Optimized smart manufacturing of plastic packaging, their characterization and validation in relevant environment

Deliverable 4.2

Author: PROPAGROUP, UDC, IVW

Date: 22 May 2025

Funded by the European Union

Project	
Action Number	GA 101058654
Action Acronym	Waste2BioComp
Action Title	Converting organic waste into sustainable bio-based components

Document	
Document Identifier	D4.2
Due date of delivery to EC	31 May 2025
Actual date of delivery to EC	30 May 2025
Dissemination level	Public
Work package	4

Contributors	
Main Contributors	Davide Crapanzano (PROPAGROUP), Renato Mortera (PROPAGROUP)
Contributors	Barbara Güttler (IVW), Hendrik Hahlbom (IVW), Mª Belén Montero Rodríguez (UDC), Anja Schmidt (UDC), Danny Robert Moncada Villalobos (UDC)

Control Sheet

Version	Date	Editor	Summary of Modifications
0	03/03/2025	Helena Vilaça	Template for the report
1	22/05/2025	Helena Vilaça	Submitted version of the report, with inputs from PROPAGROUP, UDC and IVW

Table of Contents

List of Figures

List of Tables

List of Abbreviations

Acronyms	Description
DE	Diethyl ether
DIN	Deutsches Institut für Normung
DSC	Differential Scanning Calorimetry
EN	European Standard
GA	Grant agreement
ISO	International Organization for Standardization
MPs	Microparticles
MS	Milestone
PBS	Phosphate-buffered saline
PHA	Polyhydroxyalkanoate
PHB	Polyhydroxybutyrate
PHBV	Poly(3-hydroxybutyrate-co-3-hydroxyvalerate)
PLA	Polylactic Acid
SD	Sample Standard Derivation
SD	Standard deviation
SEM	Scanning Electron Microscopy
TGA	Thermogravimetric Analysis
W2BC	**Wast2BioComp**
WP	Work package

1. Introduction

The target of this report was to demonstrate the use of PHAs, developed within the project, for the production of flexible and rigid demonstrators for packaging purpose, namely more than 100 m of a flexible plastic film, and to test their basic characteristics and performance, comparing with fossil-based benchmarks.

This report, together with D4.1, D4.3 and D4.4, show the accomplishment of MS5, with the development of fully compostable flexible and rigid demonstrators for packaging purpose and their validation in relevant environment, using PHAs developed in the project.

2. Optimized smart manufacturing of plastic packaging, their characterization and validation in relevant environment

2.1. Flexible Packaging Demonstrators

2.1.1. Brief description

As part of MS5, fully compostable flexible films for packaging applications have been successfully produced in operational environment with a continuous industrial process using PROPAGROUP's existing blow extrusion line. The demonstrator consists of over 300 meters of continuously extruded PHA-based tubular film, wound onto reels for further characterization.

2.1.2. Materials and extrusion process

The materials used are:

- polyhydroxyalkanoate PHA.K.3.3.1.4 developed by partner HSKL
- commercial Polylactic Acid (PLA) by ADBioplastics – Premium TecnoBi 26

The final film configuration, showed in Figure 1, is a triple-layer structure comprising two thin outer layers of PLA (30% by weight) and a central layer of PHA.K (70% by weight).

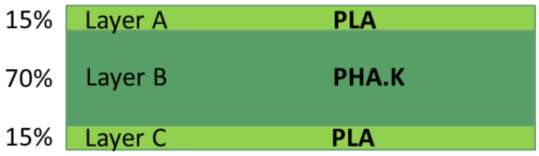


Figure 1 Triple-layer film configuration.

Table 1 summarizes the main processing parameters for the demo production showed in Figure 2.

Table 1 Blow extrusion parameters

Film type	Temperature profile	Screw and production rate	Blow-up ratio
PHA.K 70% wt. **PLA 30% wt.**	165-175 °C	Screws: 25-50 rpm Production: 180 m/h	2 to 2.5

