks, research continues to integrate monomers derived from biological sources, as reported by several manufacturers [76]. Adipic acid, a key component in the manufacture of PBAT, is conventionally derived by chemical procedures that utilise benzene as a precursor [77]. Nonetheless, the traditional synthesis of adipic acid is linked to the release of significant quantities of nitrogen oxides (NO_x), which are powerful greenhouse gases that exacerbate global warming and air pollution. To mitigate these environmental issues, scientific research has concentrated on investigating alternate biochemical routes for adipic acid synthesis, aiming to decrease emissions and enhance process sustainability [78]. Recent research, including that by Zhang et al. [79], has explored the capability of genetically engineered yeasts, particularly *Saccharomyces cerevisiae*, to transform carbohydrates like glucose into adipic acid. Babu et al. [80] developed an alternative biochemical method using lignocellulosic biomass as a substrate for the manufacture of adipic acid.

Likewise, BDO is routinely produced by proven chemical methods within the petrochemical sector. Among classic approaches, a prominent approach utilises acetylene and formaldehyde, or employs chemical intermediates like butadiene or maleic anhydride [81–83]. Recently, more sustainable manufacturing techniques for BDO have emerged through the use of novel biotechnological processes [84]. The application of modified *Escherichia coli* strains has shown significant potential [85,86]. These genetically engineered bacteria can metabolise lignocellulosic biomass or sugars from renewable sources, such as glucose and fructose, to synthesise BDO. This process is much more sustainable than conventional techniques, diminishing dependence on fossil fuels and employing agricultural waste or other renewable carbon sources. Alongside sugars and lignocellulosic biomass, glycerol, a byproduct of biodiesel production, has been suggested as an alternate carbon source for the synthesis of BDO or AA [81]. This method signifies a viable technique in the framework of the circular economy, wherein the worth of industrial by-products is augmented, and the ecological footprint of biopolymer manufacturing is diminished.

TPA is another crucial element of PBAT, serving as the aromatic portion of the polymer. The conventional process for synthesising TPA utilises p-xylene, a product of petroleum [87]. The transformation of this monomer into a biobased variant has posed a considerable challenge to the scientific community. Recent advancements have led to the discovery of innovative technology for the synthesis of bio-TPA from renewable sources [88]. Tachibana et al. [89] specifically examined the possibility of 5-hydroxymethylfurfural (HMF), a derivative of fructose, as a precursor for bio-TPA production. HMF may be oxidised to produce furandicarboxylic acid (FDCA), which can partially or entirely substitute TPA in PBAT and other polyesters [90]. FDCA demonstrates similar chemical characteristics to TPA, making it especially suitable as a biobased monomer for biodegradable polyesters.

The PBAT manufacturing encompasses shopping bags, packaging materials, mulching films, biodegradable cutlery, hygiene goods, biomedical equipment, and applications in industrial composting settings [91,92]. The material exhibits good toughness, exceptional ductility, and resistance to moisture and water, according to worldwide criteria for biodegradability and compostability [93–96]. PBAT has characteristics similar to those of a thermoplastic elastomer due to its significant elongation at break and low modulus of elasticity. The structural composition and molecular weight of PBAT significantly impact its mechanical characteristics, similar to other polymers [44,97]. Research by Lee and Herrera demonstrates that a higher fraction of BT units leads to increased polymer stiffness, while simultaneously decreasing ductility [98,99]. Furthermore, it was shown that an increased molecular mass improves tensile strength, whereas elongation at break often decreases. Consequently, the mechanical characteristics of PBAT may be optimised by modifying the synthesis parameters, including pressure and reaction temperature, which influence the molecular weight and, subsequently, the polymer performance. A comparison with LDPE indicates that PBAT demonstrates similar mechanical characteristics [100,101], characterized by significant flexibility, with a flexural strength (σ_F) above 7 MPa, a flexural modulus of around 130 MPa, and a Young's modulus (E) between 20 and 50 MPa. The tensile strength (σ_B) of PBAT varies from 20 to 35 MPa, and the elongation at break (ϵ_B) can attain 700% [102,103]. These attributes make it appropriate for applications necessitating flexibility and impact resistance, such as flexible packaging and agricultural film manufacturing. These qualities also make it compatible with standard industrial gear used for polyolefins, therefore promoting its use in many applications.

The melting temperature (T_m) of PBAT is contingent upon the ratio of stiff BA units to flexible BT units within the copolymer structure. As the concentration of BT units increases, the T_m can vary from around 40°C (at 10 % mol of BT units) to 190°C (at 80 % mol of BT units) [104]. This versatility makes PBAT adaptable for use in various operational settings. Compared to polyolefins, the T_m of PBAT can be aligned with that of other polymers, such as low density-polyethylene (LD-PE), depending on the precise composition.

The glass transition temperature (T_g) indicates the shift from a rigid to an elastic state. The T_g of PBAT is often reported to be rather low, ranging from -30°C to -20°C, as indicated by most research [105]. While PE exhibits superior flexibility at extremely low temperatures, PBAT is a suitable alternative owing to its elasticity above -30°C, making it perfect for applications necessitating low-temperature resistance.

PBAT is a polymer generated from fossil sources that is engineered for biodegradability [106,107]. It has a notably elevated degradation rate in aerobic environments, especially within composting facilities, where the process may be finalized in under six months. Nonetheless, in natural settings like soil, the deterioration process occurs at a reduced pace [108]. Yang et al. [109] found that the integration of TPA into the polymer structure significantly affects the breakdown rate. In high-temperature composting, the chemical hydrolysis of PBAT facilitates the biodegradation of long-chain aromatic oligomers, whereas oligomers containing one or two terephthalate units are biodegraded more quickly.

1.4.2 Poly(butylene succinate)

Poly(butylene succinate) (PBS) is a biodegradable aliphatic biopolymer that demonstrates mechanical and physical properties similar to those of conventional synthetic polymers, including PE and PP. The synthesis of PBS is thoroughly described in scientific literature and entails the condensation polymerization of two primary monomers: The condensation of 1,4-butanediol (BDO) and succinic acid (SA) is a standard procedure in the fabrication of this substance. This reaction can be conducted using monomers sourced from fossil fuels or materials obtained from renewable resources [110,111]. Besides BDO, succinic acid is conventionally derived from fossil sources, notably maleic anhydride (MA), a petroleum byproduct [112]. Although this procedure is well-established and prevalent in the traditional

chemical industry, the increasing emphasis on sustainability has stimulated the creation of alternative routes. Microbial fermentation serves as a feasible alternative, utilizing genetically engineered bacteria and yeasts to transform renewable biomass, such as starch, cellulose, and sucrose from agricultural or forestry byproducts, into SA [113–115]. Genetically engineered strains of *Escherichia coli* and *Corynebacterium glutamicum* have been created to improve the efficiency of SA synthesis from renewable carbon sources [116,117].

PBS is distinguished for its mechanical qualities, such as flexibility, thermal resistance, and chemical and physical stability. These characteristics render it suitable for traditional processing methods, including extrusion, injection molding, and blow molding [118–122]. The combination of these attributes, together with biodegradability, positions PBS as a competitive material across several sectors, particularly in packaging [123–125]. PBS exhibits an elastic modulus (E) ranging from 300 MPa to 650 MPa. ϵ_B can attain 650%, indicating higher ductility relative to biopolymers like PLA and PHA. The flexural modulus of PBS is around 650 MPa, and its flexural strength is about 40 MPa, characteristics that make it appropriate for components required to endure static loads without deformation [126,127]. PBS demonstrates impact resistance values ranging from 5 to 20 kJ/m² [128], in contrast to the 1.5 to 2.5 kJ/m² range noted for PLA. This improved impact resistance reduces the brittleness of PBS, making it less prone to fracture under dynamic stresses.

In terms of its thermal properties, PBS possesses a comparatively low T_m , situated between 100 to 115 °C. This lowers the energy consumption necessary for processing, hence improving efficiency and making it appropriate for low-energy-cost industrial methods [119]. The heat deflection temperature (HDT) of PBS ranges from 90 to 95 °C, making it suitable for applications requiring stability at high temperatures, such as contact with hot food or heat-exposed components. PBS demonstrates significant thermal stability, with a T_{deg} of around 300 °C, thereby ensuring strength during high-temperature processing [121]. The crystallization temperature (T_c) of PBS ranges from 70 to 90 °C, whereas the T_g spans from -40 to -10 °C [118,120]. These values are contingent upon the synthesis conditions and the raw ingredients employed. These characteristics make PBS a good material for applications requiring good performance at low temperatures.

It is suitable for use in packaging and other applications where biodegradable materials are needed since PBS is certified as compostable in compliance with ISO EN 13432 [129]. The

biodegradation rate of PBS depends on its molecular weight and environmental circumstances. In industrial composting facilities, when conditions are optimised (temperatures ranging from 50 to 70 °C, humidity, and oxygen levels), PBS can achieve full biodegradation within a few weeks due to microbial activity and hydrolysis. In natural settings like soil, the degradation process occurs at a reduced rate due to the absence of controlled environmental conditions. The completion may need several months to over a year. Although the composting process in natural settings is more gradual than in industrial facilities, PBS may nonetheless decompose organically, therefore markedly diminishing its environmental effect relative to conventional non-biodegradable polymers [130].

1.4.3 Poly(3-hydroxybutyrate-co-3-hydroxyhexanoate)

PHAs are a class of biopolymers that have garnered considerable attention owing to their biocompatibility, biodegradability, compostability, and sustainable origin [37]. These compounds are produced by diverse microorganisms via the metabolism of carbohydrates or fatty acids sourced from agri-food waste or vegetable oils. PHA macromolecules are stored within bacterial cells as water-insoluble intracellular inclusions, functioning as reservoirs of carbon and energy [39,131,132].

PHAs are aliphatic polyesters whose monomeric units differ based on the precise carbon source and growth circumstances present in the bacterial culture [133,134]. They are generally divided into two classifications:

- a) Short-chain length PHA (SCL-PHA) include monomers with carbon chains ranging from C3 to C5, including 3-hydroxybutyrate (3HB), 4-hydroxybutyrate (4HB), and 3-hydroxyvalerate (HV).
- b) Medium-chain length PHAs (MCL-PHAs) comprise monomers with carbon chain lengths ranging from C6 to C14, including 3-hydroxyhexanoate (HH), 3-hydroxyoctanoate (HO), and other longer-chain monomeric units.

The monomer structure directly influences the physical qualities of PHAs, such as crystallinity and mechanical performance. SCL-PHAs, such as PHB, demonstrate significant crystallinity, imparting stiffness and brittleness. MCL-PHAs, characterized by reduced crystallinity, have superior elasticity and flexibility [135].

PHAs provide a notable benefit due to their biodegradability, making them especially appropriate for use in environmental and health-care applications. They can fully decompose in many environmental conditions, such as compost, soil, and water, rendering them an alternative for creating sustainable packaging solutions to conventional polymers and biopolymers. Moreover, their biocompatibility makes them acceptable for biomedical applications, since the degradation products are non-toxic [136–138].

Within the PHA family, poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBH) is notably prominent, garnering interest from both industrial and academic sectors [139]. This copolymer, originating from PHB, demonstrates improved flexibility and strength. The integration of 3-HH into the polymer matrix decreases crystallinity, hence improving ductility while maintaining the material's thermal characteristics [140,141]. The monomers that comprise PHBH, namely 3-HB and 3-HH, originate from sustainable carbon sources [142,143]. 3-HB is predominantly synthesized from simple sugars, such as glucose and fructose, or other plant material, which bacteria metabolize via the acetyl-CoA cycle. Conversely, 3-HH is synthesized from medium-chain fatty acids, including hexanoate or octanoate, which can be obtained from vegetable oils, agricultural byproducts, or other renewable fatty acid sources. Bacteria metabolize these fatty acids via the β -oxidation cycle, producing the monomer 3HH [144–146].

The elastic modulus of PHBH ranges from 0.1 to 0.5 GPa, depending on the copolymer composition, with the proportion of 3HH being a critical determinant. This value is inferior to that of pure PHB (1-4 GPa) but comparable to that of LDPE (0.2-0.4 GPa). PHBH has an ϵ_B , potentially reaching 850%, proportional to the concentration of 3HH present. This value significantly exceeds that of pure PHB, which demonstrates ϵ_B ranging from 5 to 20%. Compared to LDPE, which has an ϵ_B of 100 to 800%, PHBH exhibits comparable and, in certain cases, better ductility. The σ_B of PHBH varies between 20 and 35 MPa, depending on the copolymer composition and synthesis parameters. σ_B of PHBH, equivalent to that of LDPE (8-35 MPa), suggests its adequacy for many industrial applications [146–148].

Additionally, owing to its thermoplastic characteristics, PHBH has good heat stability and impact resistance. The integration of 3HH units into the structure leads to a decrease in the T_g , thereby improving flexibility at low temperatures [149,150] Furthermore, the T_m of PHBH is often lower than that of pure PHB. Increasing the proportion of 3HH units to between 15

and 20%, T_m decreases from around 170 to 115-120°C, attaining a minimum of 54°C. This results in a reduction of crystallinity to 15%, hence improving the material's flexibility and processability at lower temperatures due to the widening of the thermal processing window [151–153].

PHBH has many uses, including medical implants because of its biocompatibility and controlled degradation ability, as well as biodegradable packaging materials because of its mechanical and biodegradable qualities, which make it a great substitute for conventional plastics. Moreover, PHBH is developing prominence as a material for biodegradable disposable items, addressing the increasing need for sustainable alternatives. The compatibility of PHBH with various industrial processes, such as injection molding [154,155], extrusion [156,157], thermoforming [158,159], and 3D printing [160,161], facilitates these possibilities.

Market forecasts suggest that PHA output would significantly rise from around 105 thousand tons in 2023 to one million tons by 2028. This rise is due to the proliferation of applications and continuous research focused on improving performance and minimizing production costs [94]. Techniques such as incorporating natural fillers, using plasticizers, and blending with other biodegradable polymers are broadening the use of PHBH and other PHAs across many sectors.

1.5 Classification of Filler and Biocomposite Materials

Considering its potential for environmental sustainability and biodegradability, the use of biopolymers presently faces many restrictions. This leads to high production costs and, in certain instances, worse mechanical, thermal, and barrier characteristics compared to traditional polymers. These constraints limit the range of possible applications. A way to mitigate these drawbacks is to include fillers into biopolymer matrices, which might differ in chemical composition (organic or inorganic) and production method (natural or synthetic), therefore creating a biocomposite system [162]. This method also diminishes the carbon impact linked to conventional polymers and material expenses. Moreover, it enhances the mechanical, physical, and thermal characteristics of biopolymers, while also affecting their degradation rates [163–167]. The improved performance of biocomposite materials points to

a possible path for the creation of sustainable materials that can compete with traditional plastics in terms of functionality. Inorganic fillers are frequently utilized in the fabrication of biocomposites, including ceramic materials such as glass microspheres [168], nanoclay [169], silica [170] and zinc nanoparticles [171], calcium carbonate [172], and magnesium oxide [173]. The use of these fillers confers significant mechanical strength and durability to the biocomposites. These fillers promote stability, making plastic materials appropriate for many uses. Moreover, specialized inorganic fillers such as silver nanoparticles, nanostructured alumina (Al_2O_3), silica (SiO_2), and titanium dioxide (TiO_2) exhibit antibacterial capabilities, UV and abrasion resistance, and improved optical attributes, making them appropriate for particular applications. However, the incorporation of inorganic fillers into biopolymers may hinder biodegradability [174].

For a transition to a circular economy, numerous studies in the literature report the use of organic fillers of natural origin. These include polysaccharides, such as cellulose nanocrystals (CNC) [160] and cellulose nanofibers (CNF) [175], hemicellulose [176], lignin [177], chitosan [178], starch [179–182], and alginate [183], as well as more complex fillers such as natural lignocellulosic fibers from agro-food waste and agricultural by-products. They include wood flour [184–189], hay, hemp [190], brewers' spent grains [191–194], almond shells, rice husks, vegetable husks, coffee grounds, eggshells, and crustacean shells provide advantages for structural reinforcement and environmental sustainability, promoting a circular economy due to their lightweight nature and natural abundance [195–199]. Moreover, these bio-based fillers are often used for applications where biodegradability is essential.

This approach, in addition to being complementary to the circular economy theme, can benefit the structural and functional properties of biopolymers. Consequently, it is becoming a primary strategy to meet the diverse needs of various applications. In alignment with environmental sustainability, biocomposites can be processed using conventional methods employed for traditional fossil-derived polymers, such as single-screw or twin-screw corotating extruders, flat-head and blown extrusion, injection molding, thermoforming, and 3D-printing [180,183,200–209].

The primary parameters affecting processing include moisture, fiber type, fiber volume fraction, and temperature. Extrusion involves melting and mixing thermoplastic polymer pellets with natural fibers using one or two rotating screws, and subsequently pushing the

blend through a die. A twin-screw extruder is preferred due to its ability to ensure uniform distribution of fibers within the matrix, a benefit that arises from the polymer melting caused by the action of the twin screw as the blend passes through the system. The flat-head extrusion process is employed to convert polymeric materials into films with a controlled thickness down to under 100 μ m. For smaller thicknesses, the preferred processing method is blow extrusion. Another conventional processing method is injection molding, which is useful for producing complex and precise parts at high production rates, making it a cost-effective process. Another method for producing polymer-based composites is thermoforming. In this method, the polymer matrix and fibers are placed at the bottom of a mold, and pressure is applied while the polymer is heated to its melting temperature. This results in high-strength products with good dimensional accuracy and surface finish. The advent of 3D printing represents the most recent technological advancement in the field, enabling the fabrication of objects with biocomposite materials.

The use of those fillers improves both the structural and functional properties of biopolymers, thereby serving as a key method to meet the demands of various applications. Biocomposites consist of two separate phases: a continuous phase (the matrix) and a dispersed phase (the filler). In natural fiber-reinforced composite systems (NFCS), the reinforcing component comprises plant fibers, but the matrix may consist of any polymeric material, either synthetic or natural. Reinforced biocomposites demonstrate enhanced stiffness, an often sought mechanical feature [162]. The addition of an extra phase to the matrix significantly modifies its mechanical characteristics, such as tensile strength. The interactions among the composite phases are crucial in determining its overall performance [210,211]. The effectiveness of the composite depends on the establishment of strong interfacial connections between the matrix and filler surfaces. Several theoretical models have been established to elucidate the characteristics of composites [212–214]. These models provide a correlation between the volume percent of the reinforcing phase and the characteristics of the material. Optimized compatibility between fillers and biopolymers can improve the mechanical strength and flexibility of the composite, while also increasing its biodegradability. Moreover, the use of fillers in biocomposites is an efficient method to lower the overall production costs of bioplastics [163].

In cases of insufficient interfacial adhesion, many solutions have been developed to improve filler-matrix compatibility. The surface treatment of fillers, encompassing plasma treatments, alkali treatments, acetylation, and coupling with compatibilizing agents (such as maleic anhydride, epoxy compounds, or isocyanates), has been shown to improve adhesion between filler and matrix, thereby ensuring the integrity and durability of biocomposites [215–217]. These procedures aim to reduce the hydrophilic properties of natural fibers by eliminating surface substances such as proteins, waxes, and lignin. The compatibilizing agents react with the functional groups of the fillers, forming a network with the polymer matrix. The presence of the compatibilizer functions as a "molecular bridge" between the hydrophobic matrix and the frequently hydrophilic filler, thereby increasing the interfacial area. This, in turn, enhances adhesion and the transfer of mechanical stresses at the filler-matrix interface during the application of external loads, leading to improved mechanical properties of the composite material, as well as increased thermal stability and processability. A prominent example is maleic anhydride (MA), a multifunctional molecule with anhydride groups that can react with various functional groups on fillers, such as hydroxyl (-OH) and amine (-NH₂) groups. This reaction enables the covalent binding of MA to the polymer matrix, thereby creating a stable bond. The efficacy of this approach has been documented in numerous studies [218–221].

1.6 Hemp Fiber

Hemp (*Cannabis sativa* L.) is among the oldest and most adaptable crops utilized worldwide. Hemp is regarded as a very sustainable crop for contemporary eco-friendly farming systems because to its capacity to vary crop rotations in intensive agriculture, diminished requirement for fertilizers and pesticides, and minimal environmental effect [222–224].

In Italy, industrial hemp is seeing a resurgence as a versatile crop, particularly with the enactment of Law 242/2016 by the Ministry of Agriculture, Food and Forestry. This material is distinguished by its adaptability and sustainability, being utilized across several industries owing to its physical, chemical, and ecological features [225,226]. Typical applications encompass construction materials, including bricks and insulation panels, textiles for fabric and apparel manufacture, as well as the automobile and food sectors. Hemp is utilized in the manufacture of bioenergy, paper, and bioplastics. Nonetheless, hemp stems frequently

constitute an underexploited resource and considerable waste, since the supply chain predominantly emphasizes seeds and inflorescences. This results in logistical, environmental, and economic issues with disposal, along with increased expenses for manufacturing enterprises [227,228].

The management of agricultural and industrial hemp wastes has emerged as a matter of worldwide importance [229]. Europe has led in establishing legislation and recommendations to minimize agricultural waste output and encourage its reuse and valorization [230]. The processing of lignocellulosic wastes for the creation of new, bioresource-based biocomposite systems, such as wood-plastic composites, or for the extraction of bioactive chemicals, is in complete accordance with the principles of the circular economy [231–235]. Lignocellulosic fibers, derived from renewable resources, need lower energy usage for manufacture compared to traditional fibers.

Emerging applications for the development of biodegradable and sustainable products from hemp fibers are reinforcing the status of this crop as a significant renewable resource, owing to its low density, renewability, abundant availability, and superior strength compared to conventional fibers. Combining with polymer matrices enables the creation of biocomposites that exhibit modified mechanical properties, as well as being biodegradable, recyclable, and renewable, making them suitable for food and cosmetic packaging applications while addressing sustainability, biodegradability, and product preservation challenges. Recently, composite materials utilizing hemp fibers as reinforcing agents, in conjunction with different thermoplastic polymer matrices, have been created for applications in the automotive and construction industries. The maximum tensile strengths in these applications have been achieved in composites including up to 50% hemp fibers [190,236].

The fibers found in hemp stems, typically discarded or incinerated, provide one of the most valuable natural sources of cellulose, comprising an estimated 70 to 74% in the outer fibers. This cellulose is incorporated into a complex structure consisting of 15-20% hemicellulose, 3.5-5.7% lignin, 0.8% pectin, and 1.2-6.2% waxes [237,238]. The elevated stiffness and strength characteristics of hemp stems, akin to those of flax or ramie fibers, along with their biodegradability and affordability, render them useful as reinforcing fillers for biocomposites with a polymer matrix. These materials can be utilized in sustainable and eco-friendly packaging as a substitute for glass or wood fibers.

Grinding hemp stems yields hemp chips or hemp flour, classified based on the particle size distribution of the constituents in the mixture. These items may be utilized to create many types of composite systems.

1.7 Brewer's Spent Grain

Brewer's spent grains (BSG) represent another promising lignocellulosic filler for making biocomposites. Unlike hemp fibers, which come mainly from agricultural crops or agro-industrial processes, BSG is a by-product of the agri-food industry [239,240]. This material is obtained during brewing, in which, after barley malt mashing, the insoluble solid fraction, known as BSG, is separated from the wort, which is the sweet liquid destined for alcoholic fermentation [241,242].

BSG constitutes the most significant by-product of the brewing industry, accounting for about 80 wt.% of the total waste generated. Beer, one of the oldest and most widely consumed alcoholic beverages in the world, is the third most popular after water and tea. It is estimated that the production of each hectoliter of beer generates between 6 and 20 kg of BSG, an amount that varies depending on the production technologies used [243,244]. According to data reported by Brewers of Europe (2018) [245], the European industry produces more than 40 billion liters of beer annually, a volume that is constantly growing. As a result, an estimated 2.5 to 8 million tons of BSG are generated in Europe each year [246].

The chemical composition of BSG is strongly influenced by the variety and type of malt grains used, the harvest period and the technological parameters adopted during the brewing process. BSG has a composition comparable to that of lignocellulosic fibers such as hemp or wood, including bio-organic fractions such as holocellulose (hemicellulose and cellulose), lignin and waxes. However, it is distinguished by its high content of protein (up to 30 percent by weight), lipids (up to 10 percent by weight) and a variety of bioactive compounds, such as vitamins, minerals and phenols, which differentiate it from traditional lignocellulosic materials [247,248].

Despite the potential of BSG, its use is limited by logistical issues related to its high moisture content, which can reach 80wt.%. This characteristic, combined with the presence of fermentable sugars, makes it susceptible to rapid decomposition due to microbial activity.

These factors pose a significant obstacle to the storage and transportation of BSG, reducing the potential for large-scale applications. In addition, its disposal in landfills is a major contributor to greenhouse gas emissions, with about 514 kg of CO₂ equivalents per ton of BSG. Drying is a viable solution for the preservation of BSG, as it allows its volume to be reduced, thereby decreasing transportation and storage costs. Studies conducted by Bartolomé et al. [249] have examined alternatives such as freeze-drying, conventional drying, and freezing, identifying approaches that could improve preservation and efficiency in BSG management.

Although BSG has historically been considered a by-product of limited economic value, its chemical characteristics make it a material with a wide range of applications. Due to its high nutritional content, BSG is mainly used as agricultural fertilizer and animal fodder, both in wet and dried form [250]. However, its properties also make it suitable for more sophisticated applications, such as organic acid and enzyme synthesis [251,252], bioenergy production (including biogas and bioethanol) [253–255], and use as an ingredient for high-fiber baked goods or cosmetics with anti-aging properties [256–258]. In addition, BSG has been explored as an adsorbent in industrial purification processes and as a substrate for the synthesis of biopolymers, such as polyhydroxyalkanoates (PHAs), due to the presence of fatty acids [259,260].

In the field of composite materials production, the use of BSG represents an opportunity to valorize residual biomass. In fact, BSG can serve as a filler in wood-plastic-based biocomposites (WPCs). Due to its unique chemical composition, which includes proteins and lipids, it differs from traditional lignocellulosic fillers, such as wood flour and other agricultural wastes [247]. In particular, the presence of proteins and lipids enhances compatibility with the polymer matrix and acts as a plasticizer, improving the mechanical and functional properties of composite materials. Proteins also participate in the Maillard reaction, producing melanoidins, which possess antioxidant and antimicrobial properties [261–263].

BSG is also the subject of innovative research projects, such as the Horizon 2020 project, which aims to use it in the production of biodegradable and compostable disposable utensils (such as forks, spoons, and knives) to replace traditional plastic utensils [264]. For example, Cunha et al. [265] have developed PHBV/BSG composites for food packaging applications, showing that the addition of BSG reduces the stiffness and crystallinity of the polymer, improving biodegradability. However, it is reported in the literature that strong aggregation of BSG fibers

can compromise the processability of biocomposites, especially if the treatment of BSG for protein and sugar removal is not properly managed.

In conclusion, BSG emerges as a by-product with great potential, not only for its traditional nutritional value, but also as an innovative material for sustainable applications. However, its full exploitation requires advanced technological solutions to address storage and processing challenges, paving the way for new environmentally friendly applications.

1.8 Plasticizers and its Evolution

Plasticizers are chemical additives used into polymers to enhance specific mechanical qualities, including processability and ductility. These additives are among the most prevalent in both conventional plastics and bioplastics. Their utilization considerably broadens the applicability of a polymer matrix, rendering it appropriate for purposes necessitating such qualities. The efficacy of plasticizers is contingent upon the molecular interactions between plasticizers and polymer chains, as posited by plasticization theories .

The initial theories of plasticization were formulated between 1930 and 1950, encompassing lubrication theory, gel theory, and subsequently, free volume theory. Lubrication theory posits that polymer stiffness arises from internal friction among chains, whereas plasticizers function as lubricants, decreasing friction between polymer layers. The gel hypothesis posits that stiffness arises from the bonding sites between polymer chains, whereas plasticizers diminish the quantity of these bonding sites, hence enhancing flexibility [266].

The free volume theory is the latest framework, emphasizing the notion of free volume, which refers to the internal space present inside a polymeric structure. A stiff polymer possesses little free volume, whereas the use of a plasticizer enhances this volume, rendering the polymer softer and more elastic. The augmentation of free volume enhances the mobility of polymer molecules, hence enhancing flexibility. The free volume originates from three primary sources: the motion of the polymer chain ends, the motion of the side chains, and the motion of the backbone chain [266].

Furthermore, plasticizers can be categorized into internal and exterior types [267–269]. External plasticizers are low-volatile substances incorporated into a polymer without

establishing direct chemical connections with its chains. Secondary interactions guarantee their compatibility with the polymer matrix. Despite the potential loss of plasticizers through migration, evaporation, or extraction, they remain the most prevalent choice because to their cost-effectiveness. Internal plasticizers are chemically attached to polymer chains by primary bonds, either through copolymerization procedures or direct interactions with the initial polymer.

The primary function of plasticizers is to enhance the flexibility and processability of polymers by reducing the glass transition temperature (T_g) and viscosity. Simultaneously, plasticizers are compatible with traditional processing processes, including extrusion, injection molding, and calendering, so enabling reductions in processing temperatures, mixing durations, and extrusion pressures. Plasticizers also influence several mechanical qualities, including hardness, elastic modulus, and fracture and impact strength. Moreover, their utilization influences several physical material characteristics, such as crystallization and melting temperatures, water interactions, gas permeability, and degradation rates [270].

Nonetheless, over time and under specific environmental circumstances, plasticizers may leach from the polymer matrix, presenting hazards to environmental and biological health. To mitigate plasticizer migration, more intricate and higher molecular weight plasticizers have been formulated, which adhere more effectively to the polymer substrate, thereby restricting migration. This method is constrained by inadequate plasticization and elevated expenses. Consequently, apprehensions and disputes have arisen over the utilization of conventional plasticizers, especially phthalates [271–273].

Furthermore, because to the exhaustion of petroleum supplies and many environmental concerns, the polymer sector is progressively transitioning to bio-derived and eco-friendly plasticizers. Regulations in both Europe and the United States prohibit six phthalate derivatives (namely diethylhexylphthalate (DEHP), dibutylphthalate (DBP), benzylbutylphthalate (BBP), diisobutylphthalate (DIBP), diisodecylphthalate (DIDP), and di-n-octylphthalate(DNOP)) when present in amounts over 0.1 wt.% [271,274]. With the rising interest in low-migration, low-toxicity plasticizers as substitutes for phthalates, attention has increasingly shifted towards bio-based plasticizers derived from vegetable oils, citrates, and sugar derivatives, capable of plasticizing both petrochemical and biopolymer materials [275].

Bioplasticizers mostly originate from agricultural and agri-food by-products, including lignocellulosic waste, vegetable oils, and biomass rich in fatty acids. An optimal bioplasticizer, irrespective of its source, should be non-toxic, have high migration resistance, be miscible, effective, and reasonably cost-effective. The bioplasticizer industry is seeing significant growth, with a projected annual growth rate of approximately 10 % from 2015 to 2025. In 2016, worldwide demand for bioplasticizers was 887 kilotons, anticipated to increase to 1900 kilotons by 2025. By 2026, sustainable additives are projected to comprise almost 20% of the plasticizer market, propelled by the increasing need for bioplastics that maintain characteristics like flexibility and durability [276].

Glycerol and levulinic acid are promising starting materials to synthesize glycerol trilevulinate (GT), abiobased plasticizer, due to their sustainability and compatibility with polymeric materials. Both originate from renewable sources, serving as more ecologically sustainable alternatives to conventional petroleum-based plasticizers [270,275,276]. Glycerol, a non-toxic byproduct of biodiesel manufacturing, possesses three hydroxyl groups that enable its conversion into other derivatives [277]. Levulinic acid, obtained from waste cellulosic biomass, is a renewable resource that enhances sustainability and the circular economy by circumventing rivalry with food production [278].

1.9 Possible Employment in Industrial Environment: Wood Plastic Composites

Wood Plastic Composites (WPCs) are among the most established and intriguing eco-efficient composite materials now available [279]. This term refers to composite materials that integrate plant fibers sourced from wood or lignocellulosic by-products of the food or agricultural sectors with a polymer matrix, which may be either thermoplastic or thermosetting [280,281]. The finished product mimics the appearance and functionality of genuine wood. The WPC idea was first developed in Italy in the early 1970s, but it acquired prominence in the United States throughout the 1990s.

The characteristics of WPCs are influenced by several aspects, including as the kind of polymer matrix, the nature of the filler (including form and particle size), the interaction between the filler and matrix, and the employed technological processes.

In the 21st century, the use of WPCs has proliferated, being employed in sectors such as automotive, outdoor furniture, flooring, siding, fence, gardening, and outside flooring [282–285]. Nonetheless, polyolefins continue to be the most extensively utilized thermoplastics in their manufacturing [286–288]. Despite possessing commendable mechanical qualities, these composites exhibit sustainability constraints: they are non-biodegradable and pose challenges for standard recycling procedures due to their material complexity. As awareness of petrochemical resources and their finite reserves rises, there is an escalating need to substitute petroleum-derived polymers with biobased, biodegradable, or compostable plastics, including PBAT, PBS, and PHBH, in composite applications.

Furthermore, prior studies indicate that wood-plastic composites (WPCs) are predominantly manufactured with plant fiber powders sourced from various wood species (e.g., maple, pine, oak, poplar), including sawdust and shavings, which may constitute up to 70 percent of the final product [289]. In recent years, the cost of high-quality wood flour has consistently risen. The surging demand for WPC, together with escalating raw material expenses, may diminish profit margins and intensify rivalry among sectors utilizing wood for energy production, pellets, biofuels, chemicals, and other products. Estimates suggest that insufficient timber from sustainably managed forests will be accessible by 2030 to satisfy the demands of green markets [290]. An economical and more sustainable substitute for wood is non-wood plant fibers, which can satisfy increasing commercial demand and mitigate deforestation issues. These fibers, distinguished by their strength and stiffness, cost-effectiveness, low density, minimal CO₂ emissions, biodegradability, and yearly renewability, are appropriate for reinforcing polymer matrices [291]. This motivation compels the scientific community to investigate novel techniques for incorporating lignocellulosic fillers from food and agricultural waste into biodegradable or compostable polymer matrices at varying weight percentages for diverse sustainable uses.

This thesis aims to build biodegradable composites that can substitute traditional petrochemical-based wood-plastic composites by modifying the characteristics of fillers and their interaction with the polymer matrix. These advancements correspond with environmental objectives, fostering sustainability and diminishing reliance on nonrenewable resources. This method advances the development of sustainable, eco-friendly materials suitable for many sectors.

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Chapter 2 - PBAT-based biocomposites

2.1 Introduction

This chapter presents the production and characterization of biocomposite materials based on PBAT as polymer matrix, mixed with different concentrations of natural lignocellulosic fiber microparticles such as hemp flour (HF). The goal of this research is to make an environmentally friendly material and investigate the effect of the HF within the PBAT in terms of structural, morphological, mechanical, and dynamic-mechanical properties.