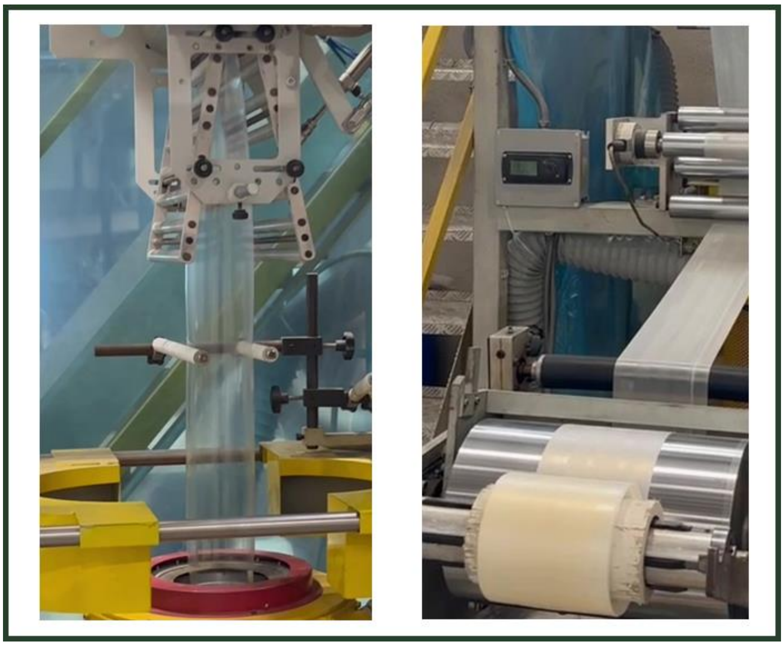


Figure 2 Production of flexible film demonstrator for packaging applications by blow extrusion.

2.1.3. Demonstrator

The pictures below (Figure 3 and Figure 4) shows the final demonstrator for flexible packaging applications.

Figure 3 Flexible packaging demonstrator.

Figure 4 Flexible packaging reels with blow extrusion lines.

2.1.4. Characterization of flexible packaging

The characterization of the flexible packaging films was carried out using Differential Scanning Calorimetry (DSC) and Thermogravimetric Analysis (TGA) to determine the films' thermal properties such as melting temperature, glass transition, and thermal stability. DSC provided insights into the degree of crystallinity and potential changes due to aging or processing. TGA not only revealed the onset of thermal degradation but also allowed for the estimation of the relative content of different polymers based on their characteristic decomposition profiles. In addition, tensile tests were performed to evaluate mechanical properties such as tensile strength and elongation at break according to ISO 527-3. The combination of these methods offers a comprehensive assessment of the structural and functional characteristics of the packaging films.

Table 2 Parameters for the DSC characterization

	Temperature program			
	Start Temperature (°C)	**Heating Rate (K/min)**	**End Temperature (°C)**	**Gas**
Step 1	-30	isotherm 2 min.	-30	N_2
Step 2	-30	10	220	
Step 3	220	-10	-30	
Step 4	-30	isotherm 2 min.	-30	
Step 5	-30	10	220	

Table 3 Parameters for the TGA characterization

Temperature program			
Start Temperature (°C)	**Temperature ramp (K/min)**	**End Temperature (°C)**	**By mass loss reduced temperature ramp (K/min)***
30	10	540	0.5
* Automatic adjustment of heating rate when mass loss is detected			

Figure 5 presents the DSC cooling graph of the PHA/PLA film, while Figure 6 shows the DSC heat flow graph obtained during the second heating cycle. The DSC analysis revealed consistent thermal properties across all films. Both PHA and PLA exhibited similar melting ranges, indicating comparable thermal transitions. The occurrence of crystallization just before melting suggests that the films are predominantly amorphous. Furthermore, all samples demonstrated a well-defined and stable heating and cooling behaviour, reflecting uniform thermal performance.

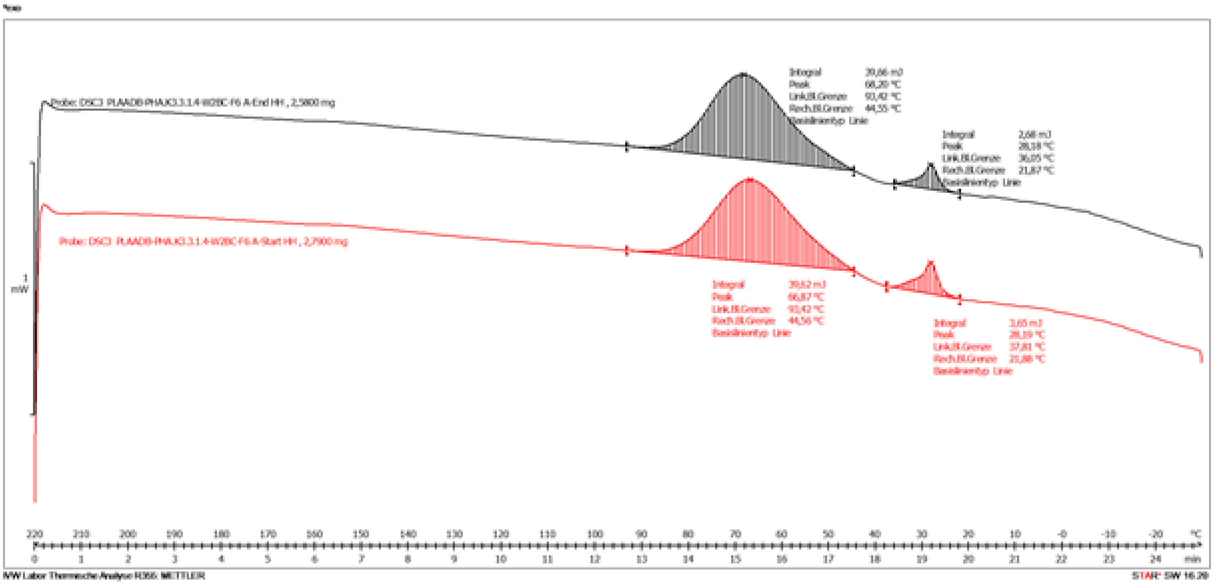


Figure 5 DSC Result for PHA/PLA film (cooling) listed results can be found in Table 4.

Table 4 DSC Results Cooling

DSC Results Cooling				
Sample	**Weight (mg)**	**Heat capacity (mJ)**	**Start temperature (°C)**	**End temperature (°C)**
PLAADB-PHA.K3.3.1.4-W2BC-F6 A End	2.5800	39.66	96.42	44.55
PLAADB-PHA.K3.3.1.4-W2BC-F6 A Start	2.7900	39.62	93.42	44.56
PLAADB-PHA.K3.3.1.4-W2BC-F6 A End	2.5800	2.68	36.05	21.87
PLAADB-PHA.K3.3.1.4-W2BC-F6 A Start	2.7900	3.65	37.81	21.88

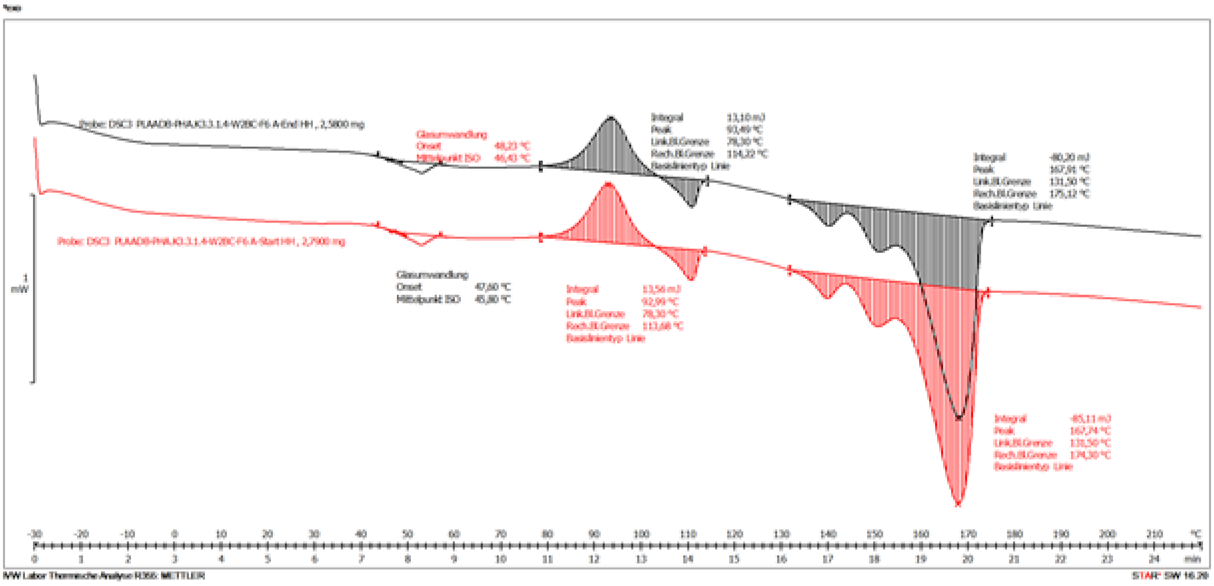


Figure 6 DSC graph for PHA/PLA film with analysis of glass transition, crystallization and melting (2nd heating) listed results can be found in Table 5.

Table 5 DSC Results 2nd Heating

<table>
<tr><th colspan="5">DSC Results 2nd Heating</th></tr>
<tr><th>Sample</th><th>Weigth (mg)</th><th>Glass transition Onset (°C)</th><th colspan="2">Glass transition Middlepoint ISO (°C)</th></tr>
<tr><td>PLAADB-PHA.K3.3.1.4-W2BC-F6 A End</td><td>2.5800</td><td>48.23</td><td colspan="2">46.43</td></tr>
<tr><td>PLAADB-PHA.K3.3.1.4-W2BC-F6 A Start</td><td>2.7900</td><td>47.30</td><td colspan="2">45.80</td></tr>
<tr><th>Sample</th><th>Weigth (mg)</th><th>Heat capacity (mJ)</th><th>Start temperature (°C)</th><th>End temperature (°C)</th></tr>
<tr><td>PLAADB-PHA.K3.3.1.4-W2BC-F6 A End</td><td>2.5800</td><td>13.10</td><td>78.30</td><td>114.22</td></tr>
<tr><td>PLAADB-PHA.K3.3.1.4-W2BC-F6 A Start</td><td>2.7900</td><td>13.56</td><td>78.30</td><td>113.68</td></tr>
<tr><td>PLAADB-PHA.K3.3.1.4-W2BC-F6 A End</td><td>2.5800</td><td>-80.20</td><td>131.50</td><td>175.12</td></tr>
<tr><td>PLAADB-PHA.K3.3.1.4-W2BC-F6 A Start</td><td>2.7900</td><td>-85.11</td><td>131.50</td><td>174.30</td></tr>
</table>

Figure 7 presents the TGA graph of an exemplary PHA/PLA film, revealing two distinct degradation steps. The first weight loss corresponds to the thermal degradation of PHA, while the second is attributed to the degradation of PLA. These degradation events were identified based on the temperature ranges and confirmed by the first derivative of the TGA curve, which is not shown in the figure.