HF biofiller was derived from the woody fraction of hemp stem and is mainly composed of cellulose, hemicellulose and lignin and therefore classified as a ligno-cellulosic fiber [1,2]. It was used in the form of micrometer particles (sieved below 50 μm) as a reinforcing filler. The compounding procedure was done by using the twin-screw extruder to maximize the mixing of the two different components. The pellets obtained were used to produce standard 5A specimens according to UNI EN ISO527 [3] by injection molding and to realize biocomposite films by the use of flat-head extruder. Samples with different HF concentrations, up to 40 wt.% for dumbbell specimens and up to 20 wt.% for films, were prepared.

The effect of the fillers on the structural and morphological properties of the composites was studied using electron microscopy (SEM) and Fourier transform infrared spectroscopy (FT-IR). To evaluate the mechanical and viscoelastic properties of the composites, uniaxial tensile testing (UTT) and dynamic mechanical analysis (DMA) were performed on both samples prepared by the two different processing methods.

The advantages of these environmentally friendly materials make them viable alternatives to traditional thermoplastic polymers with numerous potential applications in different industries. Finally, by reducing the amount of PBAT, the final composite will be less tied to the use of fossil-derived raw materials, contributing to a lower environmental impact and offering significant economic benefits in the application context, promoting a circular economy model [4–6].

2.2 Materials and Methods Preparation

2.2.1 Materials

PBAT was supplied by the company MAgMa Spa (Chieti, Italy), under the trade name “PBAT Ecoworld”. Hemp flour (HF) was supplied by Italian National Research Council (thanks to Dr. Pietro Russo), under the trade name of “Isocanna.” This is a solid by-product consisting of hemp shavings resulting from the separation by mechanical process of the woody part from the fibrous part and then reduced to lumps of about 6 mm in length for use in construction with natural hydraulic lime.

2.2.2 HF Filler Preparation

For use as a biofiller, the received HF (Fig. 2.1a) was initially dehydrated in an oven at 80 °C until it reached a constant weight. It was then sieved by means of a discontinuous analytical vibro-sieving instrument (Retsch AS 200 Control, Germany) on which a series of progressively finer sized sieves (150, 100, 75, 50 μm) was attached, depicted in Fig. 2.1b. An oscillation amplitude of 10 mm/g and a process duration of 15 min with 5-second intervals every 1 min of oscillation was set. To produce the composite materials, the filler collected below 50 μm was selected. Reducing the size of the biofiller allows, for the same weight, a larger surface area, creating the possibility of more interactions at the filler-matrix interface for more effective stress transfer [7].



Figure 2.1 - Representation of hemp flour (a) and discontinuous analytical vibro-sieving instrument (b)

Tab. 2.1 below shows the technical data for the hemp chipboard used in the thesis project.

Table 2.1 - Biofiller HF technical data

Product type	hemp chips
Particle size distribution	0 – 50 μm
Slurry pH	> 12.5
Apparent volumetric mass of fresh mortar (EN 1015-6)	300 ÷ 1200 Kg/m^3
Apparent volumetric mass of dried mortar (EN 1015-10)	150 ÷ 900 Kg/m^3
Water vapor	< 10 %
Sound absorption coefficient	50 dB with 30 cm thickness
Thermal conductivity (EN 1745:2002 prosp. A12)	0.07 ÷ 0.20 W/mK – according to mixing

2.2.3 PBAT Polymer Matrix Preparation

The pure PBAT granules (Fig. 2.2) have an average diameter of about 3 mm, and due to the constraints in terms of geometry and size of the screws, they were ground to enable their extrusion through the lab-scale twin-screw extruder. The samples were ground up under cryogenic conditions utilizing an analytical mill (IKA A11 Basic). Utilizing liquid nitrogen (N_2) efficiently decreases the temperature below T_g , enhancing the brittleness of the polymeric material and facilitating the reduction of particle size. The PBAT chemical and physical properties are presented in Tab. 2.2.



Figure 2.2 - Representation of the pure granule PBAT biopolymer

Table 2.2 - PBAT characteristics from the technical data sheet

Property	Value
Melt flow rate [g/10min]	≤5
Density [g/cm ³]	1.26
Tensile strength [MPa]	≥17
Melting temperature [°C]	110-120
Elongation at break [%]	≥600
Vicat softening temperature [°C]	89

2.2.4 Preparation of Batches of Biocomposite Materials

Following the milling of HF and pure PBAT, the two components were premixed at room temperature and at different filler concentrations using the analytical mill to achieve preliminary homogenization between polymer and biofiller. The compositions achieved are shown in Tab. 2.3.

Table 2.3 - Representation of the wt.% amounts of PBAT biopolymer and HF biofiller contained in each batch

Sample name	PBAT content [wt.%]	HF content [wt.%]
PBAT	100	-
PBAT + 5% HF	95	5
PBAT + 10% HF	90	10
PBAT + 20% HF	80	20
PBAT + 40% HF	60	40

Before extrusion, the prepared blends were dehydrated in an oven at 40 °C for 48 h to remove the residual moisture present, which could cause degradation of the biopolymer matrix during the extrusion process.

2.2.5 Melting Compounding

All the prepared compositions were subjected to an extrusion process to produce the various biocomposite polymer filaments. Melting compounding was performed with a RES-2 P/12A Explorer Extruder (Zamac Mercator, Poland) corotating twin-screw with an L/D ratio of 40 and a screw diameter of 11 mm (Fig. 2.3a). The profile differences of the screws along their length can allow by their rotation in concordant direction and heating, homogenization of the melt and its transport from the loading zone (hopper) to the extrusion head (Fig. 2.3b).

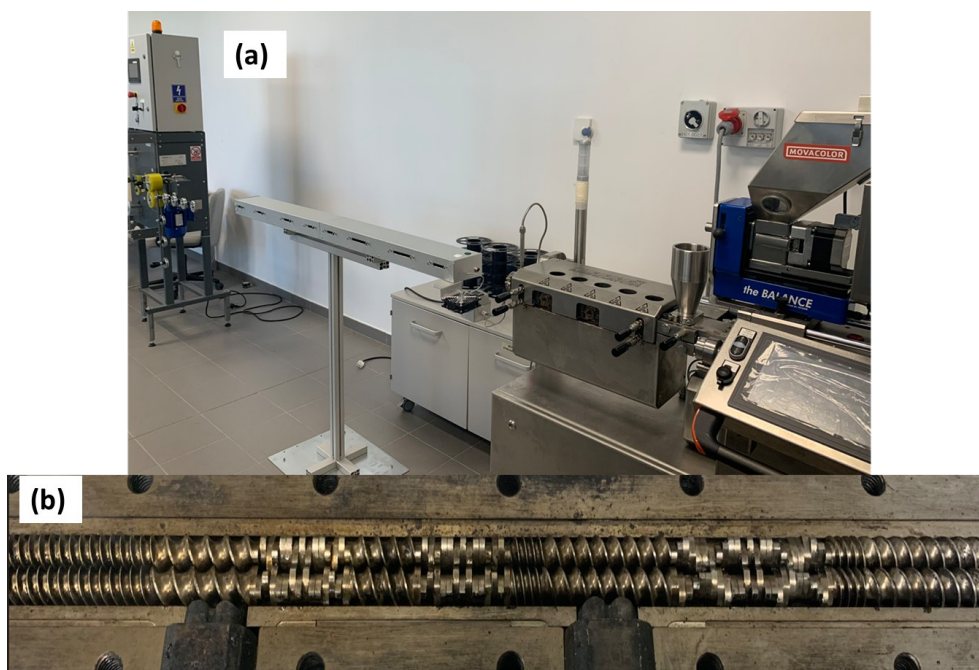


Figure 2.3 - Graphic representation of extrusion system (a) and corotating screws (b)

The optimized extrusion parameters for the pure PBAT biopolymer and related biocomposites are shown in Tab. 2.4. The differences in extrusion parameters may be related to the change in viscous behavior as the HF content increases. A screw speed between 13 and 15 rpm was used for all PBAT biocomposites to optimize material flow and flow rate.

The temperature profile should be increasing in the first zones, where the material is pushed forward and melted. In the zones where the maximum temperature is reached, mixing takes place. Therefore, in zones 3 and 4, the maximum temperatures have been set, corresponding to the screw mixing section. Then, the temperature must be lowered to cool the material and achieve the right consistency for filament formation.

Table 2.4 - Temperature profile of the twin screw extruder

Formulations	Temperature Profile [°C]							
	Hopper	Zone 1	Zone 2	Zone 3	Zone 4	Zone 5	Zone 6	Zone 7
PBAT	40	110	118	125	120	117	117	115
PBAT + 5% HF	40	110	118	125	120	117	117	115
PBAT + 10% HF	40	110	118	125	123	121	121	118
PBAT + 20% HF	40	110	118	125	123	121	121	118
PBAT + 40% HF	40	125	140	142	140	135	133	130

The extruded compounds were air-cooled and collected using the Zamac Mercator RNZ-16 winder (Fig. 2.4). Tab. 2.5 shows the winding parameters used for all compounds. Starting from the same batch quantity of the different extruded materials, the winding procedure yielded filaments with different total lengths (200-250 m) as the HF biofiller content varied. This phenomenon is presumed to be due to the decrease of the biopolymer matrix volume as the HF biofiller content increases.



Figure 2.4 - Winder at the end of the extrusion process

Table 2.5 - Winding process parameters of PBAT-based biocomposites

Preset meters [m]	Winding torque [%]	Feeding speed [m/min]	Guiding [m]
500	30	1.4	2.0

All produced materials were pelletized and converted into the form of fine granules, which were obtained following shredding cycles carried out at room temperature with the “Felfill Shredder” tool (Fig. 2.5), equipped with 12 stainless steel blades with 4 different cutting profiles. The granules produced represent the raw material used partly to produce specimens by injection molding procedure and the remaining part to obtain biocomposite films by flat-head extrusion.



Figure 2.5 - Representation of the pelletizing system “Shredder”

2.2.6 Injection Molding

The obtained pellets were dried in an oven at 40 °C overnight before being subjected to the injection molding process. The same was carried out using a Babyplast 10/12 injection molding machine (manufactured by Cronoplast) equipped with a mold for standard type 5A specimens, according to UNI EN ISO527-2 (Fig. 2.6) [3].

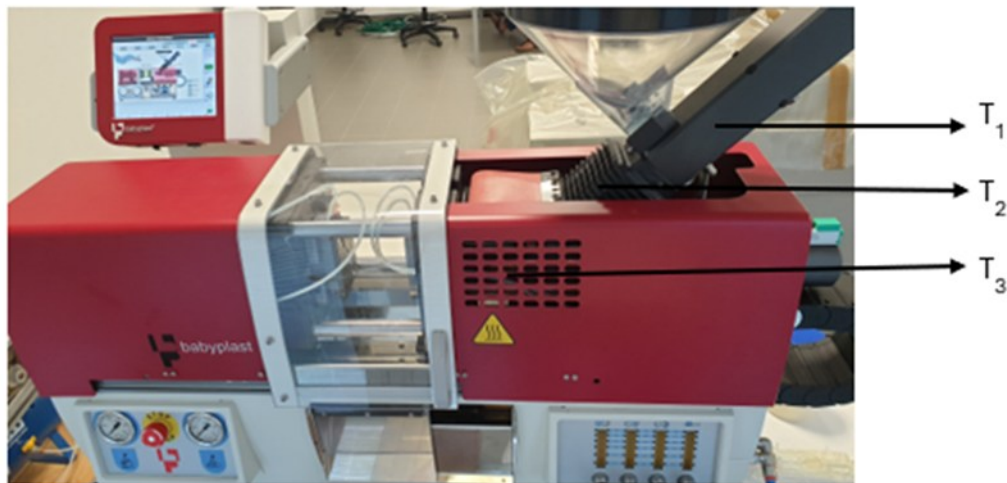


Figure 2.6 - Representation of Babyplast 10/12 injection molding system

Table 5 lists the parameters utilized for injection molding. For all PBAT-based formulations, the samples were produced at a temperature of 125 ± 5 °C, varying according to the processed material. The lowest temperatures ($T_1 = 120$ °C, $T_2 = 120$ °C, $T_3 = 125$ °C) were used for the pure

PBAT polymer and the related 5 wt.% HF biocomposite mix, while HF at higher concentrations made the material less fluid in the pre-injection build-up phase, so production was conducted by slightly varying pressures and increasing temperatures. A detailed representation of the temperatures used is given in Tab. 2.6, while other parameters related to material charge, pressures, injection times and speeds are given in Tab. 2.7.

Table 2.6 - Process temperatures for each composition (T1: plasticizing, T2: storage, T3: injection)

Composition	T ₁ [°C]	T ₂ [°C]	T ₃ [°C]
PBAT	120	120	125
PBAT + 5%HF	120	120	125
PBAT + 10%HF	122	122	127
PBAT + 20%HF	124	124	129
PBAT + 40%HF	128	128	131

Table 2.7 - Main injection parameters of PBS-based composites

Injection Parameters	PBAT	PBAT+5%HF	PBAT+10%HF	PBAT+20%HF	PBAT+40%HF
Charge materials [mm]	19	19	19	19	19
Cooling time [s]	5	30	30	30	30
1st Injection pressure [bar]	30	40	40	45	45
1st Time pressure [s]	4	4	4	4	4
2nd Injection pressure [bar]	28	30	30	30	30
2nd Time pressure [s]	4	4	4	4	4
1st Injection speed [%]	100	100	100	100	100
2nd Injection speed [%]	100	100	100	100	100

Representative specimens of each composition are shown in Fig. 2.7. The color changing, from left to right, reflects the increase in wt.% of HF. The dumbbell specimens, about 40 replicas produced for each composition, were used for subsequent structural, mechanical, and dynamo-mechanical characterization.

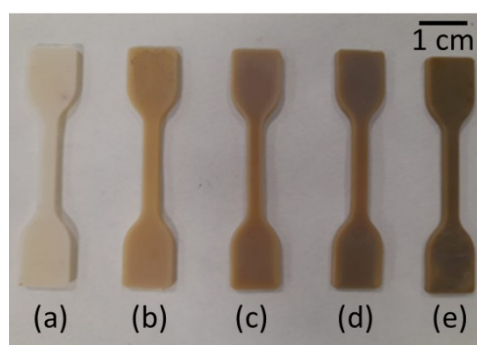


Figure 2.7 - Representation of dumbbell specimens obtained by injection molded: (a) pure PBAT, (b) PBAT + 5% HF, (c) PBAT + 10% HF, (d) PBAT + 20% HF and (e) PBAT + 40% HF

2.2.7 Flat head Extrusion of Biocomposite Films

Pellets obtained from the compounding procedure was used to make the biocomposite films. The formulations tested are given below:

- PBAT
- PBAT + 10% HF
- PBAT + 20% HF

The films were cast filmed using a Teach-Line E20T flat head extruder equipped with a CR72T calender (Collin GmbH, Ebersberg, Germany). All the pellets of the different biocomposites were loaded into the hopper right away after being pre-dried for at least 12 hours at 60 °C to eliminate any absorbed moisture. The screw was then set to run at a speed of 100 rpm and a thermal profile from the hopper to the flat filming die, as indicated in Tab. 2.8. In contrast to the extrusion parameters of the biocomposite filament, the same conditions were maintained for all biocomposite materials to make the process as homogeneous as possible.

Table 2.8 - Temperature profiles used in the flat-head extrusion procedure

Temperature [°C]				
Zone 1	Zone 2	Zone 3	Zone 4	Head
110	115	120	110	105

The adopted process conditions provided samples of biocomposite films (Fig. 2.8) with HF content of 0, 10 and 20 wt.%, named FILM-PBAT, FILM-PBAT+10%HF and FILM-PBAT+20%HF, respectively. The films had thicknesses not strictly constant either for each formulation or as the filler content varied. Critical operational issues appeared most pronounced during the filming of the pure matrix and the compound with the lowest hemp powder content

(PBAT+10%HF). These difficulties resulted in flat films with ill-defined or seemingly uneven edges, compared to the PBAT-based biocomposites with the highest HF content (PBAT+20%HF). This behavior could be caused by rheological aspects not explored in this research.

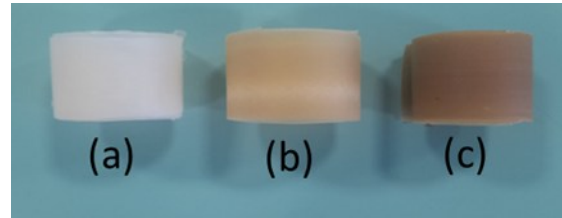


Figure 2.8 - Representations of films obtained by flat-head extrusion: (a) PBAT film, (b) PBAT+10%HF film, (c) PBAT+20%HF film

2.3 Characterization of Biofillers, Polymer Matrices and Biocomposite Materials

2.3.1 Particle Size Analysis

A Mastersizer 3000 laser diffraction particle size analyzer (Malvern Instruments Ltd., Malvern, UK) equipped with an Aero S dry powder dispersion unit was used to characterize size distribution curves and particle sizes, including mean diameter (D_{mean}) and D_{x10} , D_{x50} , and D_{x90} percentiles. The analysis was performed by applying the Fraunhofer approximation as the scattering model. HF particles were dispersed by applying a flow of compressed air at a pressure of 4 bar and a feed rate of 40 %. When the powder is larger than 20 μm , as in this case, this method can be applied. The standard percentiles, D_{x10} [μm], D_{x50} [μm], and D_{x90} [μm], which represent the diameters of the particles in the sample below which 10, 50, and 90 % of the volume density fraction of the sample lie, respectively, were used to express the particle size distribution. D_{mean} [μm], the corresponding number of the average volume diameter, was used to represent the average volume particle size.

2.3.2 Scanning Electron Microscopy

The morphology of HF biofiller and its distribution and dispersion within the PBAT biopolymer matrix, as well as charge-matrix interfacial adhesion, were observed with a scanning electron microscope (SEM) field emission cannon (FEG-SEM, Nova NanoSEM 450, FEI Company, USA) with a backscatter detector. For injection-molded specimens, cross-sections were prepared by

immersing the specimens in liquid N₂ for an optimal time (30 s) to allow total embrittlement of specimen under test. Subsequently, the samples were cryogenic fractured, and a coating of gold, about 10 nm thick, was sputter-deposited on their surfaces to give them conductivity. All images were acquired at a working distance of 6 mm, a spot size of 4 (a.u.) and an accelerating voltage of 25 kV, at different magnifications.

2.3.3 Fourier Transform-Infrared Spectroscopy

The Perkin Elmer Spectrum Two FT-IR spectrophotometer (Shelton, CT, USA) was used to perform FT-IR (Fourier Transform-Infra Red) spectroscopy measurements in attenuated total reflectance (ATR) mode with a diamond crystal in order to gather data on the chemical structures of the PBAT biopolymer, the HF biofiller, and any modifications due to filler-matrix interactions (Fig. 2.9).



Figure 2.9 - Perkin Elmer Spectrum Two FT-IR spectrophotometer

FT-IR analysis consists of irradiating sample with a beam of electromagnetic radiation having a wavenumber between 4000 and 400 cm⁻¹, which makes this test non-destructive towards the material. The energy absorption causes substance under investigation to move from a lower to a higher vibrational level. The amount of energy absorbed, and thus the wavelength of the incident radiation, depends on the characteristic bond strength of each functional

group, so by analyzing the wave numbers at which absorption is observed, the corresponding functional group can be recognized [8].

As analysis output, a graph is returned by the instrument having in abscissa the wave number (ν) representing the inverse of electromagnetic radiation frequency expressed in cm^{-1} and in ordinate the transmittance (T), i.e., the fraction of radiation transmitted (I) by the sample with respect to the intensity of the incident monochromatic radiation (I_0), expressed in arbitrary units [a.u.], respectively defined by the following equations 2.1 and 2.2:

$$\nu = \frac{1}{\lambda}$$

Equation 2.1 - Wavenumber algebraic formula

$$T = \frac{I}{I_0}$$

Equation 2.2 - Transmittance algebraic formula

The parameter λ indicates the wavelength of radiation (cm), and specifically the intensity (I) can be understood as the number of photons passing through the unit section of the sample in the unit time.

An additional parameter is the absorbance (A), which indicates the power fraction of the monochromatic electromagnetic wave absorbed by the analyte (Equation 3). A is inversely proportional to the parameter T .

$$A = \log \frac{1}{T}$$

Equation 2.3 - Absorbance algebraic formula

Specimens from the twin-screw extrusion and injection molding were in this study. Each spectrum was performed on the cross section of the specimens and was obtained in a wavenumber range of $4000\text{--}400 \text{ cm}^{-1}$ with a resolution of 4 cm^{-1} and 16 scans acquisition. Before analysis, the measured samples were conditioned at 40°C overnight. Measurements were performed at room temperature and at atmospheric pressure. The data obtained were subsequently analyzed with PerkinElmer's Spectrum 10 software, and triplicate averages are reported.

2.3.4 Uniaxial Tensile Test

Mechanical properties were tested by performing the uniaxial tension test (UTT) on Type 5A specimens (according to ISO527-2) using the Test dynamometer (Model 112, Test GmbH Universal Testing Machine, Germany) equipped with a 2 kN load cell (Fig. 2.10).



Figure 2.10 - Test dynamometer used for uniaxial tensile testing (UTT)

The tensile test is a mechanical characterization of materials that falls under the tests recognized as “destructive” and is normed for polymeric materials by the standard UNI EN ISO 527 [3]. The test is carried out at a constant strain rate, with the increasing applied force, until the specimen fails. An engineering curve is derived from this mechanical characterization test, reporting the stress σ (MPa, Equation 2.4), obtained from the ratio between the applied force (F) and the initial cross section area (A_0), as a function of the strain ϵ (mm/mm, Equation 2.5), derived from the elongation Δl (mm) of the specimen over its starting length (l_0).

$$\sigma = \frac{F}{A_0}$$

Equation 2.4 - Formula used for calculating the stress σ (MPa)

$$\varepsilon = \frac{\Delta l}{l_0}$$

Equation 2.5 - Formula used for calculating the strain ε (mm/mm)

The specimens were clamped at both ends and subjected to a constant tensile speed of 100 mm/min until failure. Eight replications were tested for each composition, and the tests were conducted at room T and P. From the stress-strain curves, the values of Young's modulus (E), yield stress (σ_Y), stress at break (σ_B), elongation at break (ε_B), and toughness (T) were calculated. Before testing, the samples were conditioned in an oven at 60°C overnight to remove absorbed moisture. Finally, the average values of l_0 and A_0 of 51 mm and 8 mm², respectively, were chosen to be taken into account to standardize the parameters. These parameters are essential for calculating the stress and strain values and thus for normalizing the force and elongation values obtained from the tensile test.

The standard dimensions (Fig. 2.11) in millimeters are respectively given in Tab. 2.9:

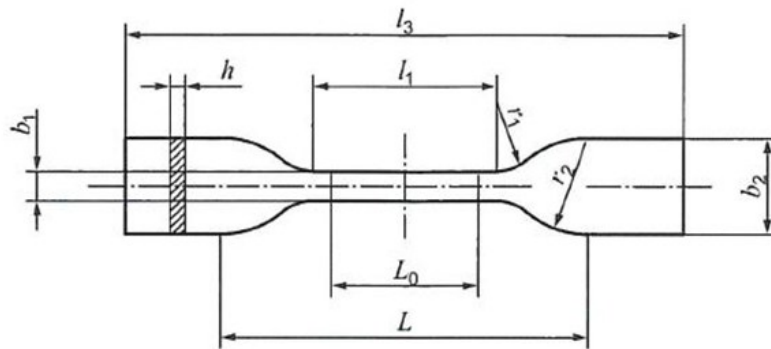


Figure 2.11 - Graphical representation of dog-bone specimen type 5A (ISO 527-2) [3]

Table 2.9 - Dimensions of type 5A specimens (ISO 527-2). All dimensions are in mm

Symbol	Characteristic dimensions	5A
l_3	Total length	≥ 75
b_2	Ride at ends	12.5 ± 1
l_1	Length narrow parallel part	25 ± 1
b_1	Width narrow part	4 ± 0.1
r_1	Small radius	8 ± 0.5
r_2	Large radius	12.5 ± 1
L	Initial distance between clamps	50 ± 2
L_0	Measuring length	20 ± 0.5
h	Thickness	2 ± 0.2

The values obtained were imported, analyzed and processed using Excel spreadsheet.

2.3.5 Dynamic Mechanical Analysis

The viscoelastic properties of the materials were studied by dynamic-mechanical analysis (DMA) with the “DMA TA Q800” instrument produced by TA Instruments company (USA) (Fig. 2.12).



Figure 2.12 - TA Instrument Q800 DMA

DMA provides the values of storage modulus (E'), loss modulus (E'') and damping factor ($\tan\delta$) defined as the ratio of loss modulus to storage modulus ($\tan\delta = E'' / E'$), reported as a function of temperature [9,10]. It is based on the possibility of applying cyclic stresses to the sample with a fixed frequency as a function of temperature and with the possibility of performing the analysis in different types of tests (bending, tensile or torsion) depending on the type of device (clamp) used. It can be used for solid or semi-solid polymeric samples, in general for samples that may be subjected to stress. The values provided by DMA, because they change with temperature, are closely related to the phase and structural changes of the composite materials tested. The material elastic component is represented by the conservative E' , also known as the storage modulus, which is correlated with the sample stiffness. Conversely, the viscous component is represented by the dissipative modulus E'' , which is associated with the sample capacity to dissipate mechanical energy through molecular motion. E' and E'' represent, respectively, the real and imaginary part of the complex modulus, characteristic of viscoelastic materials. DMA also enables the detection of the glass transition temperature (T_g), the analysis of polymer chain motions, the modification of the elastic modulus with temperature, and other secondary transitions. The maximum of the $\tan\delta$ curve, which indicates a macroscopic drop in the sample stiffness, was used to determine the glass transition temperature (T_g) [11]. Equation 2.6 was used to determine the reduced modulus (E_{red}), for assessing the HF biofiller reinforcing effectiveness in the polymer matrix, based on the E' values derived from the DMA analysis.

$$E_{red} = \frac{E'_{i-th}}{E'_{PBAT}}$$

Equation 2.6 - Equation for calculating the representative reduced modulus of reinforcement efficiency due to the addition of HF biofiller

where E'_{i-th} represents the storage modulus of the biocomposite and E'_{PBAT} represents the storage modulus of the pure PBAT biopolymer.

For DMA measurements on sample obtained by injection molding, the ASTM D7028 standard [12] was followed with a single cantilever clamp configuration (Fig. 2.13) suitable for rectangular-section thermoplastic composite specimens capable of flexing, with three replications for each composite material. In the single cantilever configuration, the specimen is fixed to two grips, one static and the other movable. The movable grip is supported by an

air bearing that allows its movement. For films produced by flathead extrusion, the analyses were carried out using the film tension clamp (Fig. 2.14).

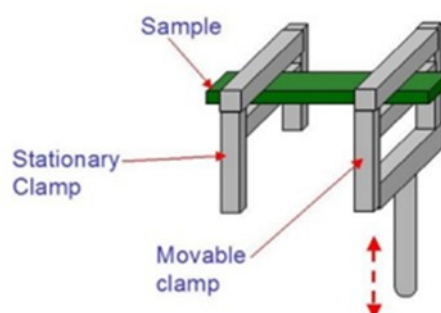


Figure 2.13 - Single cantilever clamp configuration



Figure 2.14 - Film tension clamp

The tests were carried out in "Multy-Frequency-Strain" mode with the amplitude strain of 10 μm , frequency of 1 Hz, temperature range of -60 to 55 $^{\circ}\text{C}$, initial isothermal soaking duration of 15 min, and temperature ramp of 3 $^{\circ}\text{C}/\text{min}$. The samples were vacuum stored at room temperature prior to the test.

2.4 Results and Discussion

2.4.1 Particle Size and SEM Analysis of the HF Biofiller

Following the sieving process, the HF particle size distribution and morphology was characterized, as illustrated in Fig. 2.15. SEM observation of the HF morphology (Fig. 2.15b) shows that the particles exhibit an irregular rod geometry in a wide dimensional range. Particle size distribution results in a monophasic broad curve and the main peak around 40 μm followed by a marked shoulder at lower size class values (Fig. 2.15a).

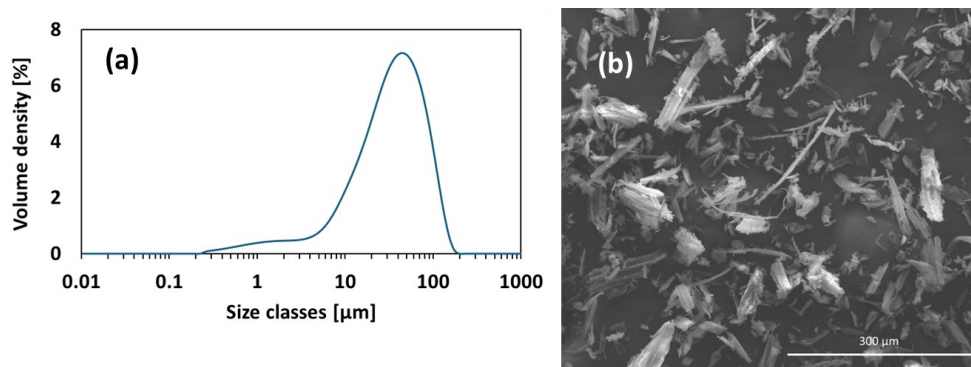


Figure 2.15 - HF Biofiller characterization: (a) Particle size distribution after sieving and (b) SEM picture at 400x

Table 2.10 - Main fractions of HF biofiller

Sample name	Dx10 [µm]	Dx50 [µm]	Dx90 [µm]	Dmean [µm]
HF Biofiller	7.4±0.3	34.5±0.2	81.4±0.3	41.5±0.5

The volume mean diameter (D_{mean} , µm) is around 40 µm, with a size distribution characterized by D_{x10} [µm], D_{x50} [µm], D_{x90} [µm] shown in Tab. 10. A D_{x90} higher than the mesh diameter of the sieve used is observed. The presence of fibers with a larger size than that predicted by the sieve can be attributed to non-uniformity in the aspect ratio of the fibers themselves. In fact, some of these fibers have a diameter lower than 50 µm, but a length significantly greater than the diameter. This morphological characteristic allows the fibers to pass through the sieve holes in the mill, despite the fact that their actual length exceeds the theoretical sieve separation limit.

2.4.2 FT-IR Analysis of HF Biofiller

The HF biofiller was also characterized structurally by FT-IR analysis, and Fig. 2.16 shows the corresponding spectrum obtained. The high-frequency band in the range of 3050 to 3600 cm^{-1} (a) corresponds to the stretching vibrations of hydroxyl groups (-OH). The signals present in the range between 2800 and 3000 cm^{-1} (b) are related to the symmetric and asymmetric stretching of the C-H bonds of methyl and methylene groups [13,14], and are similar to those observed in other studies on lignocellulosic fibers such as wood and agricultural by-products. Around 1730 cm^{-1} (c), we find the characteristic carbonyl C=O signal of esters [15]. The NH-bond adsorption band of protein amides appears around 1600 cm^{-1} (d) [16], and the peak

attributed to the stretching of the aromatic group present in lignin turns out to be at 1506 cm^{-1} (e) [17], while at 890 cm^{-1} (f) the signal related to the extension of the C-O-C bridge is present, characteristic of cellulose in the fiber [18]. The vibration signal at 1240 cm^{-1} (g) indicates the C-O stretching of acetyl groups related to hemicellulose [19]. The C-OH functional group of cell wall polysaccharides is responsible for the strongest band in the spectra [20], which is around 1030 cm^{-1} (h). Finally, the peak relative to the absorption band near 470 cm^{-1} (i) indicates the presence of silica, corresponding to the stretching and bending modes of siloxane Si-O-Si bonds [21–23].

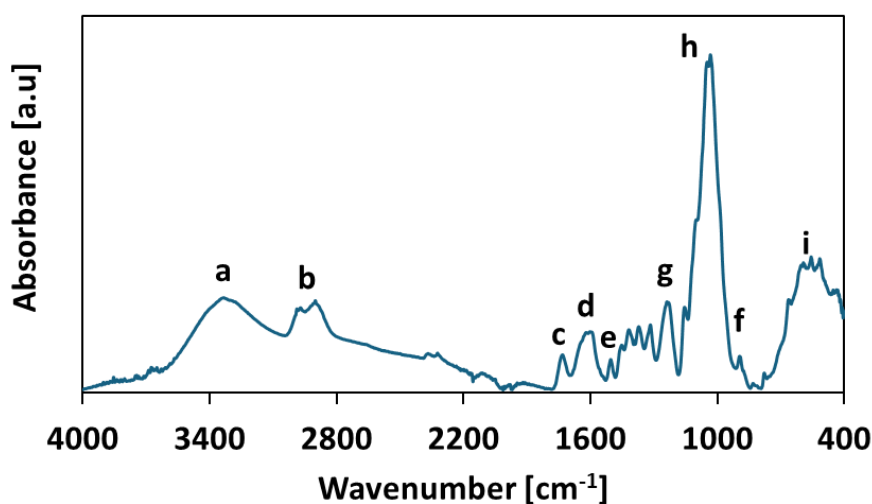


Figure 2.16 - FT-IR spectra of biofiller HF

2.4.3 FT-IR Analysis of PBAT Polymer Matrix and PBAT-based Composites

Similar to the HF biofiller, FT-IR spectra were also acquired for the PBAT and related biocomposites samples to study the structure of the polymer matrix and the potential changes resulting from the possible chemical interactions between the polymer chains and the filler particles. Fig. 2.17 shows, from bottom to top, the spectrum of pure PBAT and its biocomposites. The spectrum for the PBAT biopolymer matrix shows the characteristic signals of its chemical structure in both regions: stretching ($4000\text{--}1500\text{ cm}^{-1}$) and fingerprinting ($1500\text{--}400\text{ cm}^{-1}$), which are widely discussed in the literature [24,25].