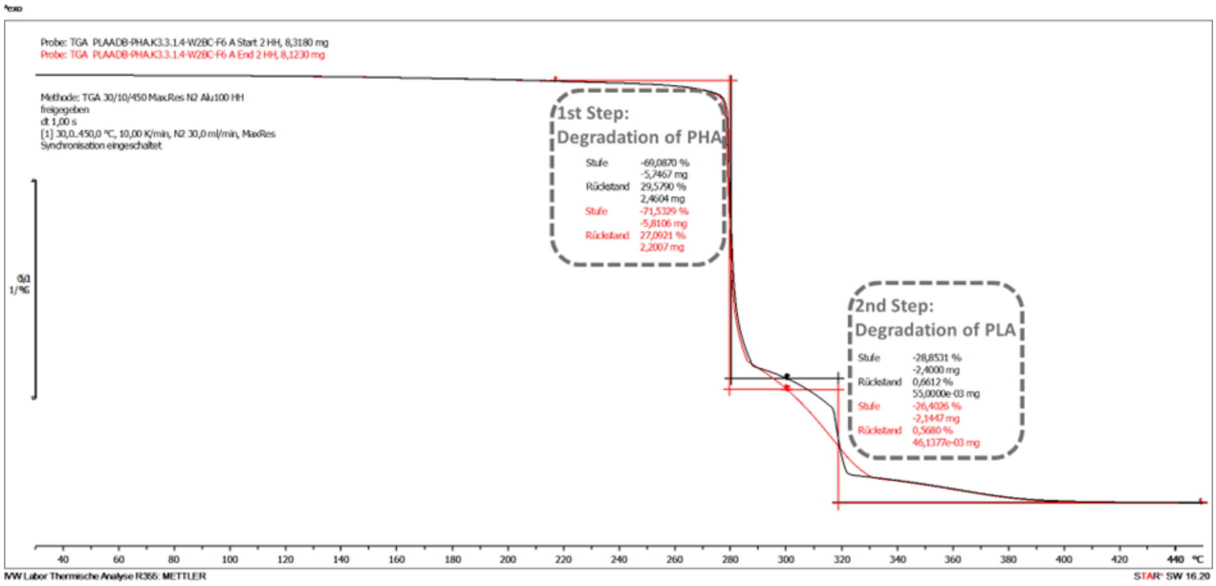


Figure 7 TGA plot of PHA/PLA film listed results can be found in Table 6.

Table 6 TGA Results for PHA/PLA film

		TGA Results			
Sample	**Weight (mg)**	**1st Degradation**	**1st Degradation rest**	**2nd Degradation**	**2nd Degradation rest**
PLAADB-PHA.K3.3.1.4-W2BC-F6 A End	8,3180	-69,0870 %	29,5790 %	-28,8531 %	0,6612 %
		-5,7467 mg	2,4604 mg	-2,4000 mg	0,0550 mg
PLAADB-PHA.K3.3.1.4-W2BC-F6 A Start	8,1230	-71,5329 %	27,0921 %	-26,4026 %	0,5680 %
		-5,8106 mg	2,2007 mg	-2,1447 mg	0,0461 mg

In summary, the thermal analysis of the PHA/PLA films confirmed that the targeted material properties were successfully achieved. The DSC results demonstrated consistent and stable thermal behaviour across all samples, with similar melting ranges for PHA and PLA and indications of predominantly amorphous structures. The TGA analysis further verified the presence of both polymers through two clearly distinguishable degradation steps. Overall, the combination of DSC and TGA provided comprehensive insight into the thermal behaviour and composition of the films, confirming that the intended structural and functional characteristics were met.

Table 7 Results of the tensile test of PHA/PLA film in accordance with ISO 527-3

	σ_x	σ_y	ε_y	σ_m	ε_m	σ_b	ε_b	b	h
	MPa	**MPa**	**%**	**MPa**	**%**	**MPa**	**%**	**mm**	**µm**
Sample 1	-	19.03	5.11	19.03	5.11	17.16	-	25.52	77.00
Sample 2	-	23.41	2.74	23.41	2.74	18.95	-	25.80	86.00
Sample 3	-	18.63	3.34	18.63	3.34	13.87	-	25.34	72.00
Sample 4	-	19.56	2.72	19.56	2.72	11.09	-	24.69	87.00
Sample 5	-	21.79	3.30	21.79	3.30	17.38	-	25.05	96.00
Sample 6	-	17.20	6.12	17.20	6.12	11.44	-	25.75	88.00
$\bar{x}$	-	19.80	3.89	19.80	3.89	14.99	-	25.36	84.33
Sample standard deviation	-	±2.12	±1.30	±2.12	±1.30	±3.19	-	±0.41	±8.30

Table 7 summarizes the tensile tests performed on the PHA/PLA films. The test provided valuable insights into the mechanical behaviour of the material. With an average tensile strength of $\bar{x}$ = 19.80 MPa (SD = ±2.12 MPa), the films demonstrated a relatively high strength that is suitable for applications in flexible packaging. These values are consistent with the mechanical properties reported for other bio-based polymer blends and confirm the structural integrity of the produced films. The relatively low variability in strength also indicates a homogeneous material distribution and reproducible processing conditions.

The average elongation at break ($\bar{x}$ = 3.89 %, SD = ±1.30 %) suggests a moderately ductile behaviour, which is advantageous for flexible applications, particularly when the material is subjected to bending, folding, or stretching during use. The relatively high elongation observed in individual samples such as Sample 6 with 6.12 % may indicate local differences in polymer phase distribution or slight process variations during production, such as uneven cooling or orientation effects. This degree of variability is typical for semi-crystalline or amorphous polymer blends and reflects an inherent characteristic of the material system.

The ultimate tensile strength values ($\bar{x}$ = 14.99 MPa, SD = ±3.19 MPa) show slightly greater variation, suggesting that some samples failed shortly after reaching maximum load, while others underwent a limited degree of plastic deformation. This behaviour may be attributed to microstructural inconsistencies, the presence of defects or voids, or varying interfacial adhesion between the PHA and PLA phases. While geometric deviations were minimal (width ≈ 25 mm, thickness ≈ 84 µm), they could have contributed marginally to local stress concentrations, though overall sample dimensions were well-controlled.

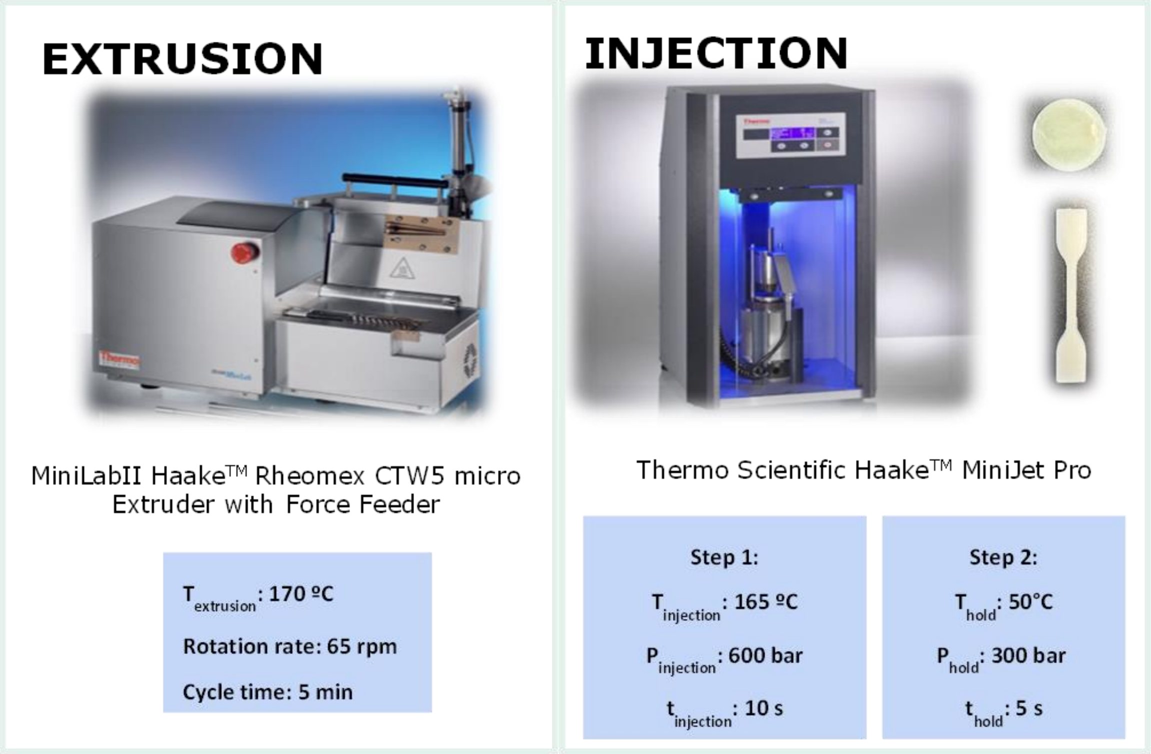
Overall, the results indicate that the PHA/PLA films met the targeted mechanical requirements. The combination of adequate tensile strength, moderate ductility, and uniform structural performance makes the material promising for use in flexible packaging applications especially in contexts where biodegradability and mechanical robustness are both essential. These findings also provide a solid basis for further optimization in terms of polymer ratio, processing conditions, or the incorporation of additives to tailor mechanical behaviour for specific end-use requirements.