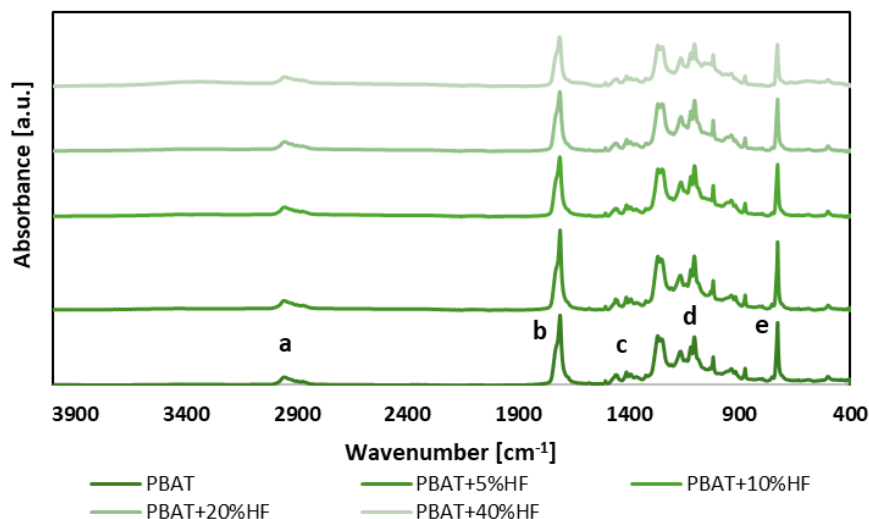


Figure 2.17 - FT-IR spectra of PBAT and PBAT-HF biocomposites

The area between 2800 and 3000 cm^{-1} (a) is related to the symmetric and asymmetric stretching of methylene ($-\text{CH}_2-$) group in adipate and 1,4-adipate and 1,4-butanediol-units. The intense band recorded at 1714 cm^{-1} (b) characterizes the stretching vibration of carbonyl $\text{C}=\text{O}$ attributed to the ester group. Specifically, the signal consists of a main peak attributed to the $\text{C}=\text{O}$ group of TPA monomers and the left shoulder characteristic of the $\text{C}=\text{O}$ of AA monomers. The bands between 1578 and 1453 cm^{-1} (c) were attributed to the stretching of the double bonds ($\text{C}=\text{C}$) in the aromatic ring of terephthalate unit. In addition, the bands in the range between 1300 and 1000 cm^{-1} (d) are attributed $(\text{O}=\text{C})\text{-O-}$ symmetric and asymmetric stretching in the adipate and terephthalate units. Finally, signals at 1016, 875, and 725 cm^{-1} (e) are associated with in-plane and out-of-plane $=\text{C-H}$ bending in the aromatic ring of the terephthalate unit.

Similar peaks were also observed for the biocomposite materials. In fact, spectroscopy analysis shows that the introduction of HF biofiller at different weight concentrations causes no change in the wavenumbers (cm^{-1}) of the characteristic signals of the PBAT matrix. This analysis suggests no detectable chemical interactions, but this does not preclude the presence of physical interactions such as entanglements between the biofiller and biopolymer matrix. Furthermore, regarding the signal intensities of biocomposite materials, it is observed that as the weight concentration of HF biofiller increases, there is a proportional decrease in the

characteristic signal of the carbonyl group $C=O$ 1714 cm^{-1} . This may be due to the gradual reduction in the amount of biopolymer matrix within the biocomposite material.

2.4.4 UTT of PBAT-based Composites

Fig. 2.18 shows the different PBAT-based biocomposite samples at different concentrations of HF after the tensile tests performed. It is observed that as the content of HF biofiller within the PBAT biopolymer matrix increases, the deformation behavior of the samples is different. Even at the lowest filler concentration, the deformation of PBAT significantly decreases.



Figure 2.18 - Specimens 5A obtained by injection molding after tensile testing, from left to right: PBAT, PBAT+5%HF, PBAT+10%HF, PBAT+20%HF, PBAT+40%HF

The stress (σ)-strain (ϵ) curves, obtained for PBAT and its biocomposites in Fig. 2.19, show typical behavior of flexible and tough polymeric materials, characterized by the presence of a well-defined yield point. To visually represent the different formulations, a color scale was adopted in which an increase in color shade correlates with an increase in filler content in the biocomposite.

Tensile curves allow us to distinguish three main phases that describe the mechanical behavior of the material. The first phase is linear and reflects elastic deformation, where stress increases proportionally to the strain. This behavior is related to the material's ability to store energy without undergoing permanent changes in structure. In this phase, the deformation of the material is completely reversible if stress were released from the specimen. The elastic

phase culminates in a peak that corresponds to the yield point, where a neck is formed in the central region of the specimen.

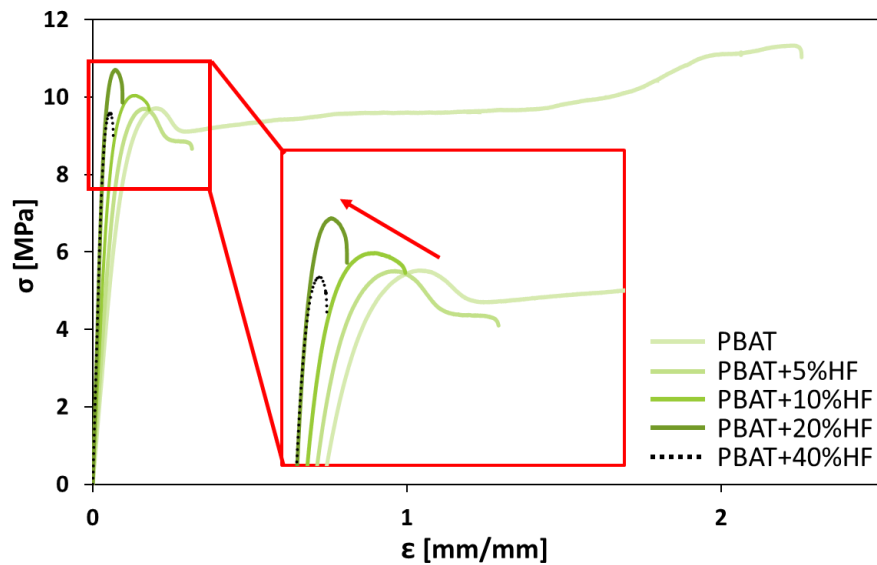


Figure 2.19 - Stress (σ) -strain (ϵ) curves of PBAT + HF

After the yield point, the material undergoes plastic deformation. In this region, the strain remains relatively constant, while the neck propagates along the entire length of the specimen. This behavior is explained by the polymer chains aligning in the tensile direction, which permits a consistent plastic deformation up to the grip zones.

The final phase is marked by a progressive increase in stress, culminating in failure of the specimen.

The variation of tensile curves related to materials at different concentrations of HF is evident compared to the curve of pure PBAT. Indeed, a transition is observed from ductile and tough behavior typical of pure PBAT, to stiffer and stronger behavior up to 20 wt.% HF. The PBAT+40%HF composite showed higher brittleness than the other materials analyzed. This composite yielded immediately after the yield point, without manifesting any plastic deformation phase.

In particular, as the percentage of FC increases, there is an increase in the slope of the initial portion of the curves, which indicates an increase in Young's Modulus (E). The increase in

slope occurs up to 20 wt.% FC and then stabilizes at 40 wt.%, implying a saturation of the polymer with respect to the percentage of added biofiller.

The increase in E modulus reflects an increase in material stiffness compared to pure polymer PBAT, an expected result when stiff fillers are incorporated into a polymer matrix [26–28]. In specimens filled up to 20 wt%, σ_y increases proportionally compared to pure PBAT, indicating that the filler contributes to improving the material's ability to resist mechanical stress. However, the increase in stiffness results in a reduction of the elastic range up to the yield point. Despite this, the increase in strength allows the material to maintain resilience similar to that of unmodified PBAT. The increase in σ_y shows more effective stress transfer from the polymer matrix, which is flexible and deformable, to the filler, which is stiffer and stronger. From an application point of view, the increase in σ_y is particularly interesting, as it allows the material to withstand higher stresses in the elastic regime, thus expanding its potential industrial applications. This trend, however, is not seen in samples containing 40 wt.% HF, which exhibit a lower σ_y but still comparable to σ_y of pure PBAT. This suggests that the maximum filler limit tolerable by the polymer matrix has been exceeded.

A reduction in the tensile parameters at break, σ_B and ϵ_B , proportional to the increase in hemp fiber content is observed. As a result, the toughness of the material undergoes a significant decrease from pure polymer to filled biocomposite.

This is mainly due to a decrease in strain at break; a somewhat expected result observing the stress (σ)-strain (ϵ) curves of the materials and in any case typical of this type of composite, which, however, does not invalidate the improvements observed in the other mechanical parameters [29,30]. Tab. 2.11 shows the values of mechanical parameters collected from tensile test for PBAT-HF samples.

Table 2.11 - Values of mechanical parameters collected from tensile test for PBAT-HF samples

Sample name	E [MPa]	σ_y [MPa]	σ_B [MPa]	ϵ_B [mm/mm]	T [MJ/m ³]
PBAT	94 ± 4	9.7 ± 0.1	10.9 ± 0.1	2.2 ± 0.1	21 ± 1
PBAT+5%HF	118 ± 3	9.7 ± 0.2	8.6 ± 0.2	0.4 ± 0.07	3.6 ± 0.6
PBAT+10%HF	169 ± 7	10.2 ± 0.1	9.2 ± 0.2	0.2 ± 0.01	1.6 ± 0.2
PBAT+20%HF	276 ± 11	10.4 ± 0.2	9.7 ± 0.3	0.1 ± 0.01	0.6 ± 0.1
PBAT+40%HF	282 ± 12	9.7 ± 0.2	9.1 ± 0.2	0.1 ± 0.01	0.4 ± 0.1

Compared with common LD-PE-based WPCs, the mechanical properties of our composites are in the same range. Using maleic anhydride as a compatibilizer and 38 wt% of wood flour, the LD-PE composites showed E around 230 MPa, strength values around 9 MPa, and elongation values below 5% [31]. In this case, based on mechanical properties alone, these samples could be a viable alternative.

The use of biopolymer matrices with a wood flour content has also been explored in the literature. In the work of Wang et al. [32] they made PBAT-based WPC composites blended up to 50 wt% wood flour. Their results show that at the same concentration of WF filler (40 wt%) they obtained a 5% lower elongation, an E modulus around 260 MPa, and when it comes to σ_B they obtained a 50% improvement compared to the samples analyzed in this study.

In further work by Ferreira et al. [33] they mixed PBAT with three different types of wood dust from the Amazon forest at 10 wt%. Their results show that again the elongation is less than 5% but at the same filler concentration in the present work the tensile strength at break was halved by 50% unlike in our work where a lower value of only 21% is observed compared to the pure polymer matrix.

2.4.5 DMA of PBAT-based Composites

DMA shown in Figg. 2.20 and 2.21, are in line with what emerged from the tensile tests.

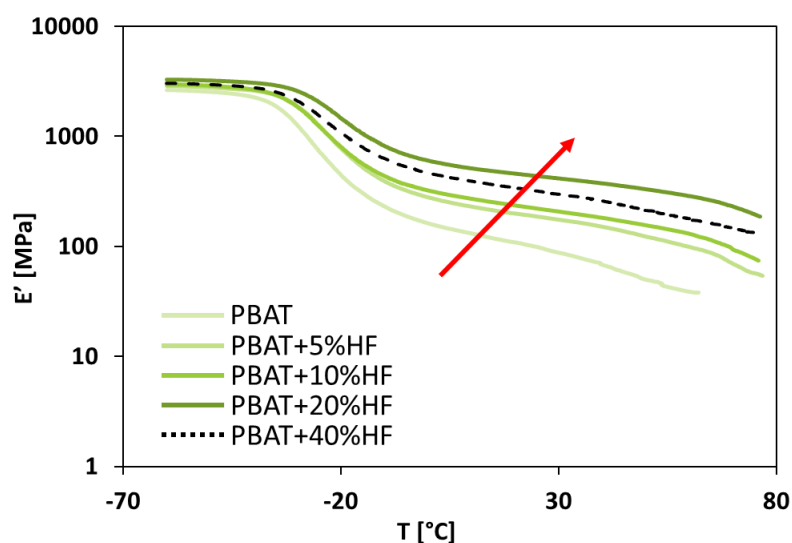


Figure 2.20 - Representation of curves inherent to modulus E' of type 5A biocomposite samples with different HF biofiller content (0-40 wt.%)

Fig. 20 displays the trend of the storage modulus (E') for the examined specimens as a function of temperature as HF content changes. The obtained DMA curves present the typical behavior of a semicrystalline polymeric material, such as PBAT [34]. At low temperatures, below the T_g , all compositions show relatively high E' values, characteristic of a rigid material. At $-60\text{ }^{\circ}\text{C}$, the values range from $2460 \pm 2\text{ MPa}$ for pristine PBAT to $2997 \pm 3\text{ MPa}$ and $2950 \pm 2\text{ MPa}$ for PBAT with 20 and 40 wt.% HF, respectively. With increasing temperature, E' undergoes a marked reduction, by about an order of magnitude. An inflection point is evident around the glass transition. This phenomenon is attributable to the increased movement of the polymer chains, which become more flexible because of the increase in kinetic energy due to the increase in temperature [35].

Above T_g , in the rubbery region, the E' modulus continues to decrease for all samples analyzed, but at a significantly reduced rate. This temperature range represents the “rubbery plateau,” where the crystalline fraction of the material remains solid, while the amorphous fraction has softened. At this stage, the material retains a leathery texture, making it suitable for applications within this temperature range [36]. Above the $40\text{ }^{\circ}\text{C}$ threshold, the slope of the curve increases again due to the progressive melting of the crystalline fraction, leading the material to lose its solid properties and making the modulus no longer measurable because beyond this temperature, the material transitions from solid to viscous liquid. This general behavior is observable for all the compositions analyzed, although significant differences emerge in relation to HF content. The results confirm what the tensile tests have shown: the E' modulus increases as the percentage of HF increases up to 20 wt.%, expanding the temperature range in which the composite material retains significant stiffness. This improvement allows the composite to retain greater mechanical properties compared to the pure matrix even at higher temperatures.

In contrast, for the material containing 40 wt.% HF, a peculiar behavior is observed, with a reduction in E' modulus over the entire analyzed temperature range. This trend confirms that the excessive filler content compromises the overall stiffness of the composite.

$\tan\delta$ of the analyzed materials is shown in Fig. 2.21. T_g was determined from the temperatures corresponding to the peaks of the $\tan\delta$ curves. As the concentration of HF in the polymer increases up to 20 wt%, the $\tan\delta$ peaks show a decrease in intensities and move toward higher

temperatures. However, a further increase in the amount of HF charge reverses this trend, as already observed in previous characterization.

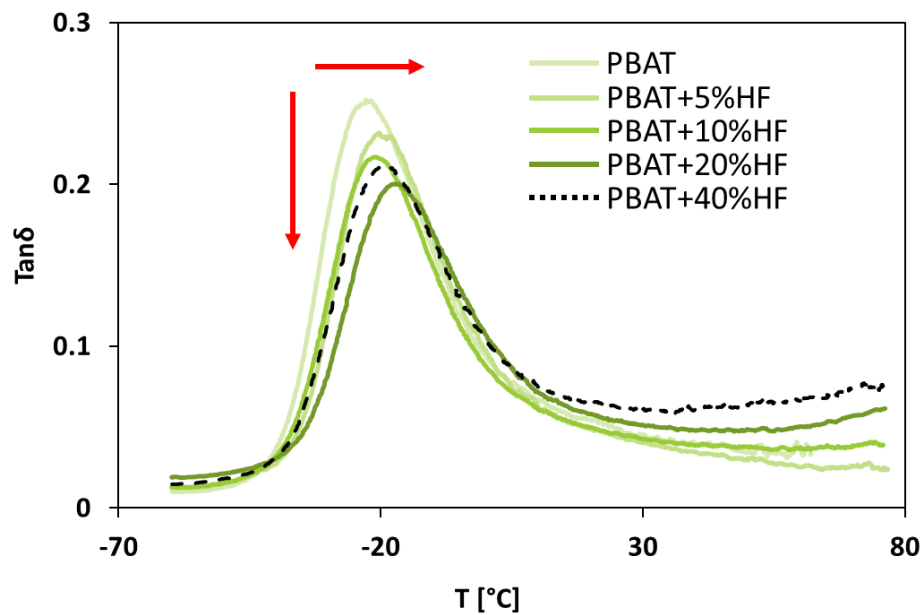


Figure 2.21 - Representation of $Tan\delta$ curves of type 5A biocomposite samples with different HF biofiller content (0-40% by weight)

The reduction in peak intensity and their shift toward higher temperatures generally indicate improved interactions at the interface between the polymer matrix and the filler [37–39]. This finding is linked to an improvement in the mechanical and thermal stability of the composite material [40,41]. Mathematically, $Tan\delta$ is defined as the ratio of the loss modulus (E'') to the storage modulus (E') [9,10]. Therefore, the decrease in $Tan\delta$ intensity can be attributed to a higher fraction of conservative energy with increasing filler concentration up to 20 wt.%, as shown in the curves in Fig. 16 [42].

Above this threshold, excessive filler content appears to compromise the performance of the material, possibly making it more brittle and thus reducing its thermo-mechanical properties.

By examining the slope of the fitting line resulting from plotting the reduced modulus (E'_{red}) against filler concentration at various temperatures (-40, -10 and 20 °C), how well fillers act as reinforcement in the polymer matrix can be assessed, in particular at temperatures above T_g (Fig. 2.22).

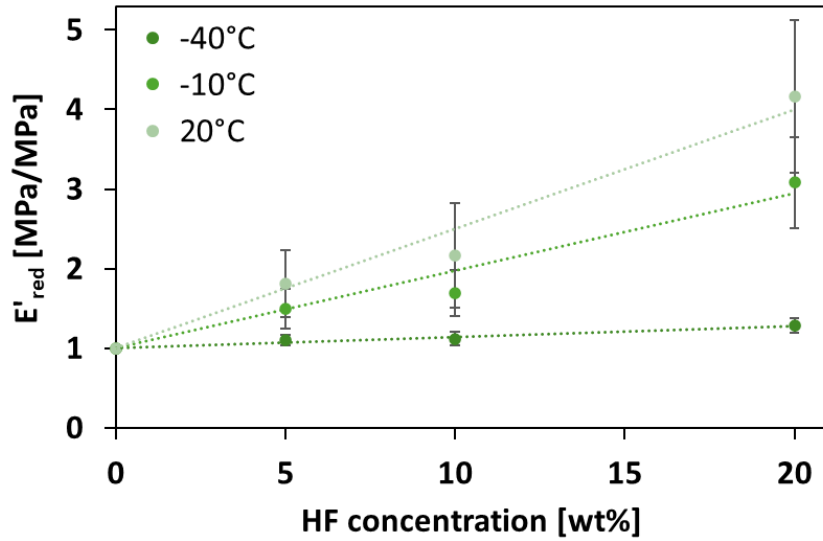


Figure 2.22 – Increase of E'_{red} for type 5A biocomposite specimens with different HF biofiller content (0-20% wt%)

Because the material is in the glassy state below T_g , where movements are mainly restricted to the stretching and bending vibrations of the atomic bonds, the mobility of the polymer chains is decreased. Any stiffening effect at this point is beneficial but limited. Conversely, the viscosity of the polymer tends to decrease in the rubbery state, which encourages viscous movement of the polymer chains and compromises the material mechanical qualities [35]. In this context, filler particles play an important role in limiting intermolecular movement, helping to maintain the mechanical performance of the composite even at elevated temperatures.

Since there is no indication of chemical interactions in the FT-IR study, the physical contact of the filler particles with the biopolymer matrix is primarily responsible for the increase in stiffness. Therefore, the inclusion of high amounts of filler allows the temperature range, in which the composites can be used effectively, to be expanded compared to pure PBAT [39].

This reinforcing effect is particularly evident in the rubbery state (approximately between -10 and 20 °C, above T_g), allowing composites to maintain their mechanical properties over a wider thermal range than the filler-free material.

The trend observed for E' modulus is consistent with results reported in studies of biocomposites reinforced with natural fibers, which show an increase in stiffness especially at

temperatures above T_g , confirming that the addition of filler materials significantly improves the stiffness of composites compared to the pure polymer matrix [35,36].

Following the results obtained from the dynamic-mechanical analysis and tensile tests on the samples made by injection molding, the films obtained from the flat-head extrusion were further analyzed with DMA, limiting the maximum HF concentration to 20 wt.% (Fig. 2.23).

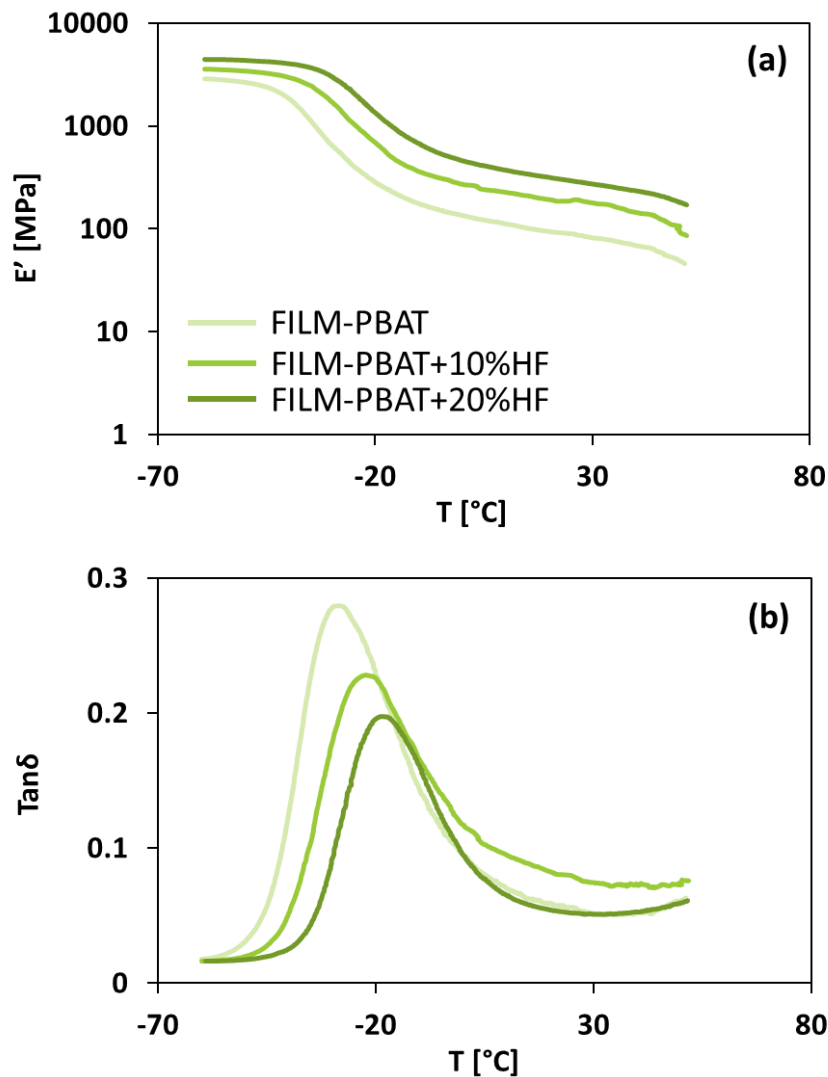


Figure 2.23 - Representation of DMA (a) and $\tan\delta$ (b) curves of biocomposite films with different HF biofiller content (0-20 wt.%)

In Fig. 2.23a, E' modulus increases with HF content for film samples too. A significant decrease in the extent of the rubbery plateau is observed in the films compared to the injection-molded samples, probably due to a decrease in the crystalline fraction, related to the different processing of the material. The analysis suggests that the inclusion of biofiller in the polymer

increases its stiffness even at elevated temperatures, thus expanding its possible use in different applications.

In the graph of Fig. 2.23b, the $Tan\delta$ curves of the films show a decrease in the intensity of the damping factor and a shift of the T_g toward higher temperatures in relation to the concentration of HF in the polymer, confirming the results obtained from the DMA tests on molded samples.

E'_{red} was also evaluated for the manufactured films to assess the effectiveness of the filler as reinforcing agent in the biopolymer matrix, and results are presented in Fig. 2.24.

Again, the E'_{red} increase becomes more evident at temperatures above T_g , as indicated by the greater slope of the fitting line, , allowing the biocomposite film to maintain their mechanical properties over a wider temperature range than the pure PBAT material.

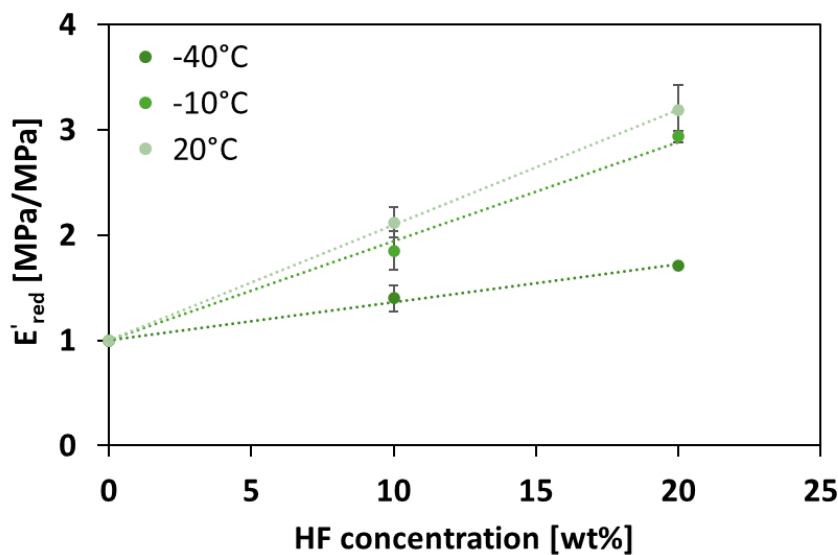


Figure 2.24 - Increase of E'_{red} for biocomposite films with different HF biofiller content (0-20% wt.%)

2.4.6 SEM Analysis of PBAT-based Composites

The distribution and dispersion of the HF biofiller within the PBAT biopolymer matrix, as well as the interfacial adhesion between the filler and the matrix, were analyzed by SEM. Fig. 2.26 presents high-resolution images of the cross sections of the samples, obtained by brittle fracture under cryogenic conditions. Such analyses are critical to support the results derived from mechanical and thermo-mechanical characterization.

Observation of the micrographs reveals a uniform distribution and homogeneous dispersion of the biofiller particles in the matrix, with no signs of aggregation or phase separation, even in the presence of high filler concentrations.

In the enlargement shown in Fig. 2.25, the surface wettability of the fibers by the PBAT matrix is clearly seen, indicative of favorable compatibility between the two composite components. This behavior results in improved mechanical properties and reduced risk of embrittlement. In particular, SEM images show the absence of significant defects or voids at the interface. This result confirms that fiber mixing in the matrix has reached an optimal level apparent impairment of structural integrity in the composite material.

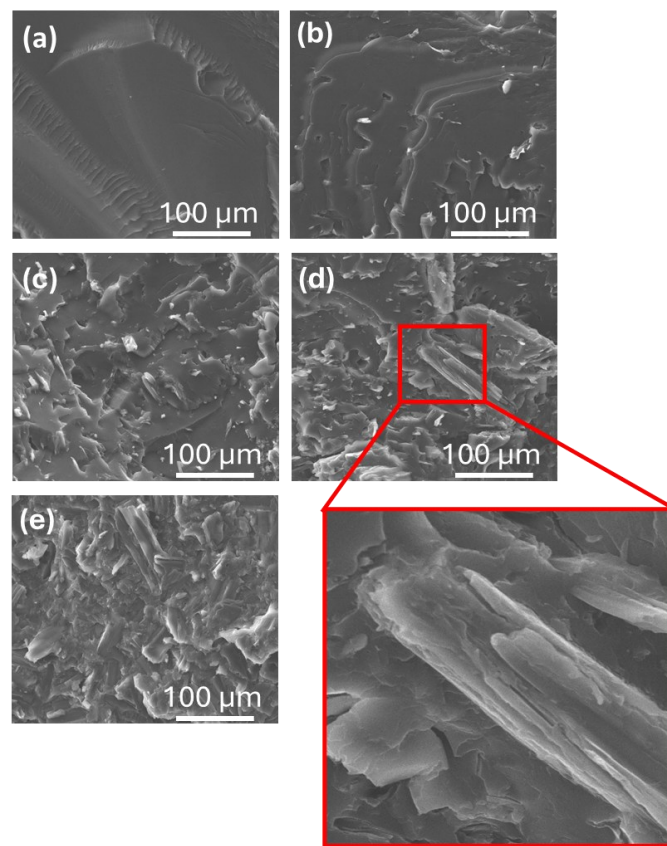


Figure 2.25 - SEM microstructures of PBAT (a), PBAT+5%HF (b), PBAT+10%HF (c), PBAT+20%HF (d), and PBAT+40%HF (e). In the inset, adhesion of the matrix to the HF fiber can be observed

2.5 Conclusions

A crucial responsibility of the scientific and industrial communities is to identify a sustainable substitute for conventional plastics utilized in different applications, such as single-use plastics, whose environmental dispersion significantly contributes to habitat contamination. The objective was to produce and characterize biocomposite materials including a biodegradable polymer matrix (PBAT) combined with varying concentrations of HF (0-40 wt.%). The blending was achieved by the melt compounding process, which enabled effective integration of the biocomposite components at varying weight concentrations by concurrently utilizing heat and mechanical treatment of the screws throughout their movement. Furthermore, it signifies a manufacturing approach that complements the notion of environmental sustainability.

The pellets acquired were originally utilized for the fabrication of type 5A specimens to investigate their structural, mechanical, dynamic-mechanical, and morphological features. Based on the first findings of mechanical and structural analyses, films with up to 20 wt.% filler were also fabricated.

FT-IR measurement indicated no alteration in the chemical structure of the biopolymer, due to the absence of discernible chemical interactions between the biopolymer matrix and the biofiller. Nevertheless, these findings do not exclude the potential existence of physical interactions, such as entanglements between the biofiller and the matrix. The decrease in the absorption intensity of signals related to the spectra of the composite specimens was observed as the weight concentration of the HF biofiller increased. This trend can be ascribed to the steady diminution of the biopolymer matrix within the composite material.

The tensile test findings for the type 5A specimens demonstrated that the addition of HF biofiller to the PBAT matrix significantly influenced its stiffness. This outcome was anticipated, given the initial polymer, PBAT, has restricted rigidity and considerable flexibility. The results clearly indicate that the material stiffness stabilizes between 20 and 40wt.% HF, suggesting that this range contains the ideal filler concentration for loading the polymer. Furthermore, in samples with up to 20 wt.%, a corresponding increase in σ_y was noted, suggesting an enhancement in the material capacity to withstand mechanical stresses, likely facilitated by the development of an interphase layer that improves the interaction between the matrix and

the filler. This tendency, however, is absent in the samples with 40 wt.% HF, which had an average σ_y value similar to that of pure PBAT. This behavior is significant from an application perspective, as it enables the material to endure greater loads in the elastic domain, hence broadening its possible industrial uses. Although the increase in stiffness leads to a decrease in the elastic range up to the yield point, the higher strength still results in a resilience similar to that of unfilled polymer.

The DMA investigation validated the enhancement in stiffness, shown by an elevated E' modulus value correlated with HF concentration at a consistent temperature, alongside a favorable interaction between the polymer matrix and the filler. Furthermore, a reduction in the E' modulus is observed above 20 wt.% HF, indicating that the ideal filler concentration is likely close to this amount. An increase in T_g is noted, transitioning from pure PBAT to PBAT + 20wt% HF, signifying a favorable interaction between the polymer matrix and the filler. To verify this, the efficacy of fillers in all biocomposite samples containing up to 20 wt.% HF was assessed by calculating the E'_{red} within the temperature range of -40 to 20 °C. The results indicated that the E'_{red} of the biocomposites increases at temperatures exceeding the T_g , throughout the entire analyzed temperature range, in comparison to the pure biopolymer matrix. Consequently, the use of the filler extends the temperature range for effective usage of the composites in comparison to pure PBAT. The results were corroborated by the analogous behaviors of the biocomposite films produced using flat-head extrusion. Consequently, this also expands the processing spectrum of this material and, thus, the applications, providing a benefit for packaging industries.

Morphological inspection of the sample cross sections corroborated the findings, revealing a consistent distribution of filler particles inside the matrix, even at elevated concentrations, without any signs of agglomeration or phase segregation. The interphase adhesion in PBAT-HF composites was confirmed, demonstrating the establishment of a good wettability between the filler surface and PBAT. This led to effective and tailoring enhancement of the unmodified polymer.

The incorporation of HF as a biofiller resulted in a composite material exhibiting desirable properties and significant market competitiveness against conventional thermoplastics and their wood-plastic composite derivatives, particularly considering the preparation method, which is readily scalable to an industrial level. Moreover, the potential for utilizing reduced

quantities of polymer diminishes the reliance on fossil-based raw resources in the production of packaging, in accordance with an increasingly effective circular economy model.

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Chapter 3 - PBS- and PHBH-based Biocomposites

3.1 Introduction

This chapter, conducted in collaboration with the Process Development Department of the Fraunhofer Institute for Process Engineering and Packaging IVV (Fraunhofer-Institut für Verfahrenstechnik und Verpackung), presents the development and characterization of biocomposite materials with a PBS [1] and PHBH [2] biopolymer matrix, combined with lignocellulosic fiber microparticles sourced from agrindustrial waste, namely brewers' spent grain (BSG) [3,4]. Considering the anticipated expansion of the wood-plastic composites (WPC) market, coupled with rised prices and reduced wood availability, the aim of this research activity is the development of environmentally sustainable composite materials, using BSG as a viable economic biofiller, alternative to wood flour in the production of WPCs [5,6].

To achieve a composition similar to wood, alkaline hydrolytic solid-liquid extractions at several pH levels (9, 10.5, and 12) and different lautering techniques (standard and alternative) were performed, considering the elevated protein content of BSG, which can reach over 25 wt.%. Three distinct varieties of biofillers were chosen from the chemical extraction: native BSG, BSG treated at pH 10.5 (BSG-S2_T2), and BSG treated at pH 12 (BSG-S2_T3). The micrometer particles were subsequently combined by melt compounding and injection molding processes with two distinct biopolymer matrices to produce biocomposites containing filler content of up to 50 wt.%. The compounding process was conducted using a twin-screw extruder, since the process temperatures and the concurrent force exerted by the screws optimize the mixing of the two distinct components.

The influence of fillers on the rheological, structural, and morphological properties of the composites was assessed using melt-mass flow rate (MFR) analysis, gel permeation chromatography (GPC), scanning electron microscopy (SEM), and Fourier-transform infrared spectroscopy (FT-IR) techniques. To evaluate the influence of filler particle incorporation on the mechanical and thermal characteristics of the polymers, uniaxial tensile testing (UTT) and dynamic mechanical analysis (DSC) were performed on all generated formulations. This made it possible to evaluate the effectiveness of the reinforcement and identify the problems associated with the use of new materials.

3.2 Materials and Methods Preparation

3.2.1 Materials

Two different types of aliphatic polyesters were utilized: PBS (BioPBS FZ91PB by MCC Biochem, Dusseldorf, Germany), and a polyester from the PHA family, specifically PHBH with a 6 mol% 3-HH content, (I am Nature B6 A13 by Gruppo Maip SRL, Turin, Italy). The BSG lignocellulosic filler was provided by Gräflisches Hofbrauhaus Freising GmbH in Freising, Germany. This solid by-product is derived from the mashing process in the creation of lager and weiss beers, comprising a blend of wasted barley malt and wheat grain husks.

3.2.2 PBS and PHBH Polymer Matrices Preparation

The granules of pure PBS and PHBH (Fig. 3.1) have different geometric shape and color, which is why they are totally distinguishable visually. In this Chapter, due to the use of an extruder with larger geometries and screw sizes than in the previous *Thesis Chapter 2*, the granules did not undergo the mechanical grinding process. In Tab. 3.1 and 3.2, the chemical and physical properties of PBS and PHBH are reported.