2.2. Rigid Packaging Demonstrators

Rigid plastics were prepared in UDC during the development of **W2BC** WP3 from M7 to M30, using several PHAs developed by HSKL partner in WP1. Lab-scale and pre-industrial scale melting procedures were used to get them. Several processing parameters were tested to be optimized for each of the materials and processing methods.

2.2.1. Lab-scale processing methods (WP3)

They were made using a mini-extrusion and mini-injection machines. As mentioned above, several processing parameters were tried and tested. Final processing parameters selected after these studies are described in Figure 8.



$T_{extrusion}$: 170 ºC

Rotation rate: 65 rpm

Cycle time: 5 min

Step 1:

$T_{injection}$: 165 ºC

$P_{injection}$: 600 bar

$t_{injection}$: 10 s

Step 2:

T_{hold}: 50°C

P_{hold}: 300 bar

t_{hold}: 5 s

Figure 8 Lab-scale melting processing equipment used to optimize rigid plastic prototype manufacturing.

As an approach to mechanical properties of the tested PHAs, the dynamo mechanical analysis in an analyser DMA7 from Perkin Elmer was performed to filaments from miniextrusion process. Dumbble-shaped specimens according to DIN EN ISO 527-2, 5Q were produced as final rigid plastic lab-scale prototype from the mini-injection machine. These specimens were used to test mechanical properties in an Instron 566 Universal Testing.

The processing methods and the results of all the analysis mentioned are described in Deliverables 3.2 and 3.4, respectively. Commercial PHAs were tested also and used as a reference to compare the properties of **W2BC** PHAs.

2.2.2. Pre-industrial scale processing methods (WP3 & WP4)

From the lab-scale melting processing studies, the PHA coded as PHA.B.2.2.0.4 was selected as the best option for melting processing at pre-industrial scale. PHA.B.2.2.0.4 was supplied in flakes. Although preliminary preparations (grinding) were initially carried out in WP3 to improve the final material, in this WP4 it was decided to optimize the processing parameters for the flakes instead of adding additional steps to the process to avoid increasing the number of steps in the process to improve the production cycle of the material.

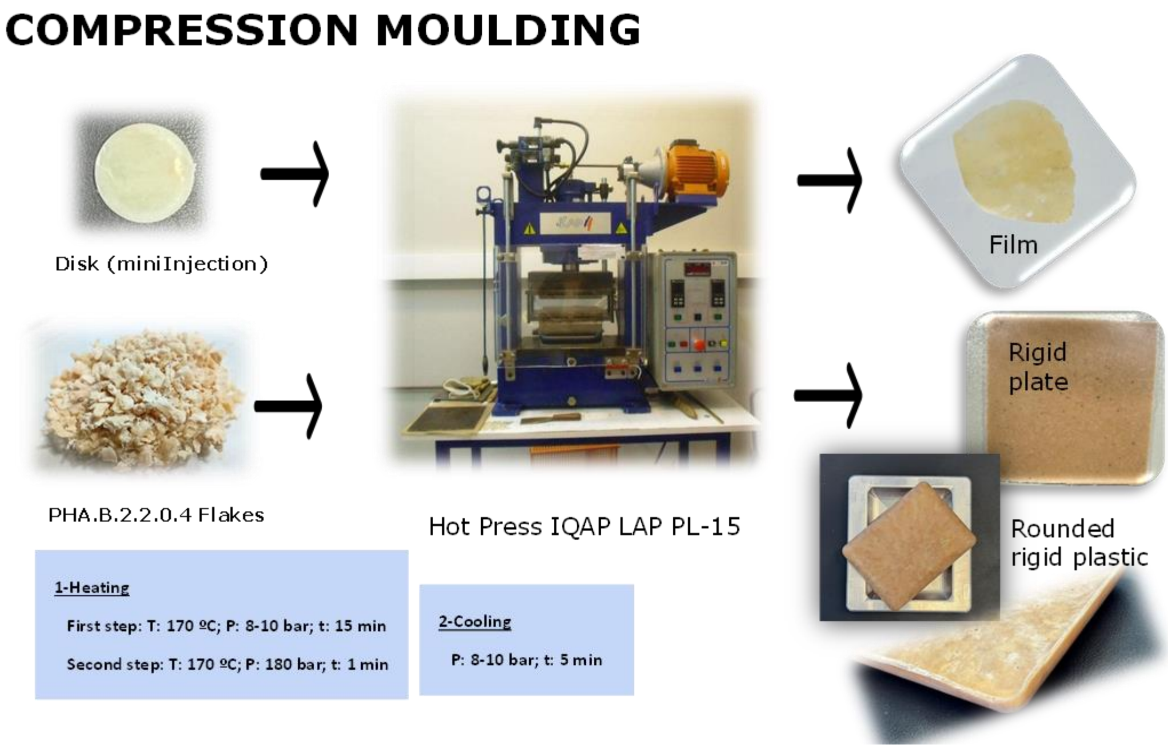
The pre-industrial melting processes developed in WP3 and after that in WP4 were the following:

COMPRESSION MOULDING in a Hot Press IQAP LAP PL-15

In WP3 several processing parameters were checked. Selected conditions as the optimal for compression moulding of this PHA.B.2.2.0.4 and used in the processing along the development of this WP4 are described in Figure 9.

Specimens in form of disk, prepared from the mini-injection machine in the previous step, were converted into films ((100-200) µm thickness) in the Hot Press. These films were used to test permeability of these materials to both, water vapour in a WATER VAPOUR Permatran-W, model 1/50 G (ASTM E-398) and oxygen gas in an OXYGEN OX-TRAN, model 1/50 G (ASTM D-3985).

Different Rigid plastics prototypes were obtained by Compression moulding in the Hot Press IQAP LAP PL-15 by using different molds, flat and rounded (Figure 9), in order to check the feasibility of performing these different forms with the **W2BC** PHA by direct melting and cooling.



1-Heating

First step: T: 170 ºC; P: 8-10 bar; t: 15 min

Second step: T: 170 ºC; P: 180 bar; t: 1 min

2-Cooling

P: 8-10 bar; t: 5 min

Figure 9 Films and Rigid plastic prototypes preparation at pre-industrial scale by compression moulding.

INJECTION MOULDING in a 35 TM Injection Machine BATTENFELD PLUS 350

PHA.B.2.2.0.4 specimens, in both forms described below, were prepared from PHA.B.2.2.0.4 flakes in a 35 TM Injection Machine BATTENFELD PLUS 350:

- Dog-bone shaped specimens (UNE-EN_ISO_527-2=2012) type 1B
- Charpy Impact test specimens (ISO 179 & ASTM D256 B)

Parameters optimized for PHA.B.2.2.0.4 flakes in injection are described in Figure 10.

Figure 10 Injection process and parameters used for PHA.B.2.2.0.4 flakes.

Specimens were used to test the mechanical properties of final rigid plastic prototypes in an Instron 566 Universal Testing machine. Charpy impact test were also made in a Pendulum impact tester. Results will be described in the next section.

Even so, during development of WP4 a new PHA (PHA.K.3.3.1.4), supplied in pellets form this time, was also tested in Injection moulding machine (Figure 11) as an attempt to increase the number of prototypes offered for rigid plastics but, results were not as good as expected so, PHA.B.2.2.0.4 flakes was the final selected as most promising for obtaining rigid plastics.

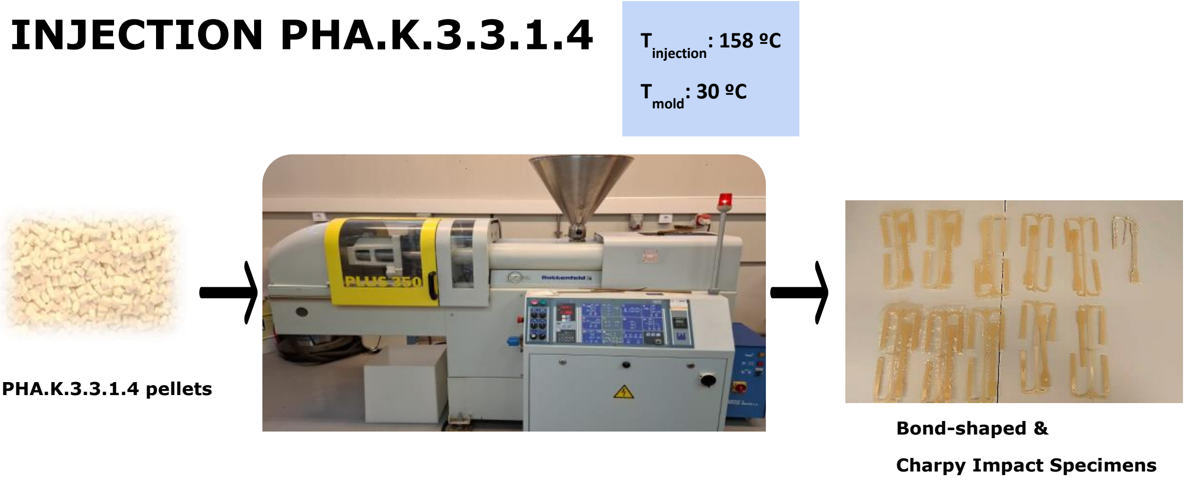


Figure 11 Injection process and parameters used for PHA.K.3.3.1.4 pellets.