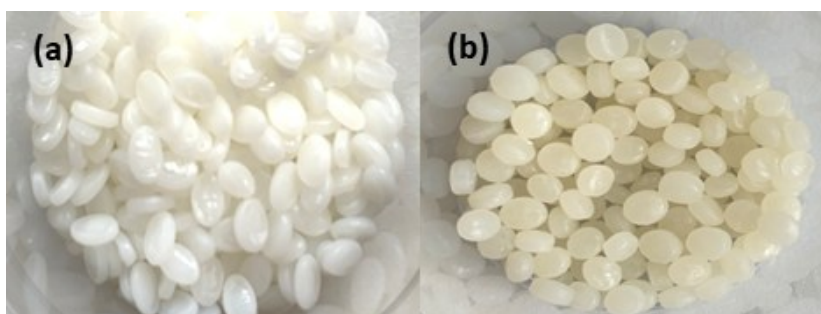


Figure 3.26 - Pellets of the polymer matrices: (a) PBS and (b) PHBH

Table 3.12 - PBS properties from the technical data sheet; Mechanical property values refer to the samples tested UTT in the machine direction (MD)

PBS Property	Value	Unit
Melt Flow Rate [190°C, 2.16Kg]	5	g/10min
Density	1.26	g/cm ³
Melting Point	115	°C
Young's Modulus	575	MPa
Yield Stress	39	MPa
Tensile Strength	42	MPa
Elongation at Break	350	%

Table 3.13 - PHBH Properties from the technical data sheet

PHBH Property	Value	Unit
Melt Flow Rate [165°C, 5Kg]	7	g/10min
Density	1.2	g/cm ³
Melting Point	145	°C
Glass Transition Temperature	2	°C
Tensile Strength	25	MPa
Elongation at Break	5	%

3.2.3 Thermal Treatment and Chemical Characterization of BSG Filler

BSG was provided in a wet state (Fig. 3.2) directly from the brewery's storage silo, following wort separation during the lautering process. Three distinct batches of BSG, designated as BSG_1, BSG_2, and BSG_3, were utilized. The dry matter content was quantified using gravimetric measurement utilizing the MA 100 moisture meter (Sartorius AG, Germany) and represented as the percentage of total biomass. To inhibit the natural fermentation processes of BSG, each batch was dried at a temperature not exceeding 60 °C using a convective air-drying cabinet (model Heraeus Type 6060, Thermo Fischer Scientific, USA) until a dry matter content of at least 90 percent was attained. Alternatively, samples were vacuum sealed in polypropylene bags and stored at -20 °C until subsequent chemical treatments. Fig. 3.3 illustrates the dried filler.



Figure 3.27 – Native BSG in the wet state



Figure 3.28 - Native BSG dried after thermal process at 60°C

For analytical reasons, the dried BSG was coarsely ground to homogenize the sample and decrease the particle size, utilizing a Braun MultiQuick 9 knife mill, model HB901AI (De Longhi Braun Household GmbH, Germany). The substrate was subsequently sieved via a stainless-steel mesh with a 2 mm aperture (Retsch GmbH, Germany). Particles above 2 mm in size were subsequently ground and screened to achieve homogeneity.

The chemical composition of the BSG was ascertained using gravimetric analysis, encompassing precise values of water extractives, ethanol extractives, soluble protein, insoluble protein, soluble ash, insoluble ash, lignin, and holocellulose. The characterization procedures are detailed in the Biomass Compositional Analysis Laboratory Procedures of the National Renewable Energy Laboratory [7]. The Kjeldahl technique was employed to quantify protein content [8]. The identical analytical techniques were utilized to characterize chemically modified BSG. The analyses were performed in duplicate, and the findings were reported as mean values.

3.2.4 Protein Extraction by Chemical Treatment

According to the results for BSG composition, the given BSG was chemically treated using a hydrolytic solid-liquid extraction procedure in an alkaline environment with the goal of eliminating non-lignocellulosic impurities, which were primarily protein components. The efficacy of this treatment was assessed by quantifying the protein extraction yield (% Yield), in accordance with equation 3.1:

$$yield_{P,\%} = \left(1 - \frac{\bar{x}_{P,R} \cdot m_R \cdot TS_R}{\bar{x}_{P,BSG} \cdot m_{BSG} \cdot TS_{BSG}} \right) \times 100$$

Equation 3.7: Equation for calculating protein extraction yield ($yield_{P,\%}$) representative of treatment efficiency

Where R is refined (i.e., BSG filter cake); P is protein; $\bar{x}_{P,R}$ is average protein content in raffinate; $\bar{x}_{P,BSG}$ is average protein content in BSG; m_R is mass of raffinate; m_{BSG} is mass of BSG before extraction; TS_R is dry matter of raffinate; TS_{BSG} is dry matter of BSG before extraction.

Protein extractions were performed as individual experiments in a 300 l stainless-steel vessel, featuring a spiral stirrer, pH meter, double jacket with thermal regulation through a heating and cooling circuit, load cells for weight measurement, and a conventional lautering system with a perforated false bottom plate, facilitating the separation of solid and liquid phases and the removal of the filter cake. The evaluated process parameters were pH, extraction time, and solid-liquid separation method, detailed in Tab. 3.3. The influence of extraction duration was assessed by collecting samples of BSG from the extraction media at specified time intervals. All extractions were performed at ambient temperature to facilitate selective protein extraction and reduce the loss of lignocellulosic fibrous components of interest. For each experiment, 10 kg of wet BSG was utilized, establishing a solid-to-liquid (S/L) ratio of 1:9 by the incorporation of osmotic water into the tank. BSG samples, immediately frozen upon receipt from the brewery, were permitted to defrost overnight before to the commencement of the trials. The pH of the extraction system was modified to the specified value by the incorporation of a 10 wt.% NaOH aqueous solution (Carl Roth GmbH & Co. KG, Germany).

Table 3.14 - Evaluated process parameters for the chemical treatment of BSG

Process parameter	Parameter level		
	<i>Low</i>	<i>Medium</i>	<i>High</i>
pH	9.0	10.5	12.0
Extraction time [min]	30	60	120
Solid-liquid separation (lautering)	Conventional (static filtration)		Alternative (dynamic filtration)

Following extraction, the settled BSG underwent solid-liquid separation using two distinct methods. The initial method included a standard lautering procedure, where the generated

BSG cake served as a natural filter for liquid separation. The alternative method entailed the deliberate disintegration of the filter cake by gradual rotation of the spiral stirrer, along with ongoing washing using osmotic water to minimize the retention of tiny particles, including protein hydrolysates, inside the cake.

After this stage, the residual filter cakes were reallocated into osmotic water, preserving the identical solid/liquid ratio employed during extraction. After the treated BSG had settled, solid-liquid separation was conducted again using the chosen procedure. The filter cakes of BSG were collected from the perforated plate of the lautering system's false bottom, dried in a convection oven at 60 °C until achieving a dry matter content of at least 90 %, and kept in sealed HDPE containers at 14 °C until further use.

3.2.5 Mechanical Pretreatment of the BSG Filler

The composite materials were fabricated using native BSG and two derivatives obtained from the alkaline extraction procedure (pH 10.5 and 12) employing an alternative separation approach. The derivatives are designated as BSG-S2_T2 and BSG-S2_T3, where S denotes the separation procedure and T represents the pH value. Subsequently, all BSG-based fillers underwent size reduction in a ZM200 centrifugal mill (Retsch GmbH, Germany), utilizing a 0.25 mm sieve at a rotating speed of 14,000 rpm (Fig. 3.4). A secondary grinding process was conducted for BSG-S2_T2 and BSG-S2_T3 variants on particles above 0.25 mm, utilizing a 0.12 mm sieve. This guaranteed a uniform particle size distribution among the fillers.



Figure 3.29 - Centrifugal mill ZM200 used for grinding the three different fillers (BSG, S2_T2 and S2_T3)

3.2.6 Melting Compounding

Biopolymer pellets (PBS and PHBH) and filler powders (BSG, BSG-S2_T2, and BSG-S2_T3) were pre-dried at 60 °C for 24 hours to eliminate residual humidity. The melting compounding was performed using a twin-screw corotating extruder (ZK 27Tx24 D) featuring an L/D ratio of 30 and a screw diameter of 25 mm (Dr. Collin GmbH, Ebersberg, Germany). Optimized extrusion settings, shown in Tab. 4, were determined to address varying melt behaviors and polymer viscosities. This study utilized two gravimetric feeders to supply the twin-screw extruder, without premixing the components before to extrusion. The increased screw geometries and dimensions facilitated enhanced processability and material flow, with a screw speed of 150 rpm employed for this purpose. The speed was sustained throughout all composite compositions. Furthermore, the extruder in zone 4 is outfitted with a vacuum pump, calibrated to a pressure of 100 mbar, facilitating the removal of volatiles and moisture produced during the extrusion process. The extrudates were chilled in a water bath and subsequently pelletized at the conclusion of the extrusion process. The comprehensive formulations are recorded in Tab. 3.5.

Table 3.15 - Extrusion parameters for the PBS- and PHBH-based composites

Matrix		Feed	Zone 1	Zone 2	Zone 3	Zone 4 (degassing)	Zone 5	Zone 6 (adapter)	Die
PBS	T [°C]	0	115	120	145	135	130	115	115
	p [mbar]					100			
PHBH	T [°C]	0	135	140	142	145	150	145	140
	p [mbar]					100			

Table 3.16 - Formulations of PBS- and PHBH-based composites used in the study

Polymer matrix	Filler type	Sample name	Polymer wt.%	Filler wt.%
PBS	none	PBS Water	100	0
	BSG	PBS90BSG10	90	10
		PBS80BSG20	80	20
		PBS70BSG30	70	30
		PBS50BSG50	50	50
	BSG-S2_T2	PBS90S2_T210	90	10
		PBS80S2_T220	80	20
		PBS70S2_T230	70	30

	BSG-S2_T3	PBS50S2_T250	50	50
		PBS90S2_T310	90	10
		PBS80S2_T320	80	20
		PBS70S2_T330	70	30
		PBS50S2_T350	50	50
PHBH	none	PHBH Water	100	0
	BSG	PHBH90BSG10	90	10
		PHBH80BSG20	80	20
		PHBH70BSG30	70	30
	BSG-S2_T2	PHBH90S2_T210	90	10
		PHBH80S2_T220	80	20
		PHBH70S2_T230	70	30
		PHBH50S2_T250	50	50
	BSG-S2_T3	PHBH90S2_T310	90	10
		PHBH80S2_T320	80	20
		PHBH70S2_T330	70	30
		PHBH50S2_T350	50	50

The obtained pellets were subsequently dried at 60 °C for 24 hours prior to injection molding. Injection molding was conducted with the same apparatus employed in Chapter 2, Babyplast 10/12. A specific mold was utilized for the manufacturing of type 5A specimens, according to UNI EN ISO 527 for mechanical characterization [9].

3.2.7 Injection Molding

Injection molding for PBS-based formulations was performed at temperatures of 140 ± 5 °C, with adjustments made according to Melt-mass Flow Rate (MFR) analysis results. The PBS biopolymer necessitated lower temperatures for the plasticization, loading and injection zones ($T_1 = 135$ °C, $T_2 = 135$ °C, $T_3 = 145$ °C), consequently, minor adjustments in pressure and temperature were implemented during processing. Uniform conditions were upheld for the three distinct fillers. Tab. 3.6 delineates specific temperature profiles, whereas Tab. 3.7 presents supplementary data including material load, pressures, injection timings, and velocities.

Table 3.17 - Process temperatures for each PBS composition prepared (T_1 : plasticization, T_2 : storage, T_3 : injection)

Composition	T_1 [°C]	T_2 [°C]	T_3 [°C]
PBS	135	135	145
PBS90	140	140	140
PBS80	140	140	140
PBS70	145	145	145
PBS50	145	145	145

Table 3.18 - Other main injection parameters of PBS-based composites

Injection Parameters	PBS	PBS90	PBS80	PBS70	PBS50
Charge materials [mm]	19	19	19	19	19
Cooling time [s]	5	30	30	30	30
1st Injection pressure [bar]	35	40	40	45	45
1st Time pressure [s]	3	3	3	3	3
2nd Injection pressure [bar]	10	35	35	40	40
2nd Time pressure [s]	3	3	3	3	3
1st Injection speed [%]	100	100	100	100	100
2nd Injection speed [%]	100	100	100	100	100

Injection molding of PHBH-based formulations was conducted over a broader temperature range (143 ± 13 °C), depending on the material composition. The pure PHBH biopolymer necessitated elevated temperatures ($T_1 = 156$ °C, $T_2 = 155$ °C, $T_3 = 154$ °C), but the incorporation of BSG fillers enhanced fluidity during the pre-injection phase, requiring minor modifications in pressure and temperature. Uniform conditions were likewise upheld for the three distinct fillers in this production batch. Tab. 3.8 delineates the temperature profiles of the three zones of plasticization (T_1), loading (T_2), and injection (T_3), while Tab. 3.9 presents other operational characteristics.

Table 3.19 - Process temperatures for each PHBH composition prepared (T_1 : plasticization, T_2 : storage, T_3 : injection)

Composition	T_1 [°C]	T_2 [°C]	T_3 [°C]
PHBH	156	155	154
PHBH90	146	141	139
PHBH80	145	140	132
PHBH70	145	138	130
PHBH50	145	138	130

Table 3.20 - Main injection parameters of PHBH-based composites

Injection Parameters	PHBH	PHBH90	PHBH80	PHBH70	PHBH50
Charge materials [mm]	19	19	19	19	19
Cooling time [s]	250	250	200	200	200
1st Injection pressure [bar]	55	52	35	32	32
1st Time pressure [s]	11	13	13	5	5
2nd Injection pressure [bar]	55	55	35	32	32
2nd Time pressure [s]	11	11	11	5	5
1st Injection speed [%]	50	50	50	50	50
2nd Injection speed [%]	50	50	50	50	50

Fig. 3.5 shows representative specimens of each composition generated for PBS and PHBH, indicating from left to right the increasing wt.% of BSG biofiller in the corresponding polymer matrix. The alteration in the quantity of filler is perceptible to the naked eye by a shift in color tone, transitioning to deeper, more opaque hues of brown compared to unfilled samples. Approximately 40 duplicates of each dumbbell specimen were utilized for further structural, mechanical, morphological, and thermal evaluation.

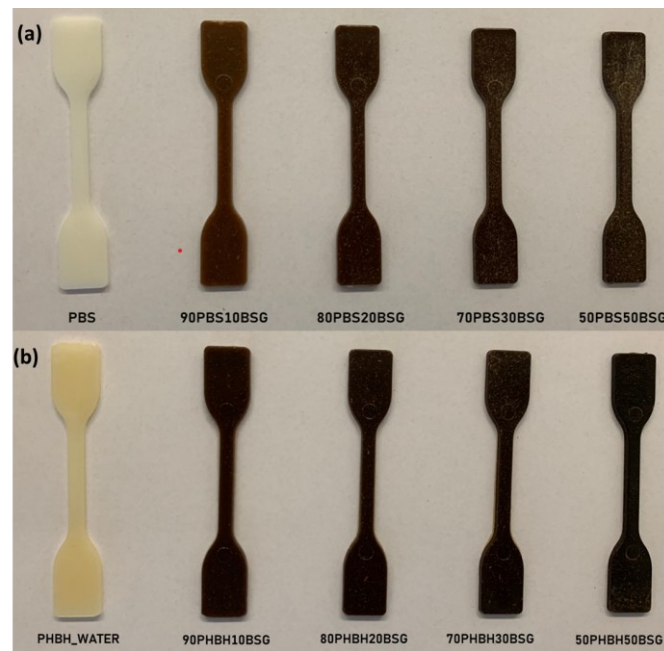


Figure 3.30 - Examples of different BSG filler contents on dumbbell specimens: (a) PBS-based composites and (b) PHBH-based composites

2.3 Characterization of Biofillers, Polymer Matrices and Biocomposite Materials

3.3.1 Particle Size Characterization

The particle size distributions of the three filler types (BSG, BSG-S2_T2, and BSG-S2_T3), including the mean diameter and percentiles Dx10, Dx50, and Dx90, were examined utilizing a Mastersizer 3000 laser diffraction particle size analyzer (Malvern Panalytical, Kassel, Germany), equipped with an Aero S dry powder dispersion unit. The particle size distribution was ascertained by Mie theory, utilizing Mastersizer v3.88 software for data processing. The refractive index (RI) for calculation was established at 1.59, representing a median value among lignin (RI = 1.61), cellulosic constituents (RI $\approx$ 1.53, derived from cellulose and hemicellulose), and protein (RI $\approx$ 1.6) [10–12]. BSG particles were disseminated by utilizing a flow of compressed air at a pressure of 4 bar and a 50% feed rate to achieve 15 repetitions.

3.3.2 Fourier Transform-Infrared Spectroscopy

The chemical structure of pure polymer matrices and different formulations was analyzed using FT-IR. The analysis utilized the Spectrum Two FT-IR spectrometer and Spectrum IR version 10 software, both provided by Perkin Elmer (Shelton, CT, USA). Measurements were conducted in ATR (Attenuated Total Reflection) mode utilizing a diamond crystal as the refractive component. Spectra were obtained from the specimen's cross section throughout the wavelength range of 4000-600 cm^{-1} , with a resolution of 2 cm^{-1} and a total of 16 scans per sample. Prior to analysis, the samples were subjected to conditioning in a vacuum oven at 60 °C for 24 hours to eliminate residual moisture.

Measurements were performed under ambient temperature (T_{amb}) and atmospheric pressure (P_{atm}) conditions. For precise comparison analysis, the FTIR spectra of the composites were normalized according to the specified peaks: 1388 cm^{-1} for PBS-based composites and 1378 cm^{-1} for PHBH-based composites.

3.3.3 Melt-mass Flow Rate

The Melt-mass Flow Rate (MFR) study is a common methodology utilized for evaluating the flow properties of thermoplastic materials under defined temperature and load conditions. This analysis offers essential insights on material viscosity, flow rate, and melt behavior, which are very important for the processing of polymer composite materials.

The MFR assessment of the examined materials was conducted utilizing a MeltFloW@on plastometer (Karg Industrietechnik, Krailing, Germany equipped with two thermal heating sources for capillary heating. The test was performed at a temperature of 165 °C, with a weight of 5 kg, and the quantity of melt extruded over an interval of 10 min was quantified in accordance with ISO 1133 [13].

3.3.4 Gel Permeation Chromatography

Average molecular weight (M_w) measurements using Gel Permeation Chromatography (GPC) were performed using a Dionex system, utilizing a solvent combination of 1,1,1,3,3,3-hexafluoro-2-isopropanol (HFIP) and trifluoroacetic acid (0.02 mol L^{-1}). Sampling was conducted using a DIONEX Ultimate 3000 injector, functioning at a steady flow rate of 1 mL min^{-1} , using a Gynkotek M480 gradient HPLC pump. The detection was conducted with a Gynkotek SE-61 refractive index detector.

Analysis utilized PSS-PFG columns ($7 \text{ }\mu\text{m}$, $300 \times 8 \text{ mm}$), regulated at a constant temperature of 40 °C (PSS, Mainz, Germany). Samples were generated by dissolving $5000 \text{ }\mu\text{g/mL}$ of material in HFIP and subsequently filtered through a polytetrafluoroethylene (PTFE) membrane having a porosity of $0.20 \text{ }\mu\text{m}$. A volume of $100 \text{ }\mu\text{L}$ was injected for each analysis. Calibration was conducted utilizing a PET standard characterized by a limited molecular weight distribution (PSS, Mainz, Germany).

The acquired curves were examined using WinGPC® UniChrom software (version 8.1, PSS, Mainz, Germany). Mean values of weighted molecular weight (M_w) were documented for all samples, exhibiting a standard variation of 5%, which reflects instrument sensitivity. The relative accuracy of the results was assessed to be under 5%, consistent with standard performance for the estimation of M_w [14].

3.3.5 Uniaxial Tensile Test

Tensile tests were conducted using the uniaxial tensile test (UTT) on type 5A specimens, following ISO 527-2 standards, with a Test dynamometer (model 112, Test GMBH Universal Testing Machine, Germany with 2 kN load cell. The specimens were gripped at the ends and subjected to a load at a continuous rate of 10 mm/min until failure occurred. Each specimen underwent eight replications, with the tests performed under room conditions.

Young's modulus (E), stress at break (σ_B), and elongation at break (ϵ_B) were derived from the acquired stress-strain curves. The shown data are the mean values of the replications. Prior to testing, the samples were preconditioned in an oven at 60 °C overnight to eliminate absorbed humidity.

A nonparametric Kruskal-Wallis test was employed for statistical analysis to identify significant differences among sample groups for various formulations, as the prerequisites for a linear model were not satisfied. Secondly, Dunn's test was employed to identify which groups exhibited significant differences from one another, following the statistical indication of an overall difference revealed by the Kruskal-Wallis test ($p < 0.05$).

3.3.6 Scanning Electron Microscopy

The surface morphology and interfacial compatibility of composite formulations were examined using scanning electron microscopy (SEM). The injection-molded specimens fractured under cryogenic conditions, and the resultant cross-sections were polished using the JEOL IB-19530CP polisher (Freising, Germany) with an Argon (Ar) beam. Ar ions produced during the process infiltrate the sample, imparting energy to the atoms and inducing their displacement by a mechanism termed “knock-on.” The regularity of such occurrences results in cascade collisions that create smooth surfaces, so revealing indications of wettability or non-wettability between the filler and the matrix. The cross sections were polished using Ar ion acceleration at 3.5 kV for a duration of 9 hours.

Due to the composite nature of these blends, comprising a rubbery component (polymer) and a stiff component (filler), the Ar beams may exhibit varying processing rates, leading to

detrimental consequences of streaking roughness. To address this limitation, swing mode was employed during processing, enabling the sample and the aperture traversed by the Ar ion beam to spin at irregular intervals within a 360°C range.

The cross sections were coated with a tiny coating of gold to provide superior SEM picture quality. The investigation utilized a JEOL 7200F SEM (Freising, Germany) in backscattered electron mode to emphasize compositional difference. The specifications for the picture capture were an accelerating voltage of 1.5 kV, a spot size of 4 (a.u.), and a working distance of 4 mm. The acquired SEM images were analyzed utilizing PC-SEM software version 7.1.0.9 (Jeol, Freising, Germany).

3.3.7 Differential Scanning Calorimetry

The thermal properties of PBS- and PHBH-based materials were examined using differential scanning calorimetry (DSC) utilizing a DSC Q100 calorimeter (TA Instruments, New Castle, DE, USA), calibrated with indium and mercury standards. The analysis of pure PBS and related formulations was conducted through three dynamic temperature cycles (two heating and one cooling). PBS samples were first heated from -60 °C to 150 °C (first cycle), thereafter cooled from 150 °C to -60 °C, and maintained under isothermal conditions for 5 minutes to eradicate the thermal history. The samples underwent a second heating cycle at 150 °C. A comparable methodology was utilized for pure PHBH and its formulations, with the exception that the samples were heated to 180 °C, due to the higher melting temperature (T_m) of PHBH (145 °C) compared to that of PBS (115 °C). All analyses were performed at a heating/cooling rate of 10 K/min in a nitrogen environment (50 mL/min), utilizing samples with an average weight of 10 ± 0.5 mg, contained in aluminum crucibles.

The curves acquired were analyzed using Universal Analysis Software 3.9 (TA Instruments, New Castle, DE, USA). The thermal parameters, including melting temperature (T_m) and glass transition temperature (T_g), were determined from the second heating cycle, when crystallization temperature (T_c) was computed during the cooling cycle.

In addition, the enthalpy of melting (ΔH_m) and degree of crystallinity ($\% \chi_c$) were also derived from the thermogram analysis.

The degree of crystallization ($\% \chi_c$) was calculated with the following equation 3.2:

$$\% \chi_c = \frac{\Delta H_m}{\Delta H_m^0 * (1 - w_{add})} * 100$$

Equation 3.8 – Equation for calculating the degree of crystallinity (% χ_c)

where ΔH_m is the fusion enthalpy (J/g) obtained from the DSC measurement; ΔH_m^0 is the fusion enthalpy at 100% crystallinity, reported to be 110.5 J/g for PBS [15] and 146 J/g for PHBH [16]; w_{add} indicates the weight fraction of the polymer matrix in the biocomposite. Equation 2 allows taking into account the melting enthalpy of the sample, normalized to the effective mass of polymer matrix in the composite material ($\Delta H_{m,norm}$).

Therefore, taking into consideration $\Delta H_{m,norm}$, Equation 3.2 can be summarized in the following equation 3.3:

$$\% \chi_c = \frac{\Delta H_{m,norm}}{\Delta H_m^0} * 100$$

Equation 3.9 - Equation for calculating the degree of crystallinity taking into account the enthalpy of melting of the sample, normalized to the effective mass of the polymer matrix ($\Delta H_{m,norm}$)

3.4 Results and Discussions

3.4.1 FT-IR Analysis of BSG Biofillers

BSG showed a dry matter concentration of 20.47 wt.%, since no drying procedure is conducted in the brewery following the separation of BSG from fermentable sugar wort in the lautering tunnel. Consequently, the remaining 79.53 % comprises water, underscoring the necessity of implementing conservation techniques to mitigate or decelerate microbial destruction of the biomass. All BSG components mentioned above pertain to the dry matter portion of the studied biomass.

Holocellulose (hemicellulose and cellulose) was identified as the primary component, comprising 47.64 wt.% of the dry matter, whereas protein represented 25.22 wt.%. These values align with those documented in the literature [3,17]. Of the proteins, 79.42 % (equivalent to 20.03% of the total dry matter) were identified as insoluble in both water and

ethanol, indicating that the mashing process in brewing denatures most of the proteins in the malt grains. During mashing, which takes place at temperatures of up to 78 °C, depending on the type of beer, the barley and wheat proteins decrease some of their activity [18,19]. 20.85% of the protein (equivalent to 5.9 % of the total dry matter) was identified in the water and ethanol extractives, which collectively represented 20.88 wt.% of the BSG dry matter.

Water extractables constituted 12.83 wt.% of the total dry matter and, with water-soluble proteins (such as residual barley albumins and globulins) [18], may include residual starch, sugars (including β -glucans), and partially self-hydrolyzed hemicelluloses [20,21]. The ethanolic extractives comprised 11.05 wt.% of the total dry matter and, alongside ethanol-soluble proteins (such as residual barley hordein) [18], may encompass lipids and polyphenolic chemicals [3,17,22,23]. Lignin was recognized as a minor component, comprising 6.92 wt.% of the total dry matter, whereas ash, indicative of the inorganic elements of BSG (including salts and minerals), was determined to be the least prevalent fraction, at 3.13 wt.% of the total dry matter. Of this, 51.11 % (equivalent to 1.60 wt.% of the total dry matter) was identified as soluble ash.

Fig. 3.6 shows the graphic representation of the average results relating to the chemical composition of BSG. The comprehensive characterization was conducted on the initial batch provided (designated as BSG_1). In the second two batches (BSG_2 and BSG_3), just the total protein content was assessed, considered as a critical criterion for process selection and further material development. The protein composition of batches BSG_2 and BSG_3 was determined to be 25.42 wt.% and 26.42 wt.% of total dry matter, respectively. The results indicate a notable compositional homogeneity of BSG regarding protein level across three distinct batches of beer, exhibiting a standard deviation of 0.52 wt.%.

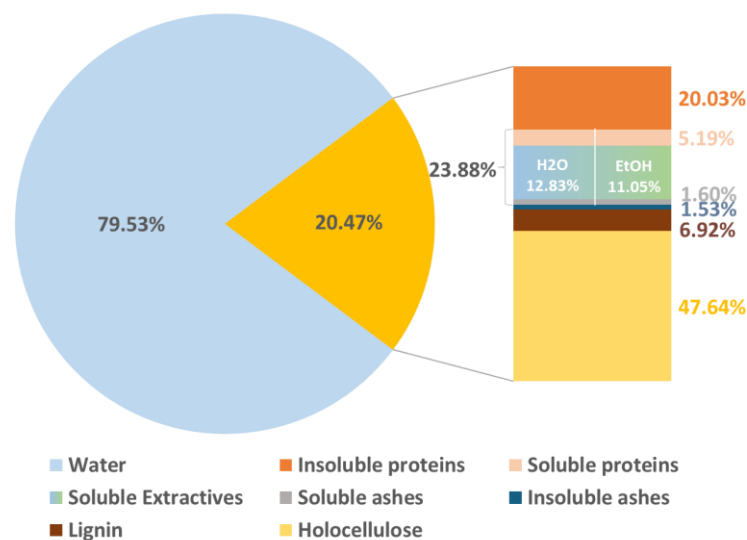


Figure 3.31 - Chemical composition of BSG as received from the brewery

3.4.2 Protein Extraction by Chemical Treatment

Due to the lack of visible trends in protein reduction at various extraction times by aliquot sampling, the data reported refer only to the complete extractions (i.e., 120 minutes). Fig. 3.7 shows the absolute protein content, on the dry matter, of BSG samples exposed to protein extractions at different pH levels and then separated from the extraction medium by different filtering procedures (lautering).

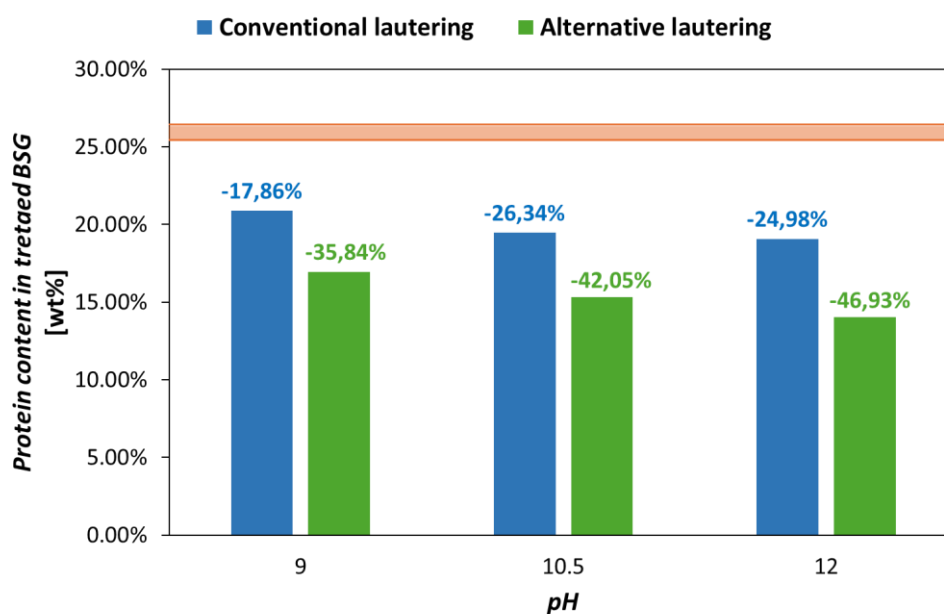


Figure 3.32 - Protein concentration of processed BSG in comparison to BSG obtained from the brewery (orange). Data related to complete extraction methods lasting 120 minutes

The protein concentration in the treated samples varied from 20.88 wt.%, achieved with the lowest alkalinity and traditional lautering, to 14.02 wt.%, observed with the greatest alkalinity and alternative lautering. The data indicate a decrease in protein content, relative to untreated BSG, ranging from 17.86 % to 46.93 %, respectively. The results indicate that the protein extraction procedures were generally efficient, since the residual protein level in the treated samples was markedly lower than in the initial material. The chemical treatment exhibited two primary trends:

1. An increase in pH value correlated with a reduction in absolute protein concentration, irrespective of the lautering method utilized.
2. Alternative lautering shows superior efficacy compared to conventional lautering in isolating the extracted proteins from the solid BSG residue, regardless of the applied pH value.

Fig. 3.8 shows the efficiency of the method, quantified as protein extraction yield. This parameter indicates the proportion of proteins removed in the dry mass of the treated BSG, hence reflecting the quantity of protein effectively extracted.

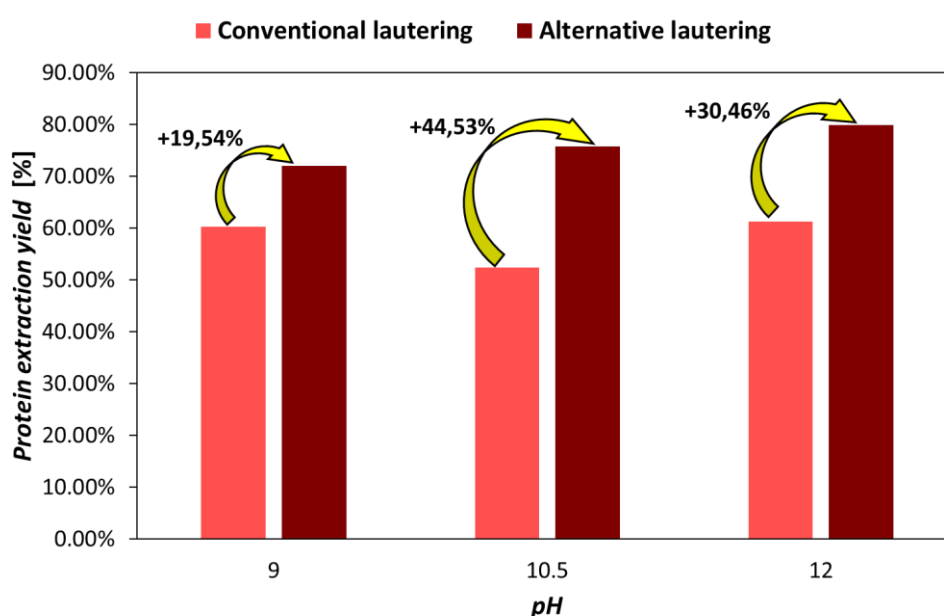


Figure 3.33 - Yield of protein extraction in treated BSG. The reported percentage denotes the enhanced yield achieved using alternative lautering compared to the usual method, at a specified pH value. The data corresponds to the total extraction process lasting 120 minutes

The extraction yields observed varied from 52.40 %, attained with medium alkalinity and conventional lautering, to 79.84 %, realized with the highest pH and alternative lautering methods. A substantial association between elevated pH and extraction yield was noted just for alternative lautering. Conversely, conventional lautering demonstrated reduced efficacy in isolating hydrolyzed proteins from the solid BSG residue, irrespective of the extraction medium alkalinity, leading to a decrease in extraction yield.

The qualitative validation of this inefficiency was evidenced by the development of a gelatinous layer on the filter cake of the BSG during standard lautering at pH 10.5 (Fig. 3.9). The protein level of the deposit was quantitatively determined at 36.50 wt.%, above that of the equivalent initial BSG (BSG_3). Conversely, alternative lautering yielded between 19.54 and 30.46 % more than traditional lautering.



Figure 3.34 - BSG filter cakes after the conventional lautering process with a presence of a gelatinous film (left) and after the alternative lautering process with no visible presence of such film (right)

Based on the selection criteria of minimal protein content in residual dry matter and maximal protein production, BSG samples treated at pH 10.5 and 12 by alternative lautering were selected for future material development and designated as S2_T2 and S2_T3, respectively. Their chemical composition is illustrated in Figg. 3.10 and 3.11, respectively.

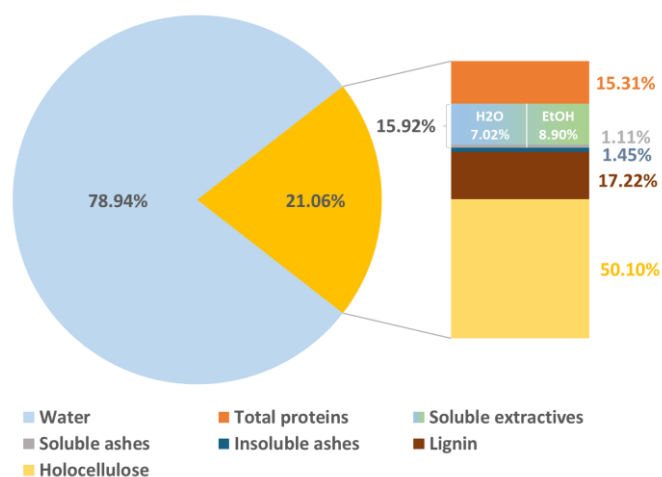


Figure 3.35 - Chemical composition of BSG-S2_T2 treated at pH 10.5 as wet filter cake from extraction process

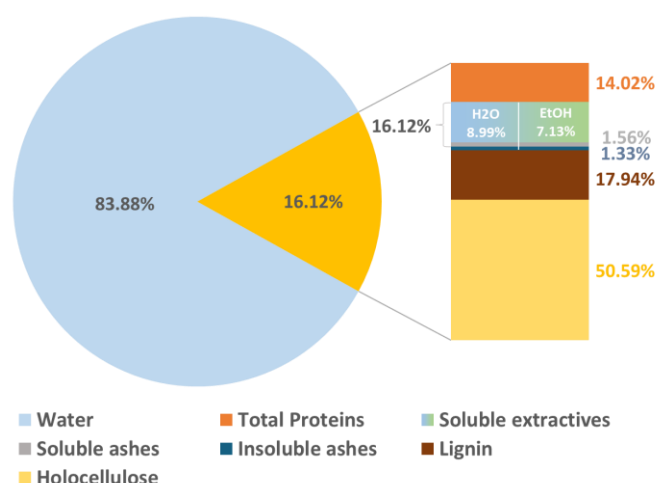


Figure 3.36 - Chemical composition of BSG S2_T3 treated at pH 12 as wet filter cake from extraction process

It is important to highlight that in the chosen samples, the overall protein content, encompassing both soluble and insoluble proteins that were not experimentally assessed, was markedly diminished, although the relative concentration of holocellulose remained almost constant compared to the original BSG supplied by the brewery. Conversely, the relative lignin concentration was shown to rise, indicating a general decrease in biomass during the extraction process. This outcome indicates that the fibrous lignocellulosic element of the untreated BSG was qualitatively maintained in the treated samples.

3.4.3 Particle Size Analysis of the BSG Biofillers

After the grinding and sieving operation, the native BSG biofiller and its two derivatives were analyzed in terms of particle size distribution. The corresponding curves are illustrated in Fig. 3.12.

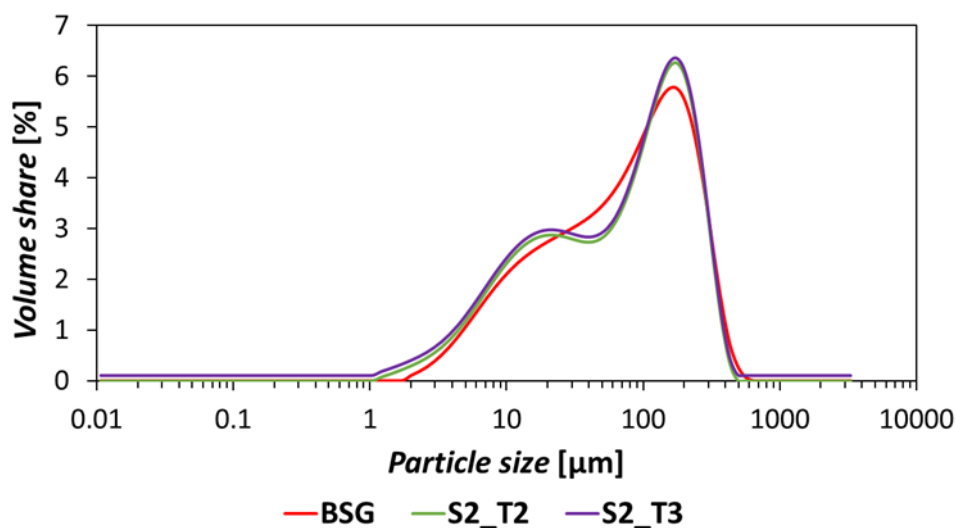


Figure 3.37 - Distribution of particle sizes of fillers post-milling: BSG (red), S2_T2 (green) and S2_T3 (violet)

The dimensions of the filler substantially influence its dispersibility and interaction with the polymer matrix, hence affecting the ultimate characteristics of the composite material. Smaller particles possess greater surface areas, leading to possibly enhanced mechanical performance [24]. The size distribution curves of the various BSG fillers, depicted in Fig. 3.12, exhibit a uniform trend across all mechanical treatments applied to the distinct lignocellulosic fillers. Bimodal curves were seen for all fillers, with an average volumetric diameter, D_{mean} , of around 110 μm , as indicated in Tab. 3.10.

Like the HF biofiller examined in *Chapter 2*, D_{x90} value exceeds the mesh diameter of the sieve employed (250 μm) for the different BSG fillers. The existence of fibers above the sieve size can be ascribed to the irregularity in the aspect ratio of the fibers. Indeed, several fibers possess a diameter of less 110 μm , although their length is considerably longer than their diameter.

Table 3.21 - Primary components of different ground fillers

Sample name	D_x (10) [μm]	D_x (50) [μm]	D_x (90) [μm]	D_{mean} [μm]
BSG	9.8 $\pm$ 0.2	76 $\pm$ 2	246 $\pm$ 21	105 $\pm$ 7
BSG-S2_T2	8.4 $\pm$ 0.1	76 $\pm$ 1	238 $\pm$ 3	102 $\pm$ 1
BSG-S2_T3	9.9 $\pm$ 0.1	91 $\pm$ 1	261 $\pm$ 2	115 $\pm$ 1

3.4.4 FT-IR Analysis of BSG Biofillers

Fig. 3.13 shows the complete FT-IR spectra of BSG, BSG-S2_T2, and BSG-S2_T3 fillers acquired post-grinding, emphasizing the characteristic signals of lignocellulosic materials. Nonetheless, variations in the intensity of some bands are noted. All spectra exhibit a large peak between 3000 and 3680 cm^{-1} (a), attributed to the stretching vibrations of hydroxyl groups (-OH) inherent in the structures of holocellulose, lignin, and starch [25,26]. The absorption bands shown between 2830-2990 cm^{-1} can be ascribed to the symmetric and asymmetric stretching vibrations of the C-H bonds in the methyl and methylene groups [27–29].

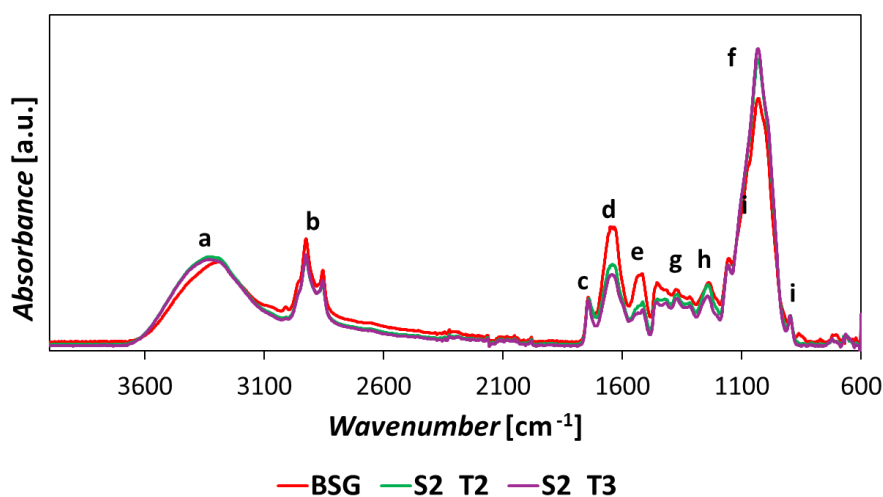


Figure 3.38 - FT-IR spectra of different ground fillers. Red : BSG; Green: S2_T2; Violet: S2_T3

The peak at around 1730 cm^{-1} (c) is indicative of the carbonyl group (C=O), which is characteristic of the lignocellulosic system [30]. The bands at 1634 cm^{-1} (d) and 1518 cm^{-1} (e) correspond to the asymmetric and symmetric bending of the N-H amine group derived from protein and the stretching vibrations of the aromatic C=C bonds in lignin, respectively [31–33]. The intensity of the bands (d) and (e) in BSG-S2_T2 and BSG-S2_T3 samples decreases compared to untreated BSG, attributable to the partial elimination of protein during extraction.

The band at around 1030 cm^{-1} (f), associated with the C-OH functional group of polysaccharides, exhibits an increase in intensity in the treated samples, indicating improved detectability of cellulose following protein removal [34,35]. The weaker signals detected between 1300 and 1470 cm^{-1} (g) are ascribed to the bending vibrations of the C-H hydrocarbon chain [30]. The band within the range of 1190 - 1280 cm^{-1} (h) is indicative of C-O stretching associated with the acetyl groups in hemicellulose [36]. The signal at around 890 cm^{-1} (i) corresponds to C-O-C stretching, indicative of cellulose in fibers [35].

3.4.5 FT-IR Analysis of PBS and PHBH Polymer Matrices

FT-IR analysis was used to examine the chemical composition and structure of the biopolymer matrices PBS and PHBH. This study is essential for investigating the biopolymer characteristic signals of interest, which will then be compared with the characteristic signals of the BSG fillers to determine if there are any alterations in the polymer structure. The resultant spectra are shown in Fig. 3.14.

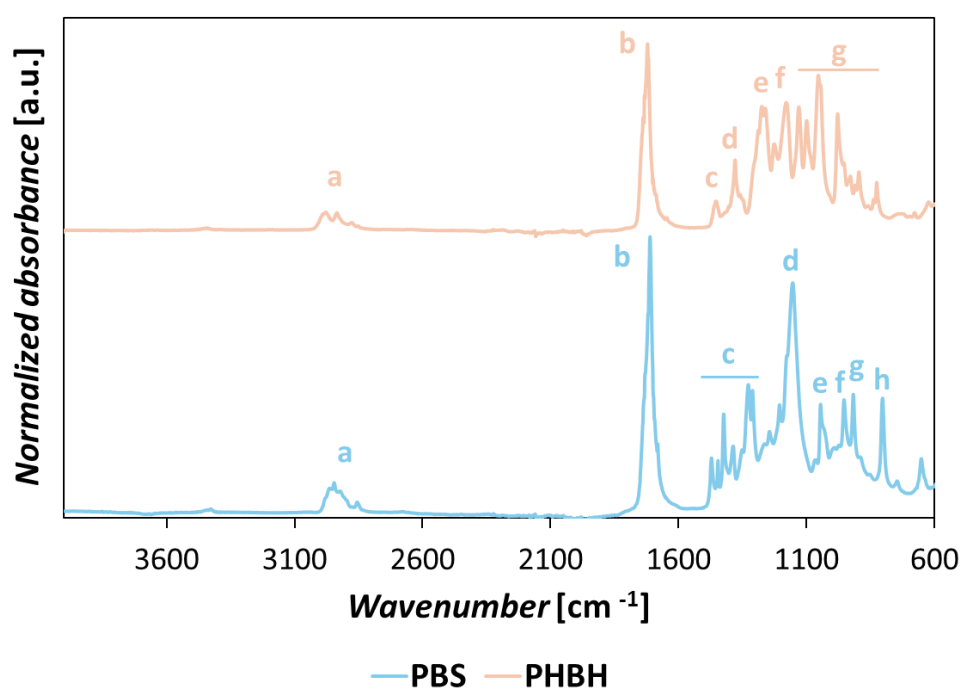


Figure 3.39 - FT-IR spectra of the polymer matrices PBS (light blue) and PHBH (pink)

The FT-IR spectrum of pure PBS (light blue) exhibits distinctive bands between 2830 and 3000 cm^{-1} (a), corresponding to the symmetric and asymmetric bending of the C-H bonds in the methylene groups ($-\text{CH}_2$) of the BU and SA monomers [37]. The band at 1713 cm^{-1} (b) is

ascribed to the stretching of the carbonyl (C=O) within the ester group [38]. This signal exhibits two shoulders attributable to the distinct phases of the polymer matrix. The amorphous and amorphous-rigid (intermediate) phases correspond to the shoulders at 1732 and 1720 cm^{-1} , respectively [39,40].

The various bands between 1300 and 1500 cm^{-1} (c) result from the bending vibrations of the C-H bonds in the -CH₂ groups [41]. A further signal, detected at 1155 cm^{-1} (d), can be ascribed to the C(=O)-O stretching vibration of the esters [42,43]. PBS exhibited characteristic absorption peaks at 1047 (e), 957 (f), 918 (g), and 806 (h) cm^{-1} , attributable to the CO stretching of O-(CH₂)₄-O group, symmetrical C-O stretching, C-OH bending, and C-H in-plane bending, [44–47].

Therefore, the FT-IR spectra of pure PHBH (pink) exhibits characteristic signals of polyesters, comparable to those of PBS, but with minor variations in wavenumber and signal intensity values. Bands are notably seen between 2800 and 3040 cm^{-1} , corresponding to the C-H vibrations of the -CH₂ and the methyl group (-CH₃), which is completely missing in the chemical structure of the PBS biopolymer [48]. The increased intensity signal corresponding to the C=O group of polyesters is similarly noted here, but with a wavenumber variation of 8 cm^{-1} (1721 cm^{-1} , b), respectively [49,50]. The signals at 1453 cm^{-1} (c) and 1379 cm^{-1} (d) correspond to the bending of the C-H bond in the -CH₂ and -CH₃ groups, while the peak at 1274 cm^{-1} (e) has been attributed to the stretching of the C-O alkoxy group [51]. The signal at 1179 cm^{-1} (f) denotes the stretching vibration of the C(=O)-O bond found in the polyester PBS [48]. The extensive range of 1130-800 cm^{-1} (g) is attributed to the stretching of C-O bonds [52].

3.4.6 FT-IR Analysis of PBS- and PHBH-based Composites

FT-IR investigations were also conducted on the composite specimens to elucidate the possible alterations caused by filler-matrix interactions, in Figg. 3.15 and 3.16. Fig. 3.15 (a) displays, from top to bottom, the FT-IR spectra of BSG, the PBS50BSG50 composite, and pure PBS for reference. Figs. 3.15 (b) and 3.15 (c) show the spectra of the PBS50S2_T250 and PBS50S2_T350 composites, respectively, together with their fillers. This is repeated in Fig. 3.16 (a), (b) and (c) concerning PHBH-based composite compositions.

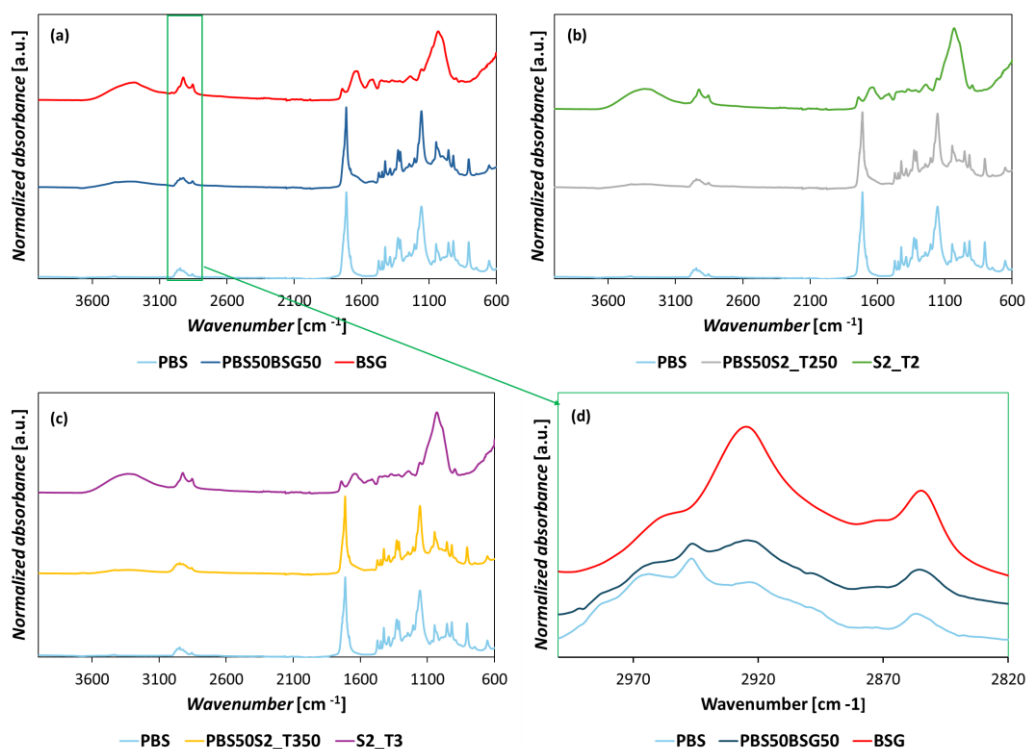


Figure 3.40 - FT-IR spectra of PBS and (a) PBS50BSG50 composite and BSG filler, (b) PBS50S2_T250 composite and S2_T2 filler, and (c) PBS50S2_T350 composite and S2_T3 filler; (d) Magnification relative to the area between 2820-3020 cm⁻¹ inherent to the BSG cluster

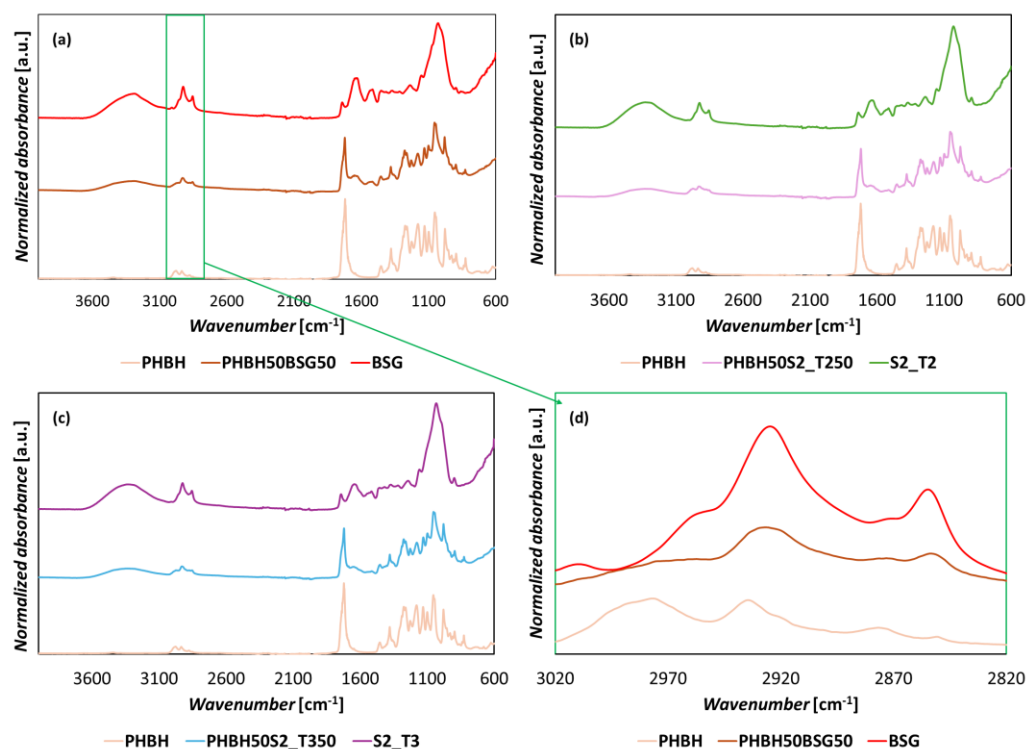


Figure 3.41 - FT-IR spectra of PHBH and (a) PHBH50BSG50 composite and BSG filler, (b) PHBH50S2_T250 composite and S2_T2 filler, and (c) PHBH50S2_T350 composite and S2_T3 filler; (d) Magnification relative to the area between 2820-3020 cm⁻¹ inherent to the BSG cluster

The FT-IR spectra of the composites containing a 50 wt.% filler exhibit a general resemblance to those of the corresponding pure polymer matrix, regardless of the type of filler used. However, the intensities of the bands vary with respect to pure polymers, a characteristic attributable to the variation in relative amounts between the components of the composite.

A signal corresponding to stretching vibrations of -OH groups, found in cellulose, hemicellulose, lignin, and starch, is observable between 3160 and 3500 cm^{-1} in all spectra. In the PBS50BSG50 and PHBH50BSG50 composites (Fig. 15 (d) and 16 (d)), two prominent peaks at 2924 and 2856 cm^{-1} indicate the presence of the filler inside the polymer matrix. The distinctive signal of the carbonyl group (C=O) at about 1713 cm^{-1} in PBS and 1720 cm^{-1} in PHBH exhibits no notable alterations following the incorporation of the filler, suggesting that the chemical structure of the polymer matrix remains unaltered. Nonetheless, a signal overlap attributable to BSG and biopolymer is seen in the range of 1400 to 800 cm^{-1} .

In agreement with other research, including investigations by Zeng et al. [53] and Hejna [54], no notable spectroscopic alterations were seen for PHBH, indicating that the incorporation of the filler does not modify the chemical structure of the polymer matrix. Nonetheless, the composites exhibit novel signals at 1651 and 1572 cm^{-1} , signifying the presence of the filler (*Section 3.4.5*).

3.4.7 MFR Analysis and GPC of PBS and PHBH-based Composites

A study of the influence of filler type and content, along with the effects of the extrusion procedure utilized for blending the components of the composite system, was performed by Melt-mass Flow Rate (MFR) and Gel Permeation Chromatography (GPC) analyses on the manufactured biocomposites. The results that were obtained are shown in Fig. 3.17. Fig. 3.17 (a) specifically presents the data for PBS-based composites, whereas Fig. 3.17 (b) shows the results for PHBH-based composites. These investigations were used to evaluate the processability of the pellets in the injection molding procedure.

The MFR results indicated a substantial impact of various filler types on the biopolymer matrices. A decrease in MFR was noted for all evaluated formulations of PBS-based biocomposites. The composites with S2_T2- and S2_T3-treated fillers exhibited a reduction in MFR of 81 % and 54 %, respectively, for the formulations comprising 50 wt.% filler. Conversely,

biocomposites including untreated BSG exhibited a 40 % decrease in MFR relative to pure PBS. In PHBH-based composites, a small upward trend in MFR values was noted, particularly in composites with the filler S2_T3 at concentrations of 30 and 50 wt.%.

The results underscored the problem of thermal instability in the PHBH matrix during extrusion. The comparison of the MFR values for extruded PHBH (10.7 g/10 min) and unprocessed granule (4.7 g/10 min) indicated partial thermo-mechanical degradation of the matrix, in Fig. 3.17 (c). This phenomenon primarily results from the low thermal resistance of PHBH, a characteristic of PHA-class polymers, and the degradation of molecular chains due to elevated processing temperatures, mechanical action of the screws, and the melt residence time within the extrusion chamber [55–57]. An extrusion temperature of 165 °C was selected as the analytical condition to limit material deterioration.

No substantial alterations were seen in the molecular weights of PBS and PHBH matrices for the GPC data for BSG-containing composites (Fig. 3.17 (a,b)). In the PBS-based biocomposites with fillers subjected to varying pH treatments, a tendency of increased molecular weights was seen, but not statistically significant. Conversely, a contrasting trend was noted in PHBH-based composites, mostly due to the thermal instability of the matrix during extrusion.

The analysis of the collected data allowed the correlation between the reduction of MFR in PBS-BSG composites and the presence of the filler, which limits the mobility of the polymer chains. In the S2_T2- and S2_T3-treated composites, the significant reduction in MFR was attributed to both the presence of the filler and the increase in molecular weight, which increased by 19 ± 5 and 14 ± 5 %, respectively, compared to the PBS matrix. These findings contrast with the discoveries of Domínguez et al. [25], who noted an enhancement in MFR by incorporating up to 15 wt.% lignin into the PBS matrix, and Hejna et al. [58], who identified an increase in MFR by introducing up to 100 parts by weight (pbw) of BSG into the PCL matrix. In the current investigation, under the operating parameters outlined in *Section 3.4.7*, natural fillers functioned as reinforcing agents, resulting in a decrease in MFR [59,60].

A further MFR study was conducted at an elevated temperature of 190 °C and a diminished load of 2.5 kg, in contrast to the typical conditions of 165 °C and 5 kg. The data shown in Fig. 3.17 (d) indicates an elevation in MFR values in composites using the BSG filler. This occurrence is due to the plasticizing impact caused by protein and lignin, which enhance the

material fluidity at increased temperatures, consistent with prior findings by Domínguez et al. [25] and Hejna et al. [58].

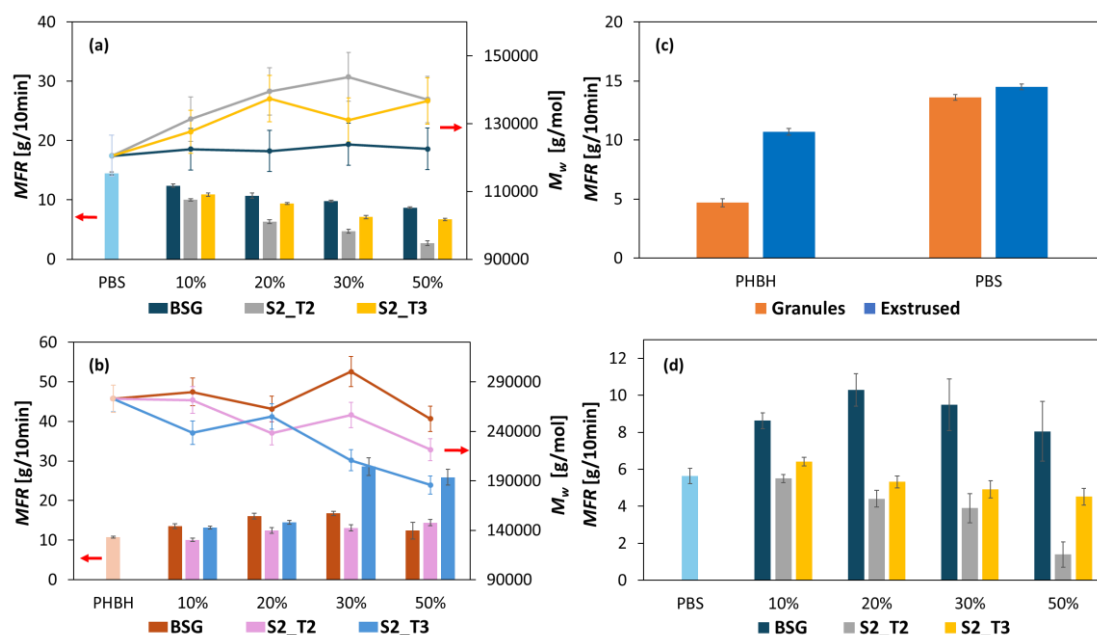


Figure 3.42 - Evaluation of MFI and GPC values for a) PBS-based biocomposites relative to biofiller content and b) PHBH-based biocomposites in relation to biofiller content; c) Comparative assessment of MFI values for the polymer matrices under examination: PHBH and PBS. The polymer matrix in granule form is depicted in orange, whereas the polymer matrix post twin-screw extrusion is represented in blue. d) Examination of MFI values of PBS-based biocomposites relative to biofiller content at 190°C and 2.5 kg

3.4.8 UTT of PBS and PHBH-based Composites

The mechanical properties of the pure polymers PBS and PHBH, as well as several formulations of biocomposites with different fillers, were evaluated using tensile testing. The Young's modulus (E) of the PBS-based composites can be observed in Fig. 3.18 (a), whereas the E values for the PHBH-based composites are shown in Fig. 3.18 (b). Figg. 3.18 (c) and 3.18 (d) show the tensile strength (σ_B) and elongation at break (ϵ_B) of the two polymer matrices, both with and without filler.

The distribution of rigid fillers inside polymer matrices leads to a gradual increase of E values with increasing filler concentration [61–64]. In PBS-based composites containing 50 wt.%, the E increases by about 98% with filler S2_T2 compared to pure PBS, whereas increases of 70% and 68% were seen for fillers BSG and S2_T3, respectively. Comparable findings were also documented in the study conducted by Kim et al. [65] conversely, in PHBH-based composites,

the addition of fillers leads to a less significant enhancement in Young's modulus (Fig. 3.18 (b)). Although a general increase in stiffness, the maximum E value reported (2215 MPa for PHBH70S2_T330) indicates a 20 % improvement compared to the pure polymer matrix (1842 MPa).

In line with the results from other studies on composite materials [61,63,64], it can be concluded that filler content significantly influences the variability of the E [66–69], whereas other mechanical properties are more affected by particle size and the presence or absence of interactions at the matrix-filler interface [70,71]. From the mechanical point of view of the bulk material, the increase in E in PBS-based composites can be mainly attributed to the introduction of a filler with higher stiffness than the polymer matrix. This result may be related at the molecular level to filler particles spatially impeding polymer chain movement [65]. While the behavior of PHBH-based composites could be the result of opposite effects: on the one hand, the increased stiffness due to the filler on the other hand, the decrease in M_w of PHBH chains, as reported by GPC analysis (Section 3.6) [55,56].

The incorporation of fillers into biopolymer matrices significantly diminishes plastic deformation and tensile strength as the proportion of natural filler increases [72–75]. Fig. 3.18 (c) illustrates that in PBS-based composites, the σ_B value diminishes progressively with increasing filler content, corroborating findings published by Hejna et al. [58] and Kim et al. [65]. With the incorporation of 50 wt.% BSG filler into the PBS matrix, there is a 56 % reduction in σ_B , from 52 to 24 MPa, while the addition of the same concentration of S2_T2 and S2_T3 determines a 62 % and 46 % decrease in σ_B (20 MPa for S2_T2 and 27 MPa for S2_T3 respectively). Concomitantly, the ϵ_B significantly diminishes in all examined composites, as seen in Fig. 3.18 (c). Comparable behavior is evident in PHBH-based composites (Fig. 3.18 (d)), wherein the pure PHBH matrix has a lower σ_B value (30 MPa) than pure PBS. The value decreases further to 11 MPa with 50 wt% BSG filler, 15 MPa with S2_T2, and 16 MPa with S2_T3. In composites with the maximum filler content (50 wt%), the intrinsic ϵ_B value of pure PHBH (0.03 mm/mm) decreases to less than half, attaining 0.01 mm/mm regardless of the filler type employed.

These results showed clear embrittlement and loss of toughness in all composite materials [76–79]. These behaviors are due to the high hydrophilicities of the different types of filler particles that form a dispersed phase in the thermoplastic matrix, allowing the formation of

aggregates and thus leading to deterioration of properties [61,67]. This dispersed phase interrupts the continuity of the polymer matrix; under these conditions, stress transfer between the particle and the matrix is not allowed [80]. Transfer of load at the matrix/particle interface requires a certain level of compatibility. In particular, when comparing the fillers, PBS showed lower compatibility with S2_T2, while the results for BSG and S2_T3 were comparable. With PHBH, the S2_T2 filler gave the best results with the lowest embrittlement and the highest elongation. This was also visible in the SEM, where the voids were much smaller for the S2_T2.

However, in general it can be assumed from the results obtained for PBS based composites that the effects of fillers on the PHBH polymer matrix are less pronounced.

This may be due to the presence of the side chains in the chemical structure of PHBH, which may favor a larger area between polymer chains and thus a smaller steric bulk, as well as the possibility of the occurrence of hydrophobic interactions at the interface. In contrast, in PBS it is more complicated due to the absence of the side chains in the chemical structure and thus a reduced interaction area for the filler particles. Compared to common WPCs based on HDPE, mechanical properties of are in the same range as our BSG composites. Using a compatibilizer and 40 wt.% of different wood fillers, HDPE composites showed E around 1.5 GPa, strength values around 30 MPa and elongation values lower 5% [81]. In that case, just based on the mechanical properties, PBS-BSG or PBS-S2T3 could be an appropriate alternative.

The use of biopolymer matrices with a wood flour content has also been explored in the literature. In the work of Weng et al. [82] they made PBS-based WPC composites blended up to a 30 wt.% of wood flour. The results showed that at the same filler concentration they obtained an ϵ_B of only 4 % compared to more than twice the elongation obtained by PBS70BSG30 and PBS70S2_T330 composites. On average, similar results were obtained when it comes to σ_B , unlike E which increased only twice as much as in Weng's work in which E tripled compared to the pure PBS matrix.

More comparable results comes from the work of Hongsriphan et al. [83] in which it is reported that loading the PBS polymer with a type of wood flour up to a 40 wt.% results in similar behaviors to our materials. In fact, the decrease in ϵ_B at volumes around 5 wt.% maximum elongation and decrease in σ_B at values around 23 MPa are observed, and an

increase in Young's modulus up to a maximum of 900 MPa compared to the average E of the PBS-based composites made in this work with a 10 wt.% higher BSG content.

Similar studies on PHA-based composites report similar behaviors. For example, in the case of PHA-wood flour blends (0-30 wt.%), results show elongation at break less than 3 % and a decrease in tensile strength from 38.7 MPa to 24.3 MPa, attributed to poor interfacial compatibility between filler and matrix [84].

An interesting comparison can be made with the work of Revert et al. [62] in which PP- and BSG-based WPCs (0-40 wt.%) with 2% compatibilizer (PP-g-MA) were developed. Composites with the highest BSG content showed a modulus of elasticity of about 510 MPa, tensile strength between 18 and 19 MPa, and elongation at break around 5 %. Although these results are similar in terms of mechanical properties to those obtained in the present work, the developed composites offer a significant advantage in that they are fully biobased and biodegradable, representing a sustainable option to replace traditional WPCs.

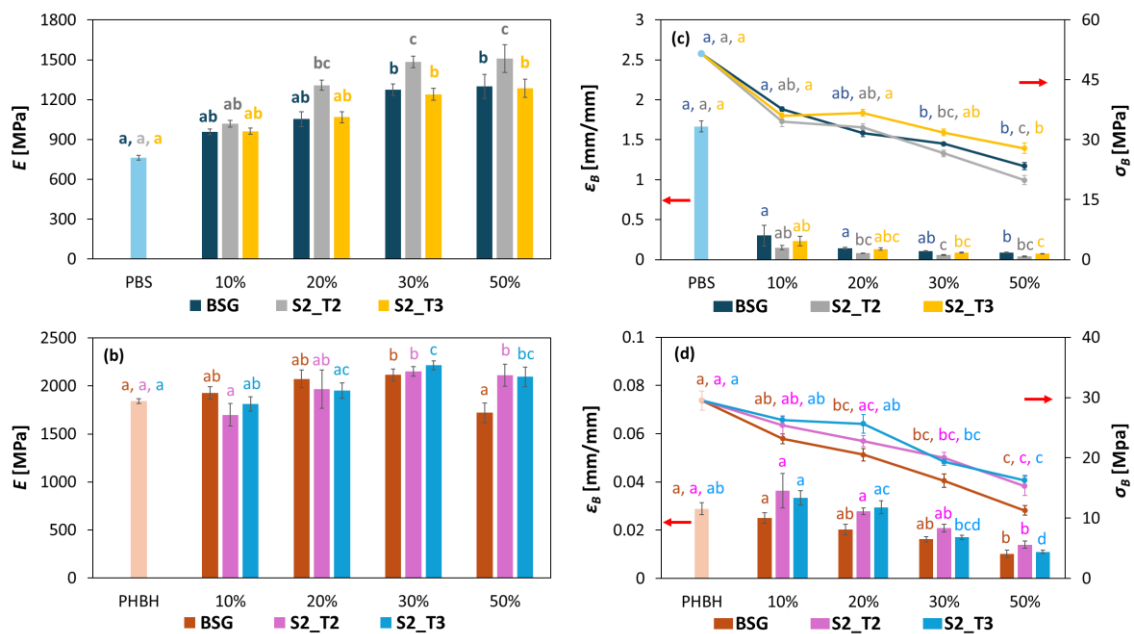


Figure 3.43 - Representation of the trend of experimental values of: a) Young's modulus (E) of PBS-based composites at varying filler content BSG (midnight blue), S2_T2 (gray) and S2_T3 (yellow); b) Young's modulus (E) of PHBH-based composites at varying filler content BSG (brown), S2_T2 (fuchsia) and S2_T3 (navy blue); c) elongation at break (ϵ_B) and stress at break (σ_B) of PBS-based composites at varying filler content BSG (midnight blue), S2_T2 (gray) and S2_T3 (yellow); d) elongation at break (ϵ_B) and stress at break (σ_B) of PHBH-based composites at varying filler content BSG (brown), S2_T2 (fuchsia) and S2_T3 (navy blue). Different letters indicate statistically significant differences within the sample series of each filler ($p \leq 0.05$)

3.4.9 SEM Analysis of PBS and PHBH-based composites

The surface morphology and interfacial compatibility of were examined analyzing the cross sections generated after polished-sputtering treatments with SEM. This study is essential to confirm the findings from the uniaxial tensile and FT-IR testing.

Figg. 3.19 (a) and (d) present SEM micrographs of the pure polymers PBS and PHBH, respectively, together with the corresponding biocomposites having 50 wt.% BSG-S2_T3. This filler was selected as representative because to the consistent findings observed across all fillers examined inside the same polymer matrix. The scans further provide magnifications of the regions of interest inside the composite materials. The PBS50S2_T350 composite is shown in Fig. 3.19 (b), with a detailed view presented in Fig. 3.19 (c). Fig. 3.19 (e) shows the PHBH50S2_T350 composite, accompanied by an enlargement in Fig. 3.19 (f).

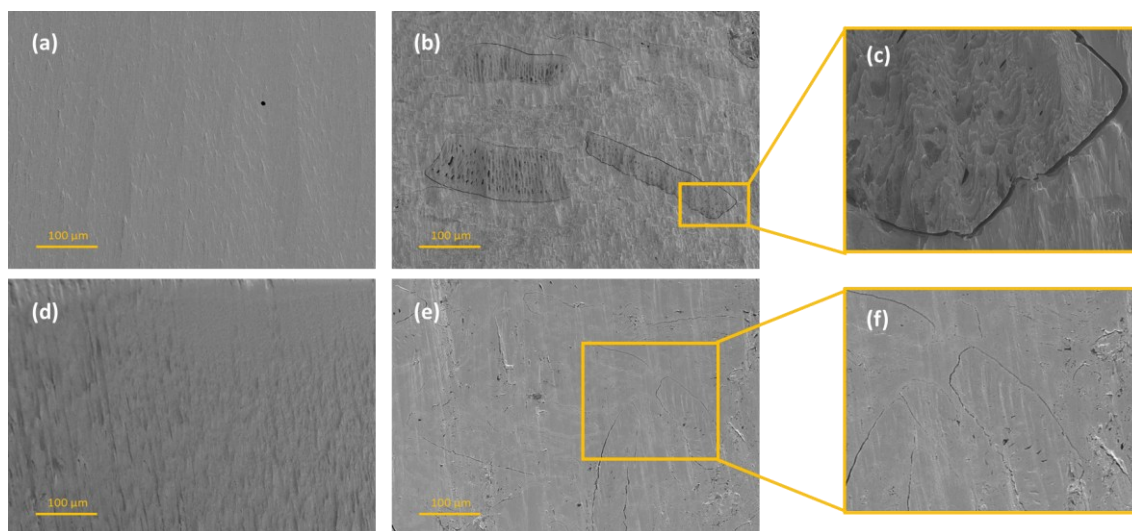


Figure 3.44 - Scanning electron microscope (SEM) images of the cross-sectional surface of: (a) PBS, (b) PBS50S2_T350 (b) and its relative magnification (c), (d) PHBH, (e) PHBH50S2_T350 and its relative magnification (f)

The pure PBS and PHBH polymers (Figg. 3.19 (a,d)) exhibited a smooth and homogenous morphology, signifying a structure devoid of inclusions or inhomogeneities. The micrographs of the composite samples indicate that the fibers of the lignocellulosic filler manifest as discontinuities on the polished surface of the polymer matrix. The existence of a micro gap surrounding the filler particles, notably seen in Fig. 3.19 (c), indicates inadequate interfacial adhesion between the filler and the matrix. This observation corresponds to results from IR spectroscopy and tensile testing. The absence of adhesion between the matrix and the fibers leads to inadequate transmission of mechanical stresses from the fibers to the matrix, causing material stiffening and diminished ductility under mechanical stress.

Compared to PBS-based composites, micrographs of PHBH-based composites (Fig. 3.19 (e,f)) exhibit enhanced matrix surface wettability on the fibers at increased filler concentrations. This behavior can be ascribed to the distinct chemical structure of the PHBH polymer matrix, which enhances compatibility with the lignocellulosic filler. In contrast, the PBS-filler system is likely to suffer from a reduced interfacial area due to the coarse size of the filler, resulting in sub-optimal morphological, structural and mechanical properties. This is a very important factor in maintaining properties and reducing excessive embrittlement caused by the incorporation of a filler that is inherently more rigid than the polymer matrix.

3.4.10 DSC Analysis of PBS- and PHBH-based composites

Fig. 3.20 provide thermograms of the heating scans and respective crystallinity changes for PBS, PHBH, and various composite formulations. The thermal characteristics of the two polymer matrices were examined by DSC analysis to assess the melting, crystallization, and glass transition temperatures variation due to BSG filler. The variance of $\% \chi_c$ was also assessed as the filler content and type of BSG varied. The complete results of the DSC analysis are included in Tabb. 3.11 and 3.12, respectively.

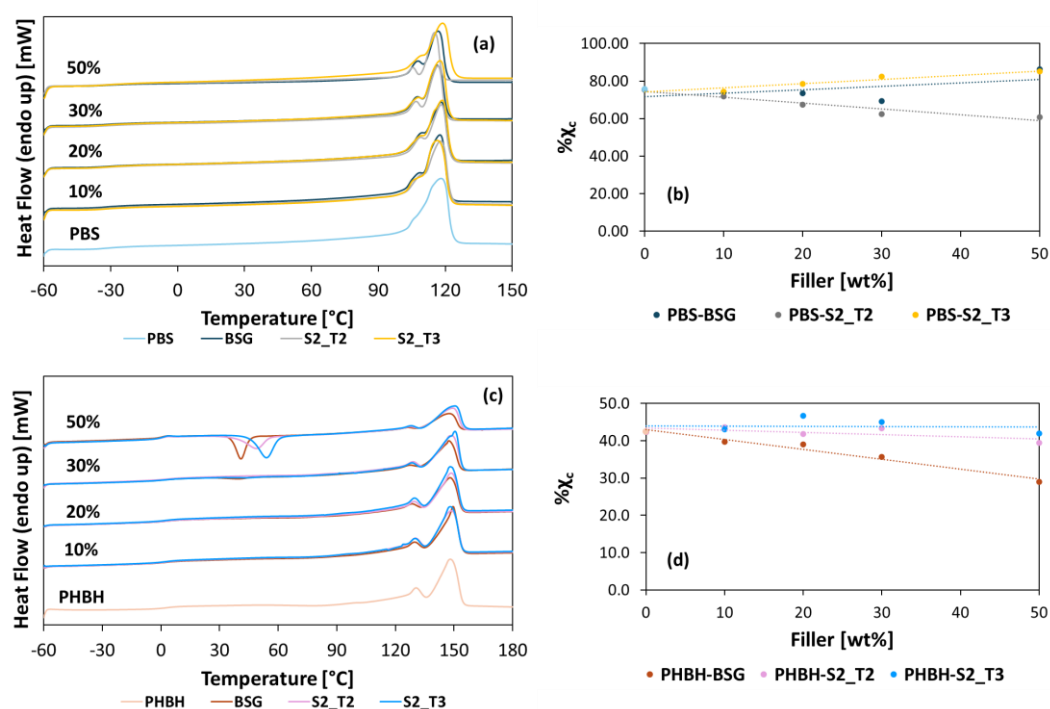


Figure 3.45 - (a) Thermogram of heating scan of PBS-based composite samples; (b) degree of crystallinity of PBS-based composites; (c) Thermogram of heating scan of PHBH-based composite samples; (d) degree of crystallinity of PHBH-based composites

Table 3.11 - Thermal properties of PBS-based composites obtained from thermograms

Sample	T_m [°C]	T_c [°C]	T_g [°C]
PBS_WATER	116±1.6	87±1.2	-33±0.7
PBS90BSG10	116±1.8	87±0.3	-33±1.03
PBS80BSG20	116±2.2	87±0.4	-32±1.7
PBS70BSG30	115±1.1	87±0.1	-33±0.9
PBS50BSG50	115±1.6	86±1.95	-35±2.6
PBS90S2_T210	117±0.7	87±0.7	-32±0.4
PBS80S2_T220	117±1.1	87±1.2	-33±3.3
PBS70S2_T230	117±0.6	87±0.3	-32±1.1
PBS50S2_T250	116±2	85±1.8	-26±7
PBS90S2_T310	116±0.3	87±0.1	-31±1.3
PBS80S2_T320	117±1.3	87±1.5	-30±1.5
PBS70S2_T330	117±0.5	87±2.8	-32±2.8
PBS50S2_T350	118±0.9	86±1.3	-32±1.3

Table 3.12 - Thermal properties of PHBH-based composites obtained from thermograms

Sample	T_m [°C]	T_c [°C]	T_g [°C]
PHBH	147±0.3	64±2.6	0±4
PHBH90BSG10	149±0.1	69±4.8	3±0.6
PHBH80BSG20	148±1	64±0.1	3±1.2
PHBH70BSG30	148±1.2	58±3.6	4±0.2
PHBH50BSG50	149±1.6	65±0.5	1±0.6
PHBH90S2_T210	149±0.8	64±0.1	3±1.5
PHBH80S2_T220	149±0.5	60±0.1	3±1.7
PHBH70S2_T230	149±0.6	55±2.4	3±0.1
PHBH50S2_T250	150±0.9	59±2.2	1±2
PHBH90S2_T310	148±1.2	69±6.5	2±1.5
PHBH80S2_T320	148±1.1	63±0.1	3±1.5
PHBH70S2_T330	150±0.8	65±1.6	2±0.1
PHBH50S2_T350	150±0.9	62±1.3	1±1

DSC findings demonstrate that the addition of three distinct types of BSG-derived fillers does not significantly modify the thermal characteristics of the PBS matrix. PBS-based composites incorporating BSG have a nearly constant T_m of $116 \pm 2^\circ\text{C}$, which is comparable to the T_m of pure PBS (116°C). Similar results were seen for PHBH-based composites, which have T_m that does not significantly differ from that of the pure polymer (147°C). Comparable behavior was

noted in the research conducted by Hejna et al. [3], which revealed no significant alteration in T_m with the incorporation of BSG into PCL matrix.

Invariance of T_c was similarly observed PHBH-based composites. As the filler content increased, peak broadening and lowering of intensity was observed due to the decrease in the weight concentration of the PHBH polymeric matrix within the composites.

No significant alterations in T_g were noted in comparison to pure PBS and PHBH. Initial investigation indicates that the incorporation of various BSG fillers into PHBH marginally elevates the T_g to a range of 1-4 °C for all composites. Given that T_g represents a range of temperatures marking the transition from a glassy to a rubbery state, it can be concluded that these minor variations in T_g are negligible, suggesting that the incorporation of BSG fillers does not substantially influence the mobility of polymer chains.

As far as % χ_c is concerned, however, it can be observed that as the filler increases, a different behavior occurs depending on the type of filler used.

3.5 Conclusions

This research evaluates the possible influence of BSG biofiller on the properties of biocomposites produced using PBS and PHBH as polymer matrices, as alternatives to conventional wood flour-based WPCs.

The BSG biofiller underwent solid-liquid hydrolytic extraction procedures at varying pH levels (9, 10.5, and 12) utilizing both conventional and alternative lautering methods. These treatments eliminated non-lignocellulosic contaminants, enhancing the wood-like characteristics of the biofiller. The results indicated a substantial decrease in protein content, although the lignocellulosic fraction was qualitatively maintained. The treatment efficacy was validated by FT-IR analysis, which indicated no substantial structural alterations. The extraction procedure with elevated pH combined with alternative lautering was effective, achieving a protein extraction efficiency of up to 79.84%.

Structural and thermal studies, designed to elucidate interactions between biofillers and biopolymer matrices, revealed no significant alterations by FT-IR or DSC. Mechanical testing and SEM indicated disparities in essential compatibility between the filler and matrix. The

optimal compatibilities for PBS were observed with BSG and S2T3 composites, although PHBH exhibited better results when paired with S2T2. Consequently, the necessity of pretreatment to reduce protein concentration may vary according on the matrix employed. Nevertheless, alterations in melt flow at elevated protein concentrations and increased temperatures must be considered throughout processing. In contrast to traditional polyolefin-based wood-plastic composites, both PBS-BSG and PBS-S2T3 exhibited similar mechanical performance.

The extrusion method significantly affects the selected polymer-matrix systems, with various biofillers exerting an influence on the MFR of both PBS- and PHBH-based biopolymer matrices. A reduction in fluidity, attributed to the restricted mobility of the chains, was seen to be particularly significant in PBS-based biocomposites incorporating treated biofillers. This is likely attributable to the reduced protein level. Conversely, PHBH-based composites exhibited MFR values with a marginal rise, which corresponded with declining GPC values as filler content rose. The results indicate the degradation issue present in the PHBH polymer matrix, attributed to inadequate resistance to thermomechanical stresses during the extrusion process. The deterioration may also result from the incorporation of excessive filler, which amplifies the total shear stress during the extrusion process.

The primary issue in integrating biofillers into polymer matrices is the significant alteration in mechanical characteristics, particularly when the compatibility between the matrix and the filler is restricted. Despite the various filler pretreatments, the overall performance suggests few interactions at the particle-polymer interfaces. Future advancements may concentrate on including plasticizers and compatibilizers to enhance the processability and characteristics of biocomposite materials. Furthermore, optimizing the dehydration and grinding processes may provide finer biofiller particles, hence enhancing the contact surface area and dispersion within the polymer matrix.

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Chapter 4 - PBS- and PHBH-based Biocomposites with Bioplasticizer

4.1 Introduction

Considering BSG as a promising economical biofiller alternative to the use of wood flour, in this study conducted in collaboration with the Department of Process Development of the Fraunhofer Institute for Process and Packaging Engineering IVV (Fraunhofer-Institut für Verfahrenstechnik und Verpackung) and the Dept. of Civil Engineering, Chemistry, Environmental and Materials Engineering of the University of Bologna, the production and characterization of new biocomposite materials based on PBS [1] and PHBH [2], compounded with BSG filler [3,4] and glycerol trilevulinate (GT) bioplasticizer [5], was presented. The addition of a plasticizer to the mix stems from the need to modulate the final properties of the composite, according to the different applications. The GT structure, characterized by a high density of carboxyl groups, together with its steric bulk, gives high plastification efficacy for polymer matrices such as aliphatic polyesters. This effect could result in a significant improvement in the processability, mechanical properties and long-term stability of the resulting compounds.

The GT plasticizer was produced by a green, solvent-free esterification method using levulinic acid and glycerol. For the preparation of the BSG filler, the grinding and sieving methods were optimized, allowing a filler with a D_{mean} below 50 μm to be obtained and the surface area of interaction between filler and polymer matrix to be improved. In addition, the ground and sieved BSG filler underwent an optimized thermal stabilization process compared to previous treatment (from 60 °C to 120 °C), in an attempt to reduce the loss of moisture inside as much as possible, while reducing the causes of degradation of composite materials. In the way polymer matrices were thermally treated as well, increasing the drying temperature by 10 °C compared to the previous tests (from 60 to 70 °C).

The composite materials were prepared by melt compounding procedure using a corotating twin-screw extruder and air-cooling system, different from the system used in Chapter 3. The obtained pellets were employed for evaluation of two possible conventional processing methods, injection molding and thermoforming.

The impact of GT plasticizer on the rheological and structural characteristics of pure polymer matrices and PBS-BSG and PHBH-BSG composites was examined by melt-mass flow rate (MFR) analysis, gel permeation chromatography (GPC), and infrared spectroscopy (FT-IR). To assess the impact of the complementary addition of plasticizer and filler particles on the mechanical and thermal properties of polymer matrices, uniaxial tensile testing (UTT) and dynamic mechanical analysis (DSC) were conducted on all formulations produced by injection molding. This allowed the effectiveness of BSG reinforcement and GT plasticizer to be evaluated simultaneously, and variations in the conditions of use of the new materials to be delineated.

4.2 Materials and Methods of Preparation

4.2.1 Materials

The polymer matrices and BSG filler, as in the previous work, are:

1. PBS, supplied by MCC Biochem (Dusseldorf, Germany), under the trade name 'BioPBS FZ91PB' ($T_m = 115\text{ }^{\circ}\text{C}$, $\rho = 1.26\text{ g cm}^{-3}$).
2. PHBH, with a 3-HH content of 6 mol%, supplied by Gruppo Maip SRL (Turin, Italy), under the trade name 'I am Nature B6 A13' ($T_m = 145\text{ }^{\circ}\text{C}$, $\rho = 1.20\text{ g cm}^{-3}$).
3. BSG, supplied by the brewery Gräflisches Hofbrauhaus Freising GmbH (Freising, Germany).

Glycerol (Gly, $\geq 99\%$), levulinic acid (LA, 98 %), sodium chloride (NaCl, $\geq 99.5\%$), ethyl acetate (EtAc, 99.5 %) and sodium sulphate (Na_2SO_4 , anhydrous to $\geq 99\%$) purchased from Sigma-Aldrich were used for the synthesis of the GT plasticizer. The initiator p-toluenesulphonic acid monohydrate (PTSA, 98.5 %) was purchased from Alfa Aesar, sodium carbonate (Na_2CO_3) was purchased from Carlo Erba (Milan, Italy).

4.2.2 Synthesis of Glycerol Trilevulinate Plasticizer

GT was synthesized in the laboratories of the University of Bologna. It is a fully bio-based plasticizer and was obtained by the esterification reaction between glycerol (GLY) and levulinic acid (LA) via a solvent-free approach under mild conditions. The reaction scheme is shown in Fig. 4.1.

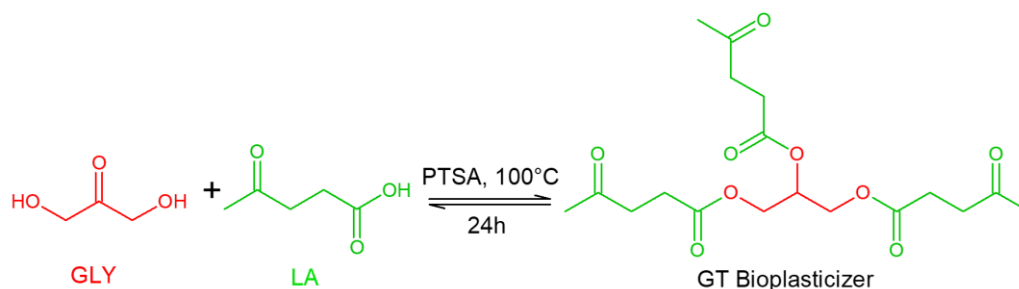


Figure 4.46 - Reaction diagram of the GT plasticizer synthesis

GLY (3.965 g/0.0431 moles), LA (25.00 g/0.2153 moles) and PTSA (catalyst, 0.249 g/0.0013 moles) were mixed in a flask under magnetic stirring. The reaction mixture has been heated up to 100 °C for 24h, allowing the water generated to leave the reaction environment. The reaction was monitored by FT-IR spectroscopy until the desired product was formed. The reaction mixture was cooled to room temperature and quenched with Na₂CO₃ saturated water solution to neutralize the excess of LA. EtAc was added and the work-up was performed. The organic layer was separated with a separating funnel, washed twice with Na₂CO₃ solution and with brine (saturated aqueous solution of NaCl), anhydridified with Na₂SO₄ and concentrated under vacuum. A yellow oil was obtained (Fig. 4.2).

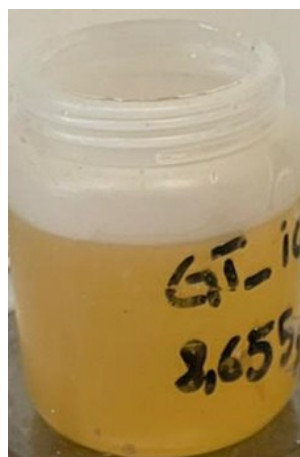


Figure 4.47 - GT plasticizer

4.2.3 Thermal and Mechanical Pretreatment of the BSG Filler

The native BSG was conditioned in an oven at 60 °C overnight to remove much of the moisture content and optimize the grinding process. Subsequently, the BSG was milled under 0.25 mm

using an SM300 cutting mill (Retsch, Germany) equipped with a “V-shape” rotor suitable for grinding lignocellulosic fibers (Fig. 4.3). Grinding was performed by setting a rotational speed of 2900 rpm.

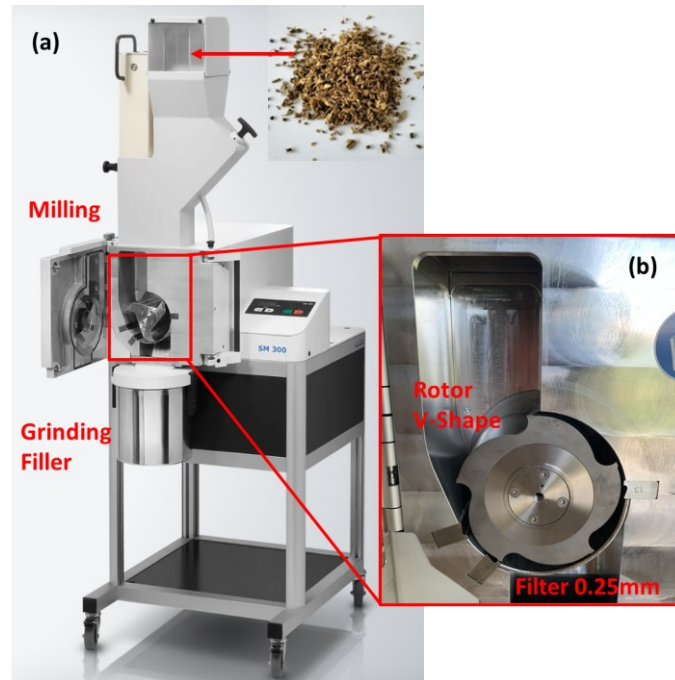


Figure 4.48 - (a) SM300 cutting mill; (b) Enlargement of the grinding zone consisting of the “V-Shape” type rotor and the filter with a mesh diameter of 0.25 mm

The ground BSG was mechanically sieved by means of the discontinuous analytical vibro-sieving instrument (Retsch AS 200 Control, Germany) with a series of progressively finer-sized sieves (250, 200, 150, 100, 75, 50 μm) (Fig. 4.4 (a)). An oscillation amplitude of 12 mm/“g” was set with the process duration of 15 min interspaced by intervals of 5 sec every 1 min of oscillation. To produce the composite materials, the BSG collected below 50 μm was used (Fig. 4.4 (b)).

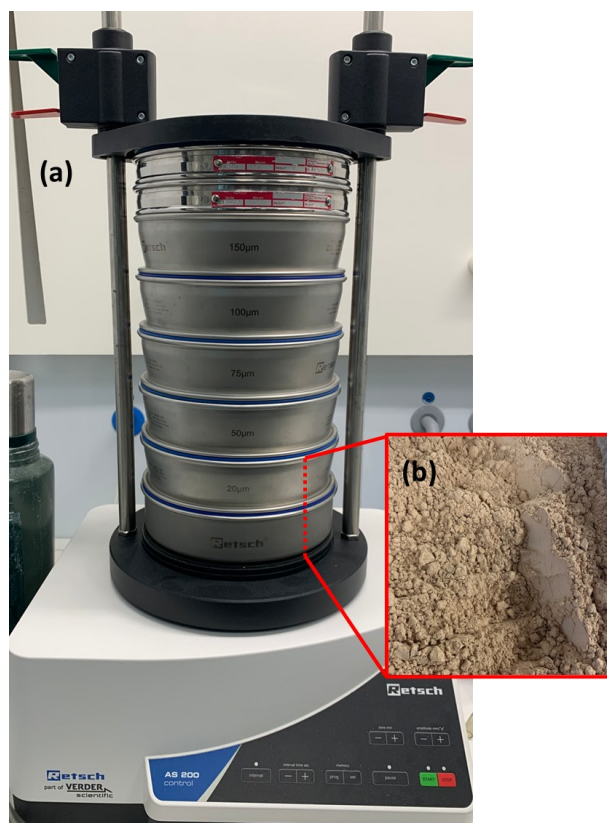


Figure 4.49 - (a) Discontinuous Analytical Vibro-Sieving instrument; (b) BSG ground and sieved under 50 μm

The 50 μm sieved BSG was subsequently dried by optimized heat treatment at 120 $^{\circ}\text{C}$ until it reached a constant weight and used as a bio-filler, in the amount of 20 and 50 wt.%, for the preparation of the various composite material formulations.

4.2.4 PBS and PHBH Polymer Matrix Preparation

The PBS and PHBH pellets were ground under cryogenic conditions using the analytical micro-mill (IKA A11). The use of liquid nitrogen results in a very brittle state of the material, which facilitates the dimensional reduction of the granules. This procedure is important for feeding the extruder (pellet size <1 mm) but also for better pre-mixing the polymer with plasticizer and filler.

4.2.5 Preparation of Biocomposite Materials Batches

Subsequently, the pellets were heat-treated at 70 °C for the dehydration process overnight. The GT plasticizer was also kept in the oven at 40 °C for the dehydration process from (ambient) water and ethyl acetate (solvent used for GT purification).

Pure ground polymers, stabilized BSG flour and GT plasticizer were dosed and mixed manually to prepare blends in various formulations shown in Tab. 4.1. The procedure was carried out at room temperature and pressure.

Table 4.22 - Formulations of composites prepared on the basis of PBS and PHBH

Formulations	PBS	PHBH	BSG	GT	Composite
	wt.%	wt.%	wt.%	wt.%	Code name
a	100				PBS
b	90			10	90PBS10GT
c	74.07		18.52	7.41	PBS-20BSG10GT
d	47.62		47.62	4.76	PBS-50BSG50GT
e		100			PHBH
f		90		10	90PHBH10GT
g		74.07	18.52	7.41	PHBH-20BSG10GT
h		47.62	47.62	4.76	PHBH-50BSG10GT

Four different formulations were therefore produced for each polymer matrix: pure polymers (PHBH and PBS), polymers with 10 wt% GT (90PBS10GT and 90PHBH10GT), composites with 20 and 50 wt.% BSG and 10 wt.% GT referred to the polymer weight (PBS-20BSG10GT, PBS-50BSG10GT and PHBH-20BSG10GT, PHBH-50BSG10GT).

4.2.6 Melting Compounding

Prior to extrusion, the composite mixes and pure polymer matrices were treated in an oven at 60 °C overnight. All prepared compositions were extruded to produce the various polymer composite filaments. The process was performed using a corotating twin-screw extruder RES-2 P/12A Explorer (Zamack Mercator, Poland), shown in Fig. 4.5, with an L/D ratio of 40 and a screw diameter of 11mm.

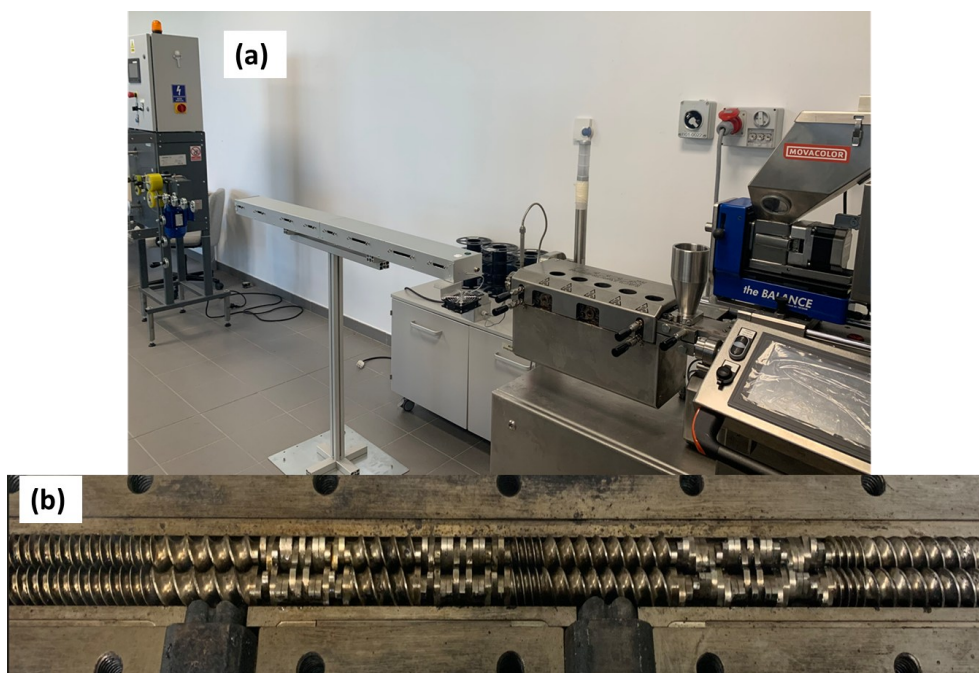


Figure 4.50 - (a) Extrusion system and (b) corotating twin-screws profile

The optimized extrusion parameters for PBS- and PHBH-based composites are shown in Tab. 4.2. Two different temperature profiles were used due to the different melt temperatures and viscosities of the two polymers. The variations in temperatures and/or screw speeds occurred in relation to the pressure and load parameters of the screws during extrusion. The screw speed was kept constant at 45 rpm for all PBS and PHBH compositions. However, for some compositions, the screw speed was increased to 80 rpm due to the addition of the GT plasticizer and BSG filler. The extruded material was air-cooled. Subsequently, all materials were pelletized and converted into granules and held in an oven at 60°C for the dehydration process. The detailed extrusion process was described in *Chapter 2 (Section 2.2.5)*. The produced granules represent the raw material used for the injection molding and thermoforming production of composite films, used for further characterizations.

Table 4.23 - Extrusion parameters of PBS- and PHBH-based composites

Sample Name	Extrusion Zone Temperatures [°C]								Head
	Hopper Zone	Zone 1	Zone 2	Zone 3	Zone 4	Zone 5	Zone 6	Zone 7	
PBS	50	125	145	145	145	135	120	120	115
90PBS10GT	50	112	132	132	132	122	117	115	115
PBS-80BSG10GT	50	112	132	132	132	122	117	115	115
PBS-50BSG10GT	50	118	138	138	138	128	123	120	115
PHBH	50	129	135	136	135	132	132	132	130
90PHBH10GT	50	121	122	123	122	119	119	119	118
PHBH-20BSG10GT	50	121	122	123	122	119	119	119	118
PHBH-50BSG10GT	50	121	122	123	122	119	119	119	118

4.2.7 Injection Molding

Material granules were injection-molded using the Babypast 10/12 printer (Cronoplast) to produce type 5A dumbbell samples (ISO 527 standard) [6]. The injection molding conditions of PBS-based composites are shown below (Tabb. 4.3 and 4.4).

For the pure PBS, the samples were molded based on extrusion conditions (Tab. 4.3). For the injection molding of the 90PBS10GT and PBS-20BSG10GT blends, the effect of the GT plasticizer decreased processing temperatures (<15 °C). In contrast to the latter and the pure polymer matrix, the higher temperatures ($T_1=138$ °C; $T_2=138$ °C; $T_3=148$ °C) were used for the PBS-50BSG10GT mixture (according to the MFR analysis).

It should be noted that during the processing of the different compositions and the pure polymer matrix, no leakage of the melt from the injection nozzle was observed during the stand-by phases.

Table 4.24 - Process temperatures (Plasticization-Accumulation-Injection)

Sample Name	T_1 [°C]	T_2 [°C]	T_3 [°C]
PBS	135	135	145
90PBS10GT	120	120	130
PBS-20BSG10GT	120	120	130
PBS-50BSG10GT	138	138	148

Other parameters relating to injection pressures, times and speeds are shown in Tab. 4.4, which were determined after several attempts to optimize the degree of filling of the mold and reduce porosity.

Table 4.25 - Principal injection parameters

Injection parameters	PBS	90PBS10GT	PBS-20BSG10GT	PBS-50BSG10GT
Material Charge	19	19	19	19
Cooling time (s)	10	20	20	10
1st Injection Pressure (bar)	35	35	35	40
Time 1st Pressure (s)	4	4	4	4
2nd Injection Pressure (bar)	10	10	10	15
Time 2nd Pressure (s)	3	3	3	3
Suction (mm)	6	6	6	6
Injection Speed (%)	100	100	100	100
2nd Injection Speed (%)	100	100	100	100
Suction Speed (%)	70	70	70	70

The injection molding conditions for PHBH-based composites are shown below in Tab. 4.5 and 6. The samples were produced at a temperature of 142 ± 10 °C, which varied depending on the material processed. For PHBH, the highest temperatures were used ($T_1=147$ °C, $T_2=152$ °C, $T_3=149$ °C), while the presence of BSG and GT plasticizer made the material more fluid, causing nozzle leakage in some cases, so efficient production was conducted by slightly varying temperatures (Tab. 4.5) and pressures (Tab. 4.6).

Table 4.26 - Process temperatures (Plasticization-Accumulation-Injection)

Sample Name	T ₁ [°C]	T ₂ [°C]	T ₃ [°C]
PHBH	147	152	149
90PHBH10GT	143	139	137
PHBH-20BSG10GT	142	141	136
PHBH-50BSG10GT	145	141	132

Other parameters for injection pressures, times and speeds are shown in Tab. 4.6, optimized for the specimen production. For all the materials produced, unlike the PBS-based composites, the suction parameter was set to 0 mm as it was seen to cause problems when detaching the final part from the injection plates.

Table 4.27 - Main injection parameters

Injection parameters	PHBH	90PHBH10GT	PHBH-20BSG10GT	PHBH-50BSG10GT
Material Charge	19	19	19	19
Cooling time [s]	460	120	150	320
1st Injection Pressure [bar]	57	59	59	57
Time 1st Pressure [s]	12.5	14	12.5	12.5
2nd Injection Pressure [bar]	52	54	54	52
Time 2nd Pressure [s]	12.5	14	12.5	12.5
Suction [mm]	0	0	0	0
Injection Speed [%]	55	55	55	55
2nd Injection Speed [%]	50	50	50	50
Suction Speed [%]	0	0	0	0

The dumbbell samples, 30 replicas produced for each composition, were used for subsequent chemical, structural, mechanical, and thermal characterization.

Fig. 4.6 shows the PBS-based biocomposite samples in order from left to right, respectively:

1. PBS
2. 90PBS10GT
3. PBS-20BSG10GT
4. PBS-50PBS10GT



Figure 4.51 - PBS-based composites specimens

Fig. 4.7 shows the PHBH-based biocomposite samples in order from left to right, respectively:

1. PHBH
2. 90PHBH10GT
3. PHBH-20BSG10GT

4. PHBH-50PBS10GT

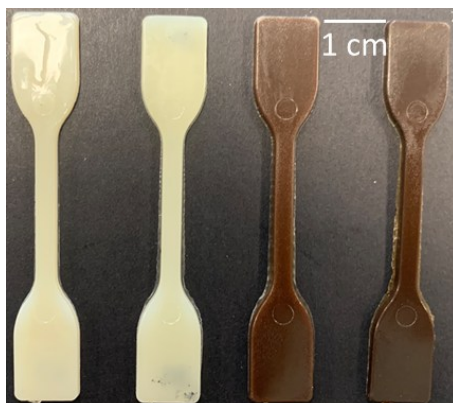


Figure 4.52 - PHBH-based composites specimens

1.4.3 Thermo Forming

The films were produced by thermoforming, using the thermal press Laboratory Press-Drive supplied by Gibitre Instrument (Bergamo, Italy), with pellets obtained from the pelletizing process distributed on the bottom plane of the instrument. This process made it possible to produce initially single-layer films and subsequently multi-layer films composed of three layers sealed together. This configuration enables the comparison between multilayer films made by pure PBS or PHBH, and those made by bio-composite materials. These multilayer films present a sandwich structure, consisting of two outer layers of pure PBS or PHBH with an intermediate layer of composite material.

The thermoforming process involved preheating the plates to a specific temperature, bringing the hot plates closer to the material and holding them almost in contact for 90 s, closing and hot pressing for 20 s, then cooling to 50 °C while maintaining pressure. The pressure was released when the sample temperature dropped below 50 °C. The PBS-based monolayer films (12x10 cm²) were produced using a temperature of 135 °C, while the PHBH-based monolayer films produced using a temperature of 155 °C. For all composites, the processing conditions of the pure polymer were maintained to standardize the production process. Tab. 4.7 presents the temperature and pressure parameters used for the production of PBS- and PHBH-based monolayer films.

Table 4.28 - Temperature and pressure parameters used for the production of monolayer films based on PBS and PHBH

Single-layer Film	Temperature [°C]	Pressing Force [Kg]	Pressure [bar]
PBS	135	22.000	160
90PBS10GT	135	22.000	160
PBS-20BSG10GT	135	22.000	160
PBS-50BSG10GT	135	22.000	160
PHBH	155	22.000	160
90PHBH10GT	155	22.000	160
PHBH-20BSG10GT	155	22.000	160
PHBH-50BSG10GT	155	22.000	160

To produce the multilayer films based on PBS and PHBH, the processing conditions are shown in Tab. 4.8. The time parameters were varied and the temperature values decreased. Again, the processing conditions remained unchanged within the same family to make the production process uniform. For the PBS-based multilayer films, a plate temperature of 103 °C was used. In the case of PHBH-based multilayer films a plate temperature of 137 °C was used. For both polymer matrices PBS and PHBH, it was preferred to use a processing temperature below the melting temperature so the layer sealing was possible without material melting. Due to the decrease in temperature, the residence time of the films within the plates increases to 210 s compared to the 90 s used to produce the single-layer films. The code name for the multilayer films is based on the composition of the intermediate layer sandwiched between two layers of pure PBS or PHBH, as displayed in Tab. 4.8. The multilayer films obtained from the thermoforming process are shown in Fig. 4.8.

Table 4.29 - Temperature, pressure and time parameters used in the production of multilayer films based on PBS and PHBH

Multi-layer Film	Temperature [°C]	Pressing Force [Kg]	Pressure [bar]	Time Close [s]
Multi PBS	103	22.000	160	210
Multi 90PBS10GT	103	22.000	160	210
Multi PBS-20BSG10GT	103	22.000	160	210
Multi PBS-50BSG10GT	103	22.000	160	210
Multi PHBH	137	22.000	160	210
Multi 90PHBH10GT	137	22.000	160	210
Multi PHBH-20BSG10GT	137	22.000	160	210
Multi PHBH-50BSG10GT	137	22.000	160	210

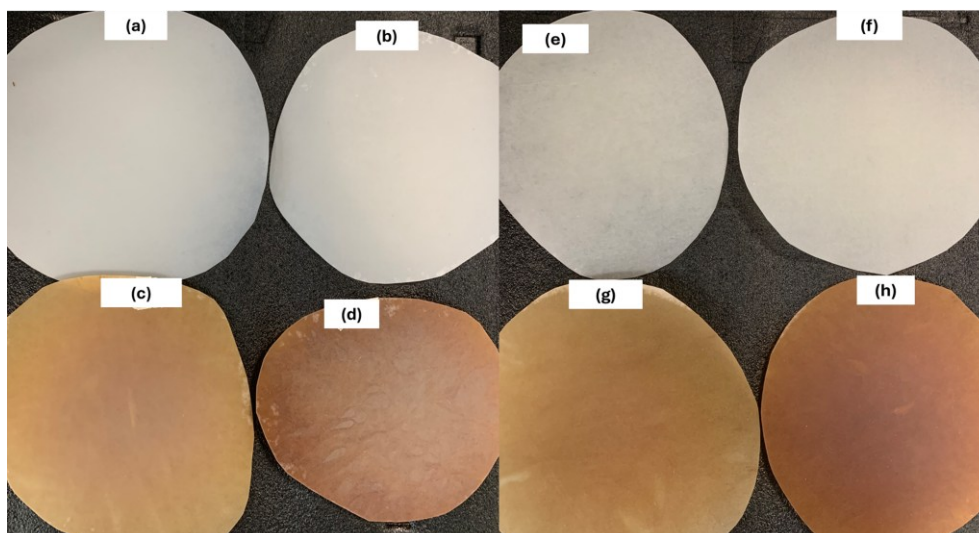


Figure 4.53 - Films obtained by thermoforming: (a) Multi PBS; (b) Multi 90PBS10GT; (c) Multi PBS-20BSG10GT; (d) Multi PBS-50BSG10GT; (e) Multi PHBH; (f) Multi 90PHBH10GT; (g) Multi PHBH-20BSG10GT and (h) Multi PHBH-50BSG10GT

The thickness of each multilayer film, useful for the permeability tests, was determined by the average of seven measurements taken at various points on the surface with a millesimal micrometer. Two multilayer films were analyzed for each composition. Tab. 4.9 shows the average thickness for each composition.

Table 4.30 - Average thickness of multilayer films

Sample Film	Average Thickness [μm]
Multi PBS	475 $\pm$ 6
Multi 90PBS10GT	493 $\pm$ 11
Multi PBS-20BSG10GT	538 $\pm$ 25
Multi PBS-50BSG10GT	560 $\pm$ 3
Multi PHBH	526 $\pm$ 12
Multi 90PHBH10GT	480 $\pm$ 6
Multi PHBH-20BSG10GT	504 $\pm$ 2
Multi PHBH-50BSG10GT	502 $\pm$ 3

4.3 Characterization of Biofillers, Polymer Matrices and Biocomposite Materials

4.3.1 Particle Size Characterization

The particle size distribution of BSG was evaluated using a Mastersizer 3000 laser diffraction particle size analyzer with an Aero S dry powder disperser from Malvern Panalytical (Kassel,

Germany). The analysis was performed with a flow of compressed air at a pressure of 4 bar and a feed rate set at 40 %. Mean distribution curves representing volume density [%] as a function of particle size [μm] were produced. In addition, mean diameter (D_{mean}) and percentiles $D_x(10)$ [μm], $D_x(50)$ [μm] and $D_x(90)$ [μm] were also evaluated.

4.3.2 Melt-mass Flow Rate

Prior to injection molding, a small aliquot of pellets from each mixture produced was used for Melt-mass Flow Index (MFR) analysis to investigate the fluidity of the different materials produced and to use these results for the study of injection molding conditions. MFR of the tested materials was determined using the CEAST MF20 plastometer (Instron®, Darmstadt, Germany). The test was performed at 165 °C with a weight of 2.16 kg, measuring the amount of material melted and discharged in 10 minutes, according to ISO 1133 [7].

4.3.3 Gel Permeation Chromatography

Average molecular weight (M_w) measurements using Gel Permeation Chromatography (GPC) were performed using a Dionex system, utilizing a solvent combination of 1,1,1,3,3,3-hexafluoro-2-isopropanol (HFIP) and trifluoroacetic acid (0.02 mol L⁻¹). Sampling was conducted using a DIONEX Ultimate 3000 injector, functioning at a steady flow rate of 1 mL min⁻¹, using a Gynkotek M480 gradient HPLC pump. The detection was conducted with a Gynkotek SE-61 refractive index detector.

Analysis utilized PSS-PFG columns (7 μm , 300 × 8 mm), regulated at a constant temperature of 40 °C (PSS, Mainz, Germany). Samples were generated by dissolving 5000 $\mu\text{g/mL}$ of material in HFIP and subsequently filtered through a polytetrafluoroethylene (PTFE) membrane having a porosity of 0.20 μm . A volume of 100 μL was injected for each analysis. Calibration was conducted utilizing a PET standard characterized by a limited molecular weight distribution (PSS, Mainz, Germany).

The acquired curves were examined using WinGPC® UniChrom software (version 8.1, PSS, Mainz, Germany). Mean values of weighted molecular weight (M_w) were documented for all samples, exhibiting a standard variation of 5%, which reflects instrument sensitivity. The

relative accuracy of the results was assessed to be under 5%, consistent with standard performance for the estimation of M_w [8].

4.3.4 Fourier Transform-Infrared Spectroscopy

The chemical structure of the samples, the BSG filler and the GT plasticizer, as well as the evaluation of a possible interaction between them, were determined using Fourier Transform Infrared Spectroscopy (FT-IR) performed with a Perkin Elmer FT-IR Spectrum Two device with PerkinElmer Spectrum IR software, version 10, both supplied by Perkin Elmer (Shelton, CT, USA). The device features an ATR (attenuated total reflection) module equipped with a diamond crystal. The spectra of each sample were obtained in a wavelength range 4000-400 cm^{-1} with a resolution of 4 cm^{-1} and 16 scans. Prior to analysis, the measured samples were conditioned in an oven at 60 °C under vacuum overnight. Measurements were performed at room temperature and atmospheric pressure.

4.3.5 Uniaxial Tensile Test

Tensile tests were performed on type 5A specimens (according to ISO527-2) using the Test dynamometer (model 112, Test GMBH Universal Testing Machine, Germany) equipped with a 2 kN load cell. The specimens were clamped and subjected to a constant tensile speed of 10 mm/min until failure. For each family of specimens, eight replicates were tested to obtain representative statistics of average values of the corresponding mechanical properties. The tests were conducted at room temperature and pressure. From the stress-strain curves, the values of Young's modulus (E), stress at break (σ_B) and elongation at break (ϵ_B) were calculated. Before testing, the samples were conditioned in an oven at 40°C overnight to remove absorbed moisture.

4.3.6 Differential Scanning Calorimetry

The thermal properties of PBS- and PHBH-based materials were evaluated using differential scanning calorimetry (DSC). Calorimetric measurements were performed with a DSC Q100 instrument (TA Instruments, New Castle, DE, USA) and related Universal Analysis software

(version 3.9 A, TA Instruments). For the thermal study of PBS formulations, an analysis of three dynamic temperature cycles was programmed. Each sample was heated from -60 to 150 °C (1st cycle), then cooled from 150 to -60 °C and kept isothermal for 5 minutes. This phase was programmed to remove the thermal history. Subsequently, the sample was heated to 150 °C. The same was done for the PHBH formulations, reaching a temperature of 180 °C due to the higher melting temperature compared to the PBS polymer. All thermal analyses were conducted at a heating/cooling rate of 10 K min⁻¹, in a nitrogen atmosphere (50 mL min⁻¹), using an average sample weight of approximately 10 mg, placed in aluminum crucibles. The analyses were carried out in triplicate, and the melting temperature (T_m), melting enthalpy (ΔH_f) and crystallization temperature (T_g) were determined in the second heating cycle. The crystallization temperature (T_c) was determined in the cooling phase of the first cycle.

The degree of crystallization (% χ_c) was calculated with the following equation 4.1:

$$\% \chi_c = \frac{\Delta H_m}{\Delta H_m^0 * (1 - w_{add})} * 100$$

Equation 4.10 - Equation for calculating the degree of crystallinity (% χ_c)

where ΔH_m is the enthalpy of fusion (J/g) obtained from DSC measurement; ΔH_m^0 is the enthalpy of fusion at 100% crystallinity, reported as 110.5 J/g for PBS [9] and 146 J/g for PHBH [10]; w_{add} indicates the weight fraction of the polymer matrix in the biocomposite.

4.4 Results and Discussions

4.4.1 Particle Size Analysis of the BSG Biofiller

Following the grinding and sieving process, the BSG biofiller was characterized from the perspective of particle size distribution. Particle size distribution is observed to have a direct influence on the final properties of a biocomposite material [11]. Smaller particles, as described in the literature [12], offer a greater specific surface area, improving interaction with the matrix and allowing for a more homogenous distribution, resulting in improvements in the mechanical performance of the composite. Fig. 4.9 shows the size distribution of the BSG filler with a monomodal curve, with the relative fraction values shown in Tab. 4.10. The

parameter D_{mean} indicates the volume weighed average particle size, which is approximately 37 μm for BSG.

90% of the particles mechanically sieved at 50 μm had an effective size of less than $70.6 \pm 0.2 \mu\text{m}$, while 10% of the particles had a diameter of less than $9.3 \pm 0.2 \mu\text{m}$ (Tab. 11). These data confirm the presence of heterogeneous particles, which is in line with previous observations on biomass-derived fillers, as pointed out by Aguado et al. [13], where the wide size distribution was related to the fibrous nature of the material and the milling process used. The observed heterogeneity in particle size could be explained by the presence of fibres with non-uniform shape ratios. Indeed, fibres with a diameter of less than 50 μm but with a significantly longer length may stack up during sieving, passing through the sieve holes despite their total length exceeding the expected size. This behavior has also been reported in studies on natural fibres such as microfibrillated cellulose where the stacking effect significantly influenced the size distribution [14]. Such dimensional characteristics could influence the mechanical properties and rheology of the composite, as highlighted by Lim et al.[15], where dispersion and homogeneity of filler distribution were identified as key factors in optimizing the performance of the final material.

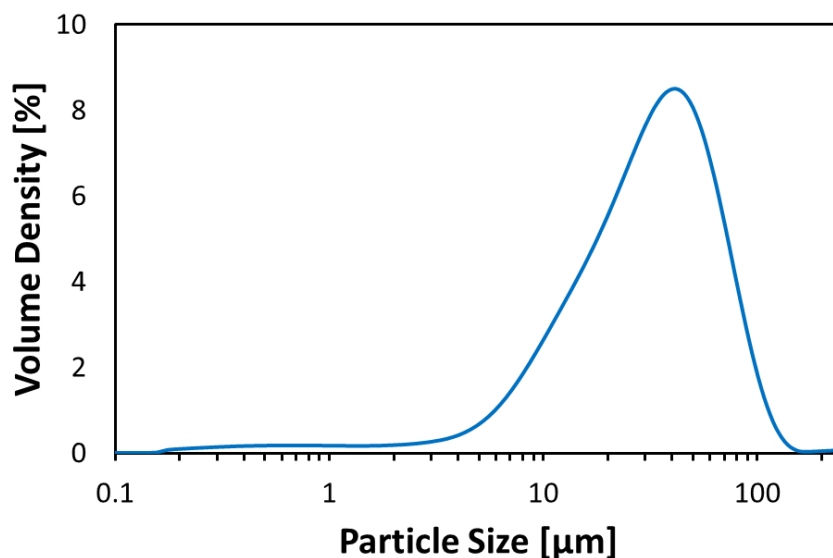


Figure 4.54 - Particle size distribution of BSG filler after pulverisation by mill and sieving

Table 4.31 - Main fractions of the BSG filler

Sample name	D _x (10) [μm]	D _x (50) [μm]	D _x (90) [μm]	D _{mean} [μm]
BSG	9.3±0.2	31.9±0.01	70.6±0.2	36.5±0.2

4.4.2 FT-IR Analysis of BSG Biofiller

Fig. 4.10 shows the full FT-IR spectra of the BSG filler obtained from the milling process in comparison with native BSG. The spectrum is consistent with those typical of lignocellulosic materials, as described in the previous *Chapter 3*. The detailed description of the signals is given in Tab. 4.11. Even after repeated thermal and mechanical grinding treatments, BSG exhibit the same signals than pristine material. This result is indicative that the fibrous lignocellulosic component of the BSG has been qualitatively preserved.

Table 4.32 - Typical signals of BSG biofiller

Code	Wavenumber [cm ⁻¹]	Signal name
a	3680-3000	Stretching vibrations of hydroxyl groups (-OH) [16–18]
b	2990-2830	Symmetric and asymmetric stretching vibrations of the C-H bonds in the methyl and methylene groups [19–21]
c	1730	Stretching of carbonyl group (C=O) which is characteristic of the lignocellulosic system [22,23]
d	1634	Asymmetric and symmetric bending of the N-H amine group derived from proteins [24–26]
e	1518	Stretching vibrations of the aromatic C=C bonds [25,26]
f	1470-1300	Bending vibrations of the C-H hydrocarbon chain [23]
g	1280-1190	Stretching C-O associated with the acetyl groups in hemicellulose[27]
h	1030	C-OH functional group of cell wall polysaccharides [28,29]
i	890	C-O-C stretching [29]

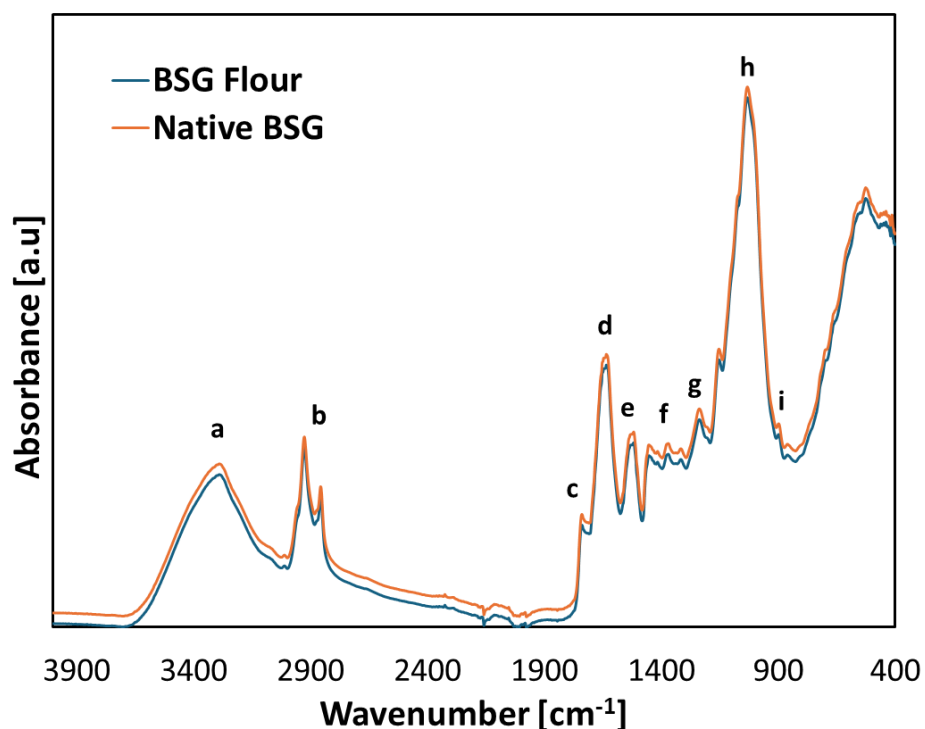


Figure 4.55 - FT-IR spectrum of: flour BSG following the milling process (blue) and native BSG (orange)

4.4.3 FT-IR Analysis of the GT bioplasticizer

The reaction progress and characterization of the GT bioplasticizer obtained by esterification between GLY and LA in the absence of solvents were monitored and studied using FT-IR spectroscopy. A large excess of LA (LA/GLY molar ratio equal to 6) was used to increase the selectivity of the desired product, since with stoichiometric amounts of LA and GLY, the reaction may lead to undesired ketalization reactions [30].

Fig. 4.11 shows the two reaction reagents (GLY and LA), as well as the reaction mixture at t_0 and the final product GT. The absorption band in the range $3600\text{--}3000\text{ cm}^{-1}$ in the GLY related to the stretching of O-H bonds is shown. LA is characterized by the signal at 1713 cm^{-1} ascribable to the carbonyl in ketone group that does not participate to the esterification reaction (Fig. 4.11). The decrease in the intensity of the characteristic signal of the -OH group is significant for the formation of the ester bond. Furthermore, the substitution of the -OH groups of glycerol for the formation of the ester bond can be evidenced by the almost total reduction of the signal at 1150 cm^{-1} inherent in the C-OH groups. An additional signal

characteristic for studying the progress of the reaction is related to the formation of the ester bond at 1733 cm^{-1} (Fig. 4.12).

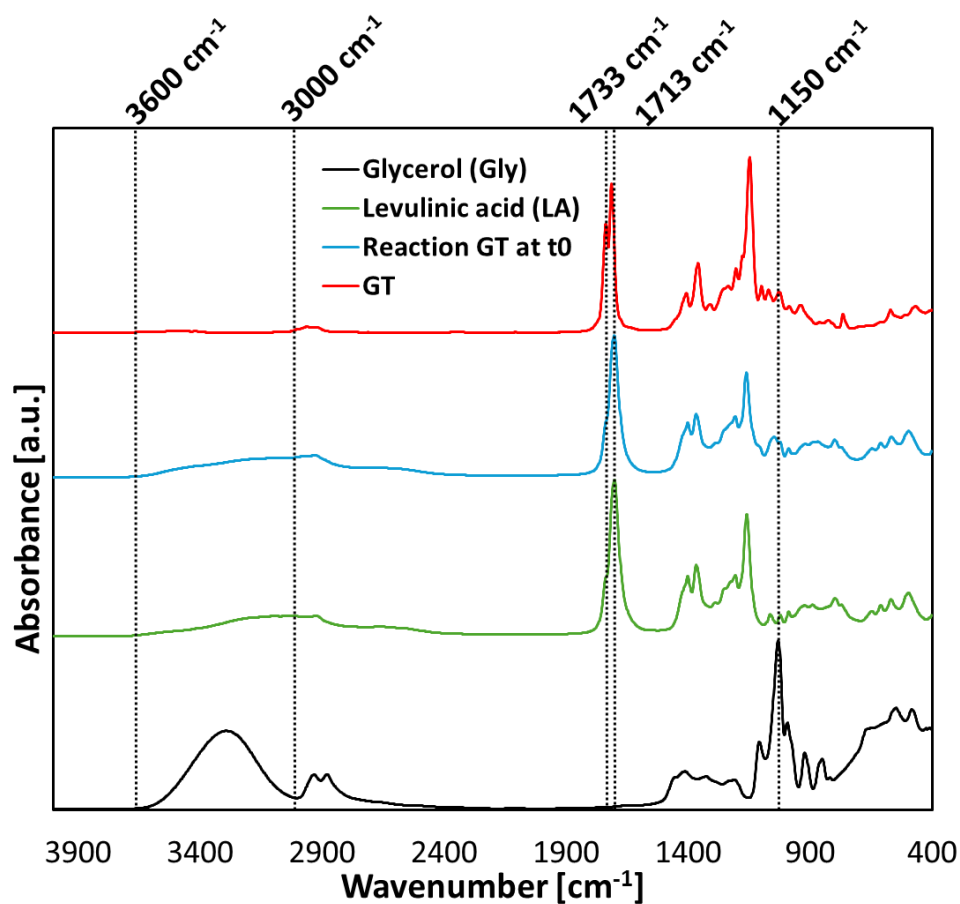


Figure 4.56 - Comparison of the FT-IR spectra of the reagents, the reaction mixture at t_0 , and the final product GT. Dashed lines indicate the areas discussed in the paragraph

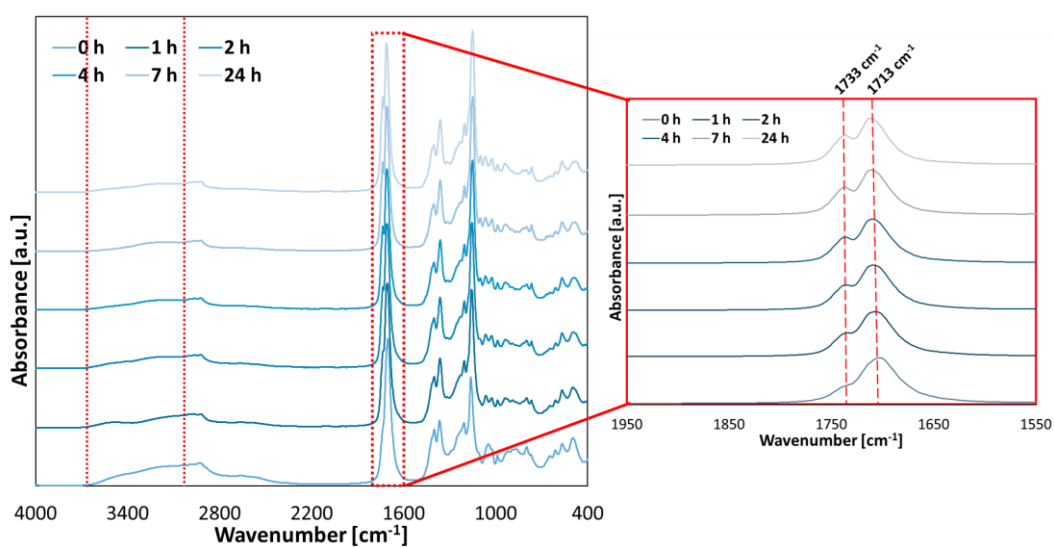


Figure 4.57 - FT-IR spectra of the reaction mixture at different time intervals. Dashed lines indicate signals associated with ester bond formation

4.4.4 FT-IR Analysis of PBS- and PHBH-based Composites

Through FT-IR analysis, the potential changes in the chemical structure of PBS and PHBH biopolymer matrices mixed to BSG biofiller and GT plasticizer for making biocomposite materials were evaluated. Fig. 4.13 shows the FT-IR spectra of the PBS and PHBH biopolymer matrices compared to the composite blends (Fig. 4.13 (a,c), respectively). The spectra for the pure PBS and PHBH show the characteristic signals found in the spectra reported in *Chapter 3* (Section 3.4.5).

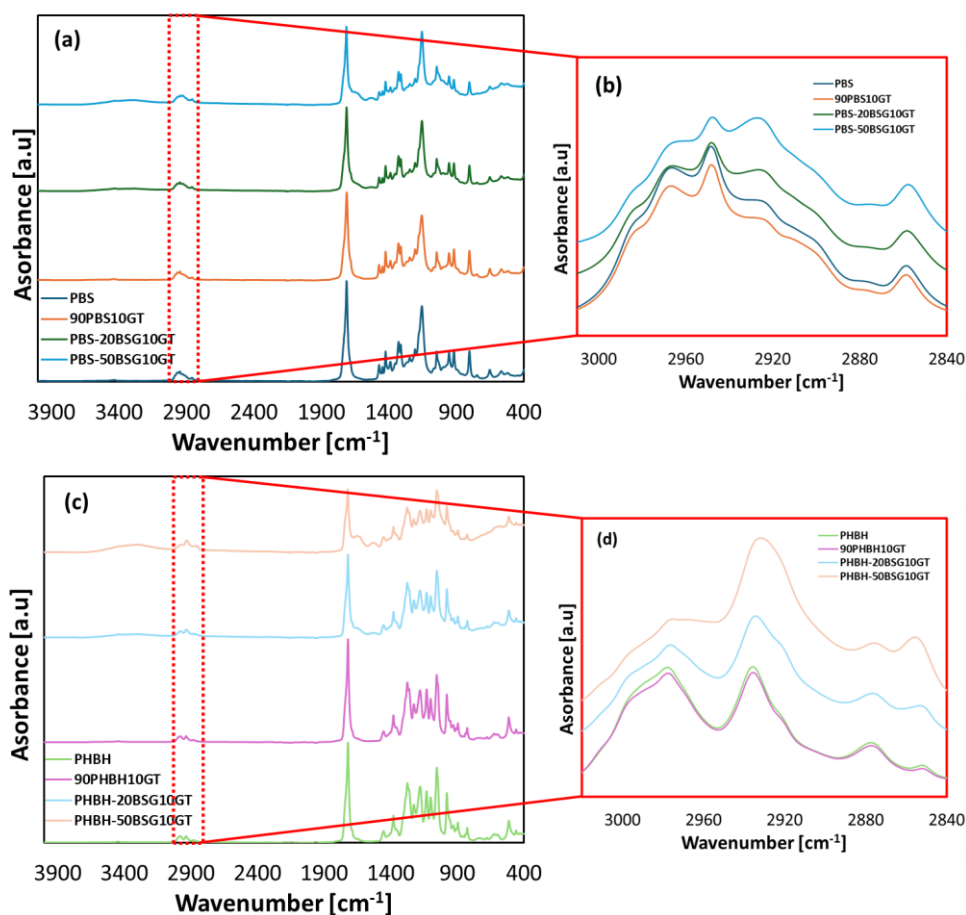


Figure 4.58 - FT-IR spectra of: a) PBS and PBS-based composites and b) relative magnifications of the band at 3000-2840 cm⁻¹; c) PHBH and PHBH-based composites and d) relative magnifications of the band at 3000-2840 cm⁻¹

The various polymer-filler-plasticizer formulations do not exhibit variations in the frequency of characteristic peaks compared to pure polymer matrices. Therefore, they share similar

functional groups and vibrational characteristics, indicating the absence of great chemical interactions between the matrix, filler and GT plasticizer. In the 90PBS10GT sample there is no appearance in the stretching region of the absorption band characteristic of the -OH group ($3600\text{--}3000\text{ cm}^{-1}$)(Fig. 4.13 (a,c)). In contrast in the composites containing BSG biofiller, an increase in intensity is observed in the same area as the BSG charge increases. The characteristic signal of the carbonyl group (C=O) (1714 cm^{-1} for PBS-based composites and 1720 cm^{-1} for PHBH-based composites) does not show signal changes. In the fingerprint region of spectra for the biocomposite materials ($1500\text{--}400\text{ cm}^{-1}$), an increase in absolute absorbance is observed as the BSG content increases. In Figg. 4.13 (b,d), magnifications of the $3000\text{--}2840\text{ cm}^{-1}$ area related to the characteristic signals of symmetric and asymmetric stretching of the C-H bonds of methyl and methylene groups are shown, and it can be seen that as BSG content increases, an increase in intensity of the signals is observed at about 2930 and 2857 cm^{-1} in all composite materials, indicative of the BSG presence.

4.4.5 MFR and GPC analysis of PBS- and PHBH-based Composite

The study of the flow behavior of composite materials and related pure polymers was conducted by measuring MFR and correlated with GPC measurements to determine the changes in molar masses as a function of different BSG and GT plasticizer content. The results for the pure polymer matrices and their biocomposites are depicted in Figg. 4.14 and 4.15, respectively.

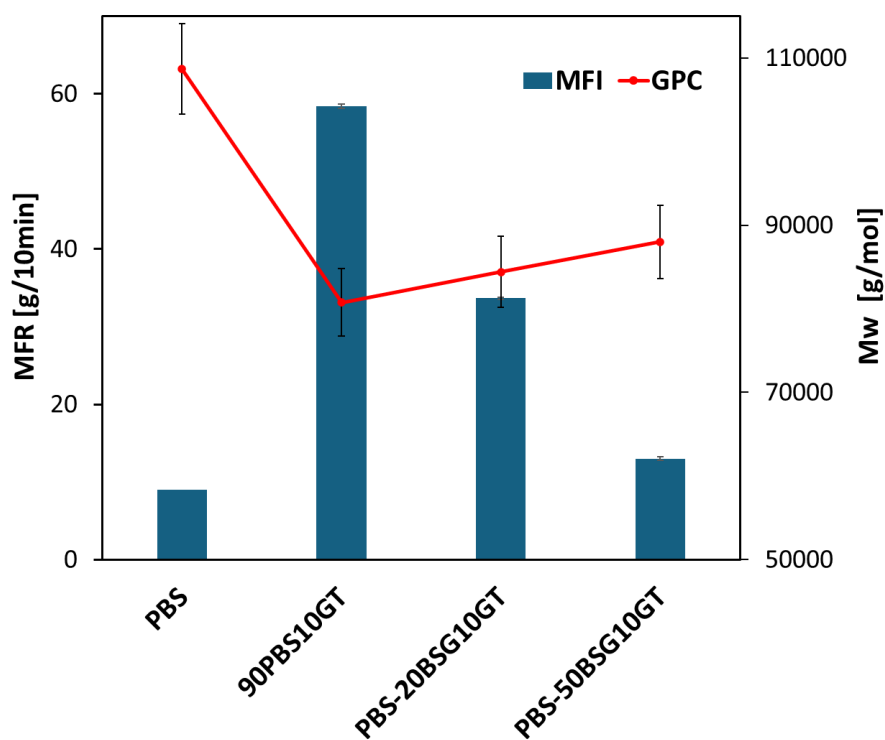


Figure 4.59 - Comparison of MFR and SEC values of PBS-based composites according to different content of BSG filler and GT plasticizer

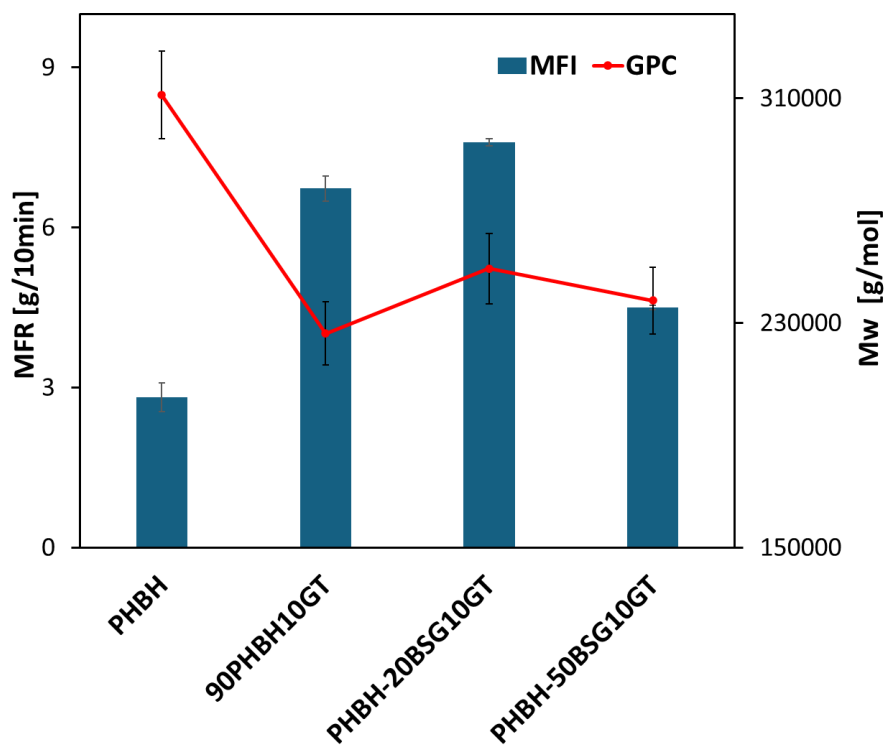


Figure 4.60 - Comparison of MFR and SEC values of PHBH-based composites according to different content of BSG filler and GT plasticizer

For PBS and PHBH, the addition of 10 wt.% of GT plasticizer induced a clear plasticizing effect, as it promotes the sliding of polymer chains, leading to a 6- and 2.5-fold increase in the MFR value, respectively. This behavior is typical of plasticized systems, as shown in other studies on semicrystalline polymers, where the addition of plasticizers showed a significant increase in the mobility of polymer chains [31,32]. In relation to the MFR results, the 90PBS10GT and 90PHBH10GT blends showed a reduction in molecular weight (M_w) compared to the pure polymers, a phenomenon that could be associated with GT plasticizer-inducing molecular fragmentation [33]. In fact, the bioplasticiser used has an experimental formulation and its thermo-chemical stability may require further optimization: it could therefore be assumed that exchange and trans-esterification reactions occur during extrusion between the GT components and the polymer chains of PBS or PHBH, resulting in a change in polymer chain length. The same phenomenon was presented for all composites, which exhibited significantly lower M_w than the those of pure PBS and PHBH. In the composites with 18.5% BSG filler, two distinct behaviors were found. In the PBS-20BSG10GT composite, there was a decrease in MFR compared to the 90PBS10GT blend, but still an approximately 4-fold increase compared to the pure polymer matrix (34 g/10 min vs. 9 g/10 min). This phenomenon, which is typical in composites with natural fillers, is attributed to the introduction of a stiff filler such as BSG into the polymer matrix, which limits the sliding of the polymer chains, as reported by Hejna et al. [34]. In contrast, the PHBH-20BSG10GT composite showed an increase in MFR (8 g/10 min), compared to both the 90PHBH10GT blend (7 g/10 min) and pure PHBH (3 g/10 min), suggesting greater flowability. The introduction of about 50 percent BSG resulted in a significant reduction in MFR, confirming that a higher percentage of filler reduces the overall fluidity of the system, in agreement with Hejna et al. [34]. In this context, GT plasticizer mitigated the effect of filler, resulting in higher MFR values than pure matrices, unlike previous work where the introduction of the BSG filler alone caused MFR values to decrease especially for PBS-based composites.

4.4.6 UTT of PBS- and PHBH-based Composites

The mechanical properties of different materials were evaluated by uniaxial tensile test (UTT). (Fig. 4.16). The addition of GT plasticizer to PBS and PHBH caused a decrease in E and a slight decrease in σ_B (Figg. 4.16 (a,b,c,d)). Specifically, the addition of GT caused a decrease in

stiffness of 26 % (Fig. 4.16 (a)) and 36 % compared to pristine PHBH (Fig. 4.16 (b)), with a discrete decrease in σ_B of 15 % (Fig. 4.16 (c)) and 24 % (Fig. 4.16 (d)) in the 90PBS10GT and 90PHBH10GT blends, respectively. An improvement of 28 % (Fig. 4.16 (e)) and 61 % (Fig. 4.16 (f)) was observed for ε_B of both the PBS and PHBH respectively. This represents a remarkable achievement from the point of view of the mechanical performance of these two biopolymers to be able to reduce the present gap with conventional polymers. Regarding the mechanical behavior of biocomposite blends, distinct behavior can be observed for the two biopolymer matrices. The introduction of about 18 wt.% BSG to the 90PBS10GT blend showed an increase in E compared to the blend with GT alone (Fig. 4.16 (a)). This effect is due to the introduction of a rigid filler with a higher E than the polymer matrix, and the effect is accentuated with the introduction of 50 wt.% BSG, with 55 % increase in E compared to the pure polymer matrix. The same behavior was noticed in PHBH-based biocomposites where there was an increase in E compared to the 90PHBH10GT but remaining at values below the matrix (Fig. 4.16 (b)). The σ_B and ε_B of both biopolymer blends decreased as the BSG filler increased due to a greater impediment of the chain movement and thus leading to early failure of the specimen during the mechanical test (Fig. 4.16 (c,d,e,f)).

In general, however, the introduction of GT plasticizer and BSG powder within the polymer matrices led to a decrease in E compared to the previously analyzed composites with BSG. Specifically for the PBS-based composites, the PBS50BSG50 sample ($E = 1300$ MPa) showed a 100 % increase in E compared to PBS ($E = 647$ MPa), in contrast to the composite containing 10 wt.% GT plasticizer (PBS-50BSG10GT, $E = 1004$ MPa), which showed only a 55 % increase in E compared to the pure polymer matrix ($E = 647$ MPa). The same can be observed for the PHBH-based composites, where the PHBH50BSG50 sample ($E = 1720$ MPa) showed an 11 % increased E value compared to pure PHBH ($E = 1547$ MPa), unlike the composite with 10 wt.% of GT plasticizer (PHBH-50BSG10GT, $E = 1284$ MPa), which showed a clear decrease in E of 17 %. For the plasticized composites, σ_B also decreased significantly compared to the composites containing only BSG filler at 50 wt.%. It is observed that the PBS-50BSG10GT composite exhibits a σ_B 53 % lower than the value recorded for the PBS50BSG50. The same behavior was observed for the PHBH-based composite, where the PHBH-50BSG10GT sample reported a σ_B reduced by 42 % compared to the σ_B of the 50PHBH50BSG. In contrast to the findings for E

and σ_B , there were no notable differences regarding ϵ_B among the different composite samples containing 50 wt.% BSG, probably due to the non optimal amount of GT plasticizer.

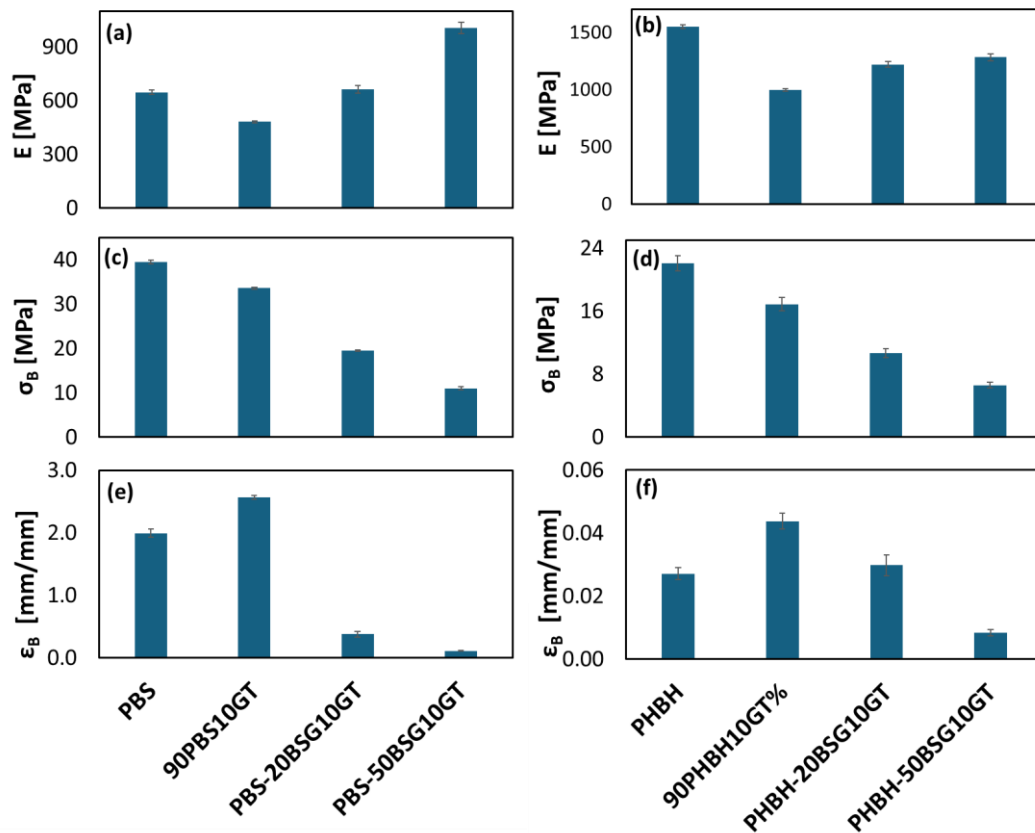


Figure 4.61 - Representation of the results inherent in the mechanical tests performed on the PBS and PHBH samples and their mixtures: a,b) elastic modulus (E), c,d) tensile strength (σ_B) and e,f) elongation at break (ϵ_B)

4.4.7 DSC Analysis of PBS- and PHBH-based Composites

The thermal characteristics of the two polymer matrices were examined by DSC analysis, and Fig. 4.17 shows the thermograms of the heating and cooling scans. The addition of GT plasticizer induced significant changes regarding the thermal properties of the produced blends as reported in Fig. 4.18.

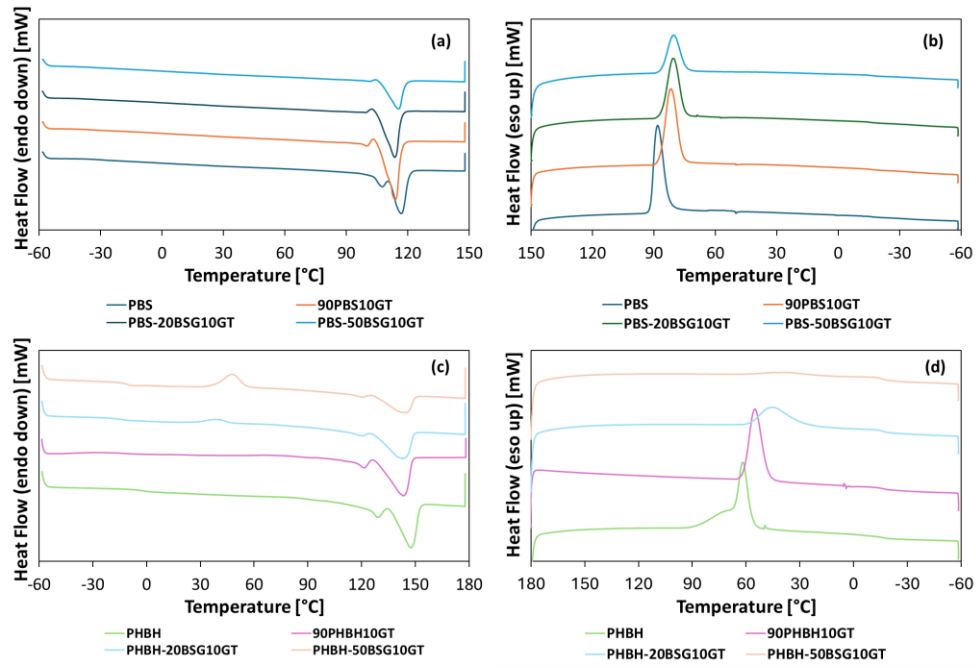


Figure 4.62 – Thermograms obtained from DSC curves: (a) second heating curve of PBS-based composite samples; (b) cooling curve of PBS-based composite samples; (c) second heating curve of PHBH-based composite samples and (d) cooling curve of PHBH-based composite samples

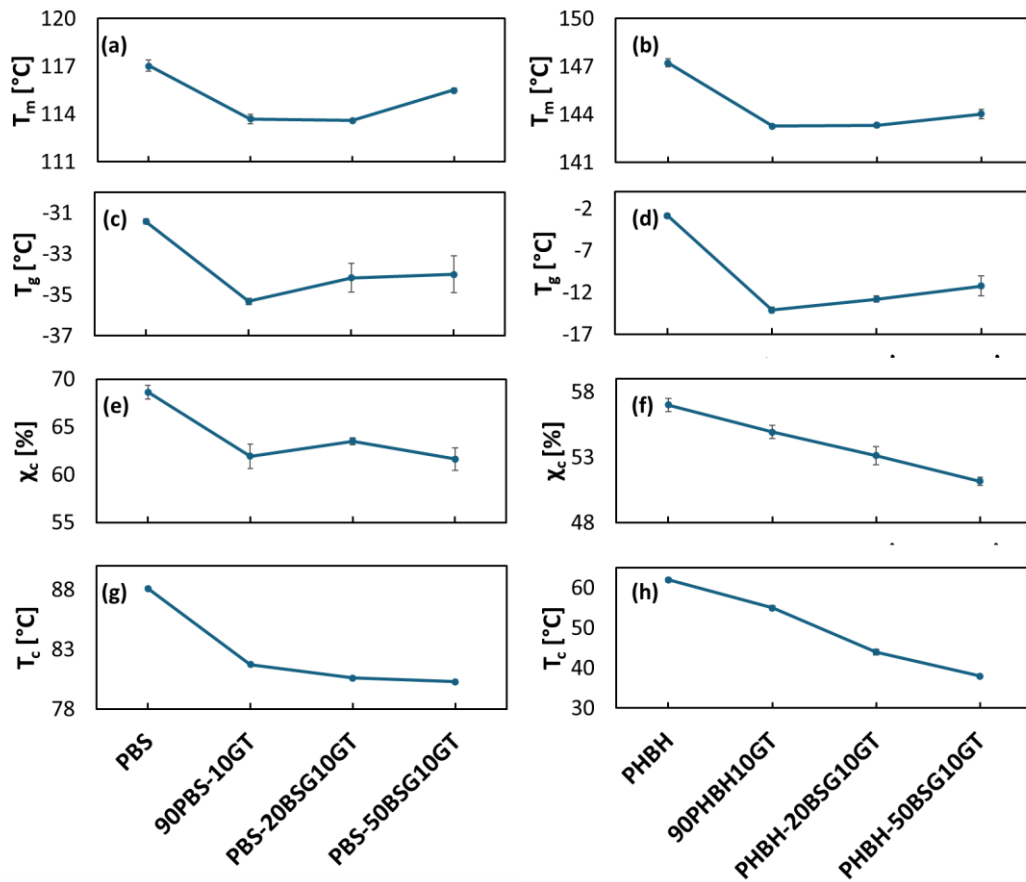


Figure 4.63 – (a,b) Melting temperature (T_m), (c,d) glass transition temperature (T_g), (e,f) degree of crystallinity ($\% \chi_c$) and (g,h) crystallization temperature of pure PBS and PHBH polymers, and related biopolymer blends

Regarding T_m , an essential parameter for studying the optimal processing conditions of materials in the fluid state, the addition of GT plasticizer at 10 wt.% led to a decrease of 3 and 4 °C compared to pure polymers PBS (117 °C) and PHBH (147 °C), respectively (Fig. 4.18 (a,b)). This decrease is maintained even with the addition of filler (116 and 144 °C, for PBS-50BSG10GT and PHBH-50BSG10GT respectively), highlighting the effect of GT plasticizer even after the addition of BSG. The decrease in T_m , albeit by a few degrees Celsius, may be a positive effect when considering the reduced window of processability of biopolymers in the molten state, especially for the PHA family. The addition of the GT plasticizer promotes increased mobility of the polymer chains by reducing the interaction forces between the functional groups of the macromolecules. In addition, increased macromolecular fragmentation may also be present, as evidenced by GPC measurements. These effects are visible not only in the crystalline fraction, with the decrease in T_m , but also in the amorphous fraction with the change in T_g : the T_g reduction in the mixtures containing the GT plasticizer at 10 wt.% is shown in Figures 4.18 (c,d). Specifically, the T_g of PBS of -31 °C and PHBH of -3 °C decreased by 4°C and 11°C with only 10 wt.% GT. The DSC curves were further processed to evaluate the effect of GT on the $\% \chi_c$ of the produced blends compared to the pure polymer matrices (Fig. 4.18 (e,f)). For both PBS and PHBH, a decrease in $\% \chi_c$ was observed. For PBS-based blends, $\% \chi_c$ decreases from 69 to 62 % (Fig. 4.18 (e)), while for PHBH-based blends, $\% \chi_c$ decreases from 57 to 55 % for the 90PHBH10GT, to 51 % for the PHBH-50BSG10GT (Fig. 4.18 (f)). Similarly, the plasticizing effect of GT was also evidenced during the cooling scan, as shown in Fig. 4.18 (g,h). The crystallization peaks of the formulations shifted to lower temperatures with the introduction of GT. Specifically, the T_c of PBS decreased from 88 to 82 °C, undergoing a further decrease in ΔT_c of 2 °C for the PBS-50BSG10GT biocomposite (Fig. 4.18 (g)). Similar behavior was reported for the T_c of PHBH, which decreased from 62 to 55 °C with the addition of GT (Fig. 4.18 (h)). However, with the BSG addition at 20 and 50 wt.%, the T_c decreased by 18 and 24 °C for the PHBH-based biocomposite materials.

4.5 Conclusions and Future Perspectives

The growing interest in the use of biopolymer matrices to replace conventional plastics has driven research toward the development of new additives, to improve their mechanical and thermal properties, and enable a broader range of applications.

This research work examined the blending by melt compounding of PBS and PHBH with 10wt% GT bioplasticizer synthesized from levulinic acid and glycerol. In addition, composite materials containing the BSG filler at two different concentrations (20 and 50 wt.%) were also made. Therefore, the presented research aims to analyze the potential impact of GT and BSG on the composite properties by rheological, structural, thermal and mechanical analyses.

For the milling of the BSG filler, the method adopted resulted in a D_{mean} less than 40 μm compared to previous work where a D_{mean} around 110 μm was obtained. Furthermore, comparison of native and milled BSG by FT-IR analysis showed that the lignocellulosic component of the filler remained qualitatively unchanged, highlighting the effectiveness of the treatment.

Regarding the introduction of the GT bioplasticizer to both pure polymer matrices PBS and PHBH, a decrease in E and σ_B for both polymer matrices was observed from the mechanical test results, in relation to an increase in ε_B of 28 % for PBS and 61 % for PHBH. From the point of view of thermal properties, GT was also an effective plasticizer, lowering the melting, glass transition and crystallization temperatures of the two polymer matrices, as well as the degrees of crystallinity, due to an increase in the mobility of the polymer chains. This represents a remarkable achievement from the perspective of the mechanical and thermal performance of these two biopolymers, which can reduce the current gap with conventional polymers. As expected, the introduction of the BSG biofiller resulted in an increase in E and σ_B in composite formulations and a decrease in maximum elongation compared to the respective polymers containing 10 wt.% GT plasticizer. When compared to the composite samples without plasticizer, a decrease in E and σ_B was observed due to the addition of the plasticizer itself, with the exception of elongation at break, which remained unchanged. Regarding the thermal properties the decrease in T_m , T_g and crystallization when compared to the pure polymer matrices were also highlighted. This effect is much more evident for PHBH-based composite samples.

These effects were also found in the processing of different formulations, with a reduction in temperatures of 13 °C and different residence times in the extrusion chamber for both polymer matrices. However, no statistically significant changes in molecular weight are observed in pure polymers under varying process conditions. The reduction in the molecular weights of the matrices was evidenced by results obtained by gel permeation chromatography (GPC) for the blends containing the GT bioplasticizer and the related biocomposites containing the BSG biofiller. This evidence suggests the absence of a direct correlation between process parameters and changes in the molecular weight of the polymer matrices.

An almost similar influence on the MFR of both PBS and PHBH biopolymer matrices with the addition of GT at 10 wt.% is noted, with increases of 6- and 2.5-fold, respectively, due to improved mobility of the polymer chains and possible molecular fragmentation induced by the plasticizer due to its thermo-chemical instability, as evidenced by the GPC results. For composites containing 18.5 and 50 wt.% BSG and 10 wt.% GT, a reduction in MFR values was observed when compared with blends containing only GT, with the exception of the PHBH-20BSG10GT composite. When considered with respect to pure polymer matrices, MFR values increase, thus highlighting the mitigation of the stiffening effect due to the filler introduction.

The structural properties investigated by FT-IR analysis showed no changes in the polymer structure but this does not exclude the presence of entanglement between the various constituents of the composite material.

Rheological and thermal characterization tests led to the identification of optimal parameters, which were then used to fabricate by thermoforming the multilayer composite films. Thermoforming represents an additional conventional processing technique that could also be exploited for these materials thus leading to an increase in the possible applications of the two different biopolymer matrices. However, comprehensive and detailed analyses of the newly produced materials are essential to accurately assess their structural, thermal, and oxygen and water permeability variations and thus be able to ensure their possible market application. The films produced are of potential importance for a wide range of applications, particularly the packaging industry.

In conclusion, GT is a viable alternative to traditional commercial plasticizers for biopolyesters, and at the same time the introduction of BSG filler results in a decrease in prices and weight

of the final composite. As already mentioned, the main problem of incorporating biofillers into polymer matrices is the drastic change in mechanical properties, especially when the compatibility between matrix and filler is limited. Although differences for different formulations were observed by DSC and UTT, the overall performance still indicates low interactions at particle-polymer interfaces. Based on these results, future developments could focus on optimizing the GT plasticizer concentration and at the same time introducing compatibilizers to improve the properties of the final composite materials.

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Chapter 5 – Conclusions and Future Prospects

This thesis project focused on the design, production and characterization of new biocomposite materials developed using biopolymer matrices of proven scientific and industrial interest, such as poly(butylene adipate-co-terephthalate) (PBAT), poly(butylene succinate) (PBS) and poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBH). Two distinct types of lignocellulosic fillers were added to these matrices: hemp flour (HF) and brewers' spent grain (BSG). In addition, a naturally derived bioplasticizer (GT) was also integrated to investigate an additional class of biocomposites.

The main objective of the work was to address environmental issues related to the high dependence on conventional plastics, which are mainly produced from fossil resources, and whose overproduction and inadequate disposal pose a significant threat to the environment and human health. According to recent studies, the lack of environmental degradation of conventional plastics, combined with deficiencies in recycling infrastructure, contributes substantially to global pollution.

In the context of the transition to a circular economy, biopolymers represent a promising alternative to fossil-based plastics. However, their large-scale use is still limited due to high costs, the often inferior thermo-mechanical properties compared to traditional materials, and the difficulties associated with their integration into established industrial production processes. This project aims to overcome these limitations through the development of biocomposites that combine biopolymers, lignocellulosic fillers and a bioplasticizer, resulting in materials with performance comparable to conventional polymers but with reduced environmental impact.

In particular, the use of hemp flour (HF) and brewing residues (BSG) as fillers was motivated by their inherent properties. Hemp flour is rich in cellulose, hemicelluloses and lignin, making it an effective mechanical reinforcement. BSGs, on the other hand, are abundant by-products of the brewing industry, consisting mainly of lignocellulosic fibers and proteins, making them a sustainable and cost-effective resource. In addition, the GT bioplasticizer showed optimal intrinsic properties as a possible substitute for traditional plasticizers.

Through an innovative approach, the resulting biocomposites have been shown to possess thermo-mechanical and chemical-physical properties comparable to those reported in the literature for similar materials. However, the used preparation method was distinguished by its simplicity and compatibility with conventional processing techniques, such as extrusion, injection molding, and thermoforming. This feature makes such biocomposites easily adaptable to industrial manufacturing processes without the need for significant modifications to existing facilities, thus facilitating an eventual transition to large-scale production.

In addition, the incorporation of lignocellulosic fillers and the GT bioplasticizer helped not only to improve some mechanical properties of the materials, but also to reduce the overall cost

of biopolymer formulations, making these materials competitive from an economic point of view as well. This achievement is of particular relevance in light of the current challenges in the field of sustainable plastics, which requires high-performance, low-cost materials to promote their large-scale adoption.

The work done represents a significant step toward the development of sustainable biocomposite materials with industrial applicability. The combination of biopolymer matrices and lignocellulosic fillers not only offers an effective solution to reduce dependence on fossil-derived polymers, but also promotes the reuse of agricultural and industrial by-products, thus contributing to a more sustainable circular economy.

Further studies could focus on optimizing the interactions between biopolymers and lignocellulosic fillers, such as through surface treatments of the fillers or chemical modifications of the matrices, to further improve the mechanical properties and thermal stability of the biocomposites.

Chapter 6 – Period of in-company research

During my doctoral studies, I engaged in a period of professional experience at Taghleef Industries Spa, a leading manufacturer of biaxially oriented polypropylene (BOPP) and biaxially oriented polylactic acid (BOPLA) films, operating in various fields, including flexible packaging for food and non-food applications, labels, and graphic arts.

Within the company, I had the opportunity to participate in a project related to the NATIVIA® brand of biobased and biodegradable films. The focus of this project was on the development of new multilayer films based on PLA blended with different biopolymer matrices, such as PBS, PBSA, PCL, and different types of PHA.

In order to familiarize myself with the subject, I first undertook a thorough literature review of PLA-based blends, which also led me to the scientific publication entitled "Poly (lactic acid) and its blends for packaging application: a review".

Subsequently, under the supervision of the Research and Development Department of Taghleef Industries, I was involved in the characterization of polymer matrices by using different types of instruments useful for studying various properties of polymer matrices. Due to the confidentiality of the results, it was not possible to include the results in the thesis. The company was interested in developing an ad-hoc material for immediate commercialization. Therefore, the activities carried out will be described.

The experimental activities encompassed the utilization of various characterization instruments, including:

1. Moisture content analysis, conducted using the Vapor Pro®.
2. Structural analysis, by Fourier-transform infrared spectroscopy (FT-IR).
3. Thermal analysis, by differential scanning calorimetry (DSC).
4. Material fluidity study, by the measurement of the Melt-mass Flow Rate (MFR).

After the characterization analyses of the various biopolymer matrices, a series of extrusion line tests were conducted at Taghleef Industries Spa.

After this, the same analyses listed above were repeated on the produced films to study how and to what extent the added polymer matrix behaved within the PLA, and whether or not there were any interactions between the biopolymer matrices. A series of mechanical tests

were performed on the produced films. These tests included the measurement of weldability at various temperatures, weld tightness, and thermal shrinkage at different temperatures.

I was able to learn the different stages of the production process on the different types of films produced by the company such as the extrusion and bi-orientation stage, then moving on to the stages following the making of the parent reel i.e. the slitting, metallization and coating areas.

In addition, I was able to learn the performance of various analysis and characterization tests under the supervision of the quality and control department. The main purpose was to draw up a technical document where reporting all the properties of the different types of films produced, useful both for tracking production progress and for gathering technical information to present to the buyer of the product. The analyses consisted of:

- Thickness measurement by an internal method depending on the type of film (cavitated or non-cavitated).
- Measurement of optical properties:
 - haze, useful for measuring scattered light i.e., transparency or otherwise of a film and observing the presence or absence of polymer flow defects;
 - gloss, related to the surface of the film i.e., the measure of how much light is reflected at an angle equal to that of the incident light (company standard of 45°);
 - optical density on metallized films, a parameter related to the thickness of the aluminum layer and, consequently, film barrier properties.
- Measurement of the coefficient of friction (C.O.F.) that provides an indication of the sliding resistance of two surfaces brought into contact with each other. The test consists of 150 mm of travel, in which a slide (having a mass of 200 g) is placed on the two surfaces in contact with each other. In the first 25 mm, the peak static C.O.F. is measured, and in the next 125 mm, the dynamic C.O.F. is measured, that is, the average measuring force of the cell useful for keeping the film in motion.
- A measure of the treatment level related to the receptivity of the film surface to inks, coatings, and adhesives. PP is mainly non-wettable as it is not polar substance, and therefore corona or flame treatment is carried out on the film to increase wettability by introducing polar species on the surface. The same is carried out on PLA-based films

to improve the wettability of the films. The verification analysis consists of applying liquids with different surface tension (mN/m) to the treated surface of the film. The measurement of the treatment level is done by going to spread a liquid, with a known surface tension value, on the surface of the film. If it maintains the spreading form for at least 3 seconds without retracting into droplets, the film possesses a treatment level corresponding to the surface tension of that liquid.

- Measurement of mechanical properties: the behavior of a film sample subjected to a tensile force is measured. The following properties were evaluated: Young's modulus, elongation at break, and tensile strength. The puncture resistance analysis was also performed. The latter measurement turns out to be important for biaxially oriented films since the orientation process induces some weakness in terms of the puncture resistance.
- Thermal shrinkage analysis, useful for measuring the dimensional stability in both directions of the film orientation. Samples are subjected to a specific heat treatment, and the dimensional change value is expressed as a percentage.
- Sealing strength analysis, in which the maximum force required to open the seal between two surfaces of a film sample is studied. Sealing occurs at defined temperature, pressure and time. In addition, minimum sealing temperature (MST) was also investigated, consisting of the temperature required to reach a minimum acceptable value of seal strength (200g/25mm).
- Analysis of water vapor barrier properties (WVTR) and oxygen barrier properties (OTR). WVTR is the permeation of water vapor through the film under specific conditions of temperature and relative humidity and is expressed in $\text{g}/(\text{m}^2\cdot\text{day})$. The OTR is the amount of oxygen that permeates through a unit area of given thickness, in unit time, under specific conditions of temperature and relative humidity, due to a difference in partial pressure between the two film sides and is expressed in $\text{cm}^3/(\text{m}^2\cdot\text{day})$. For nonmetallized films, the value of WVTR and OTR is a function of film thickness unlike metallized films where the barrier is given by metallization.
- Blocking analysis, useful for simulating in the laboratory the maximum pressure to be applied so that the film layers on a reel do not adhere to each other, preventing the unwinding of the reel and its use. The test involves the application of a pressure at a set temperature for a fixed time to films in contact with each other and then separating

the films. The instrument measures the force required to separate the films. The greater the force to be applied, the greater the tendency of the film to block. This result makes it possible to highlight the need to apply anti-blocking agents to the surface.

- Adhesion of aluminum using 3M Scotch® 610 tape.

In addition, during my time at the company, I was able to work alongside the employees of the R&D department, learning their organizational structure and different tasks:

- Overview of regulatory management and main regulations.
- Overview of raw materials management.
- Focus on biobased and biodegradable polymers: properties and characterization.
- Mix/blend/compounds of biobased and biodegradable polymers.
- Technical documentation management.
- Label product range and focus on potential applications for biobased and biodegradable polymers.
- Graphic arts product range and focus on potential applications for biobased and biodegradable polymers.
- Film properties, instruments, and methods overview.

Finally, I was given an overview of the specific markets and applications in which the films are used, the characteristics required of the films to meet the demands of different applications, and finally the development projects in which the company is investing to meet different market needs and expand the range of film characteristics and applications.

Ringraziamenti

A conclusione di questo lavoro di tesi, è doveroso porre i miei più sentiti ringraziamenti alle persone che ho avuto modo di conoscere in questo importante periodo della mia vita e che mi hanno aiutato a crescere sia dal punto di vista professionale che umano. E' difficile in poche righe ricordare tutte le persone che, a vario titolo, hanno contribuito a rendere migliore questo periodo.

Colgo innanzitutto l'occasione per ringraziare il Prof. Daniel Milanese, coordinatore di questa tesi e fonte inesauribile di conoscenza. Inoltre, volevo ringraziare il Prof. Corrado Sciancalepore, per avermi guidato nella stesura di questo lavoro trasmettendomi la passione e l'entusiasmo necessari affinché la tesi prendesse forma giorno dopo giorno e che insieme al Prof. Duccio Gallichi-Nottiani e la Dott.ssa Daiana Grimaldi mi hanno fatto sentire come entrato in una grande famiglia. Un ringraziamento speciale anche alla mia Tutor Olimpia e la mia Prof.ssa Antonella Cavazza da cui è nata questa fantastica avventura.

Inoltre, un ringraziamento speciale va alla mia ragazza, Genny Grasselli, che mi è stata sempre accanto in qualsiasi istante e mi ha dato la forza di andare avanti.

Non possono mancare da questo elenco di ringraziamenti tutte quelle persone con cui ho iniziato e trascorso questi bellissimi tre anni, con le quali ho condiviso momenti indimenticabili, instaurando una sincera amicizia e una profonda collaborazione. Non cito uno ad uno il resto degli amici, perché vi assicuro che sono talmente tanti che ci vorrebbe un'altra tesi, ma sappiate che siete tutti qui. Se ho raggiunto questo traguardo lo devo anche alla vostra continua presenza, per avermi fatto capire che potevo farcela, incoraggiandomi a "non mollare mai". Non so se trovo le parole giuste per ringraziare i miei genitori e la mia famiglia, però vorrei che questo mio traguardo raggiunto, per quanto possibile, fosse un premio anche per loro e per i sacrifici che hanno fatto. Un infinito grazie per esserci sempre, per sostenermi, per avermi insegnato ciò che è "giusto" e ciò che non lo è. Senza di voi certamente non sarei la persona che sono. Grazie per i vostri consigli, per le vostre critiche che mi hanno fatto crescere.

Ricordo per ultimo, ma non per importanza, che grazie alla rinuncia della rinuncia che oggi sono qui a poter festeggiare con voi.

Grazie di cuore a tutti! Stefano.



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