The PHA.K.3.3.1.4 pellets can be injected. Different processing parameters were tested in the injection machine with this PHA.K. As an advantage over the PHA.B flakes, the pellets work great in automatic feeding mode but, however, some problems were observed during the injection processing:

- It was observed a melted material leakage from the screw before the injection step.
- An overflow of the melt from the mould occurred. Because of that, the mould was not completely covered by PHA.K material.

- A deep material shrinkage during cooling was observed, in a higher extent than for PHA.B flakes.
- In the final product, air pockets & voids are observed in the inner part of the material. The material is heterogeneous because of that, which worsens its mechanical properties. Results with high variability are obtained.

Due to these disadvantages with respect to PHA.B flakes. We have decided to continue with our tests by using only PHA.B flakes.

2.2.3. Rigid bioplastic materials with active substances

In parallel with our test at lab and pre-industrial scale to obtain rigid plastics prototypes during WP3 development, several **Microparticles (MPs),** prepared from both, commercial and **W2BC** PHAs, were prepared in UDC laboratory as part of the work done in WP1 (Figure 12). From the screening of MPs made, the ones made from commercial PHB (from Biomer) were selected as the optimal to be loaded with active substances and embedded into the PHA.B rigid plastic prototypes in WP3 & WP4.

As said, these MPs were loaded with different active substances (antimicrobial and antioxidant) and the loading capacity as well as release behaviour of these substances to the outside media were tested as part of the work done in WP1. Two of these active substances were selected as the most promising to be incorporated to rigid plastics in WP3 & WP4. The selected active substances were **Quercetin** and **Curcumin**, which antimicrobial and antioxidant action are widely mentioned in the bibliography.

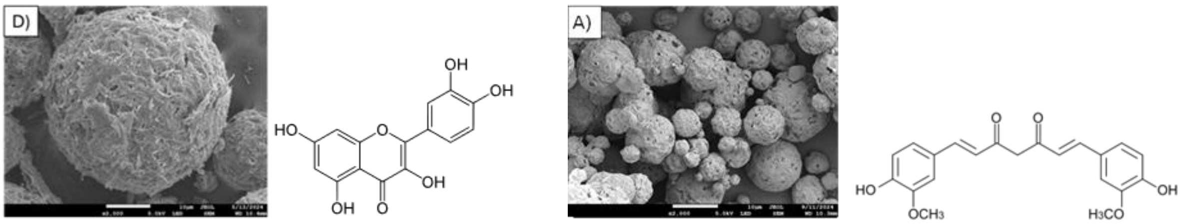


Figure 12 SEM images of PHB MPs loaded with Quercetin (left) & Curcumin (right) as well as chemical structure of both compounds.

2.2.4. Characterization of rigid packaging

Thermal & morphological analysis

Thermal analysis of both PHB MPs and PHA.B.2.2.0.4 rigid plastics neat and filled with loaded MPs, were performed by DSC to check the feasibility of incorporating the MPs into the matrix with a good dispersion and without losing the MPs because of melting, degradation or breakage during processing.

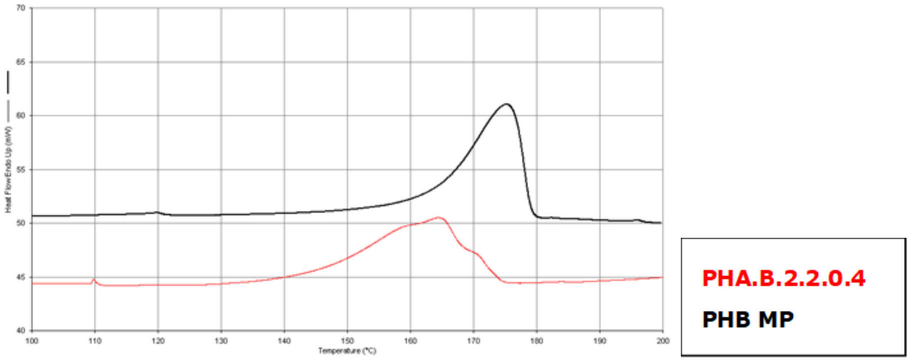


Figure 13 DSC thermograms of PHA.B.2.2.0.4 flakes and PHB MP.

The difference in melting temperatures of PHB MPs and PHA.B.2.2.0.4 flakes are about 10 ºC (Figure 13). The question here is to check if this difference is enough for the MPs to survive into the matrix the injection process without suffering from melting or breakage. To verify this SEM images (Figure 14) were obtained from the bone-shaped injection probes after a cryogenic fracture. The dispersion of MPs was also checked from these images.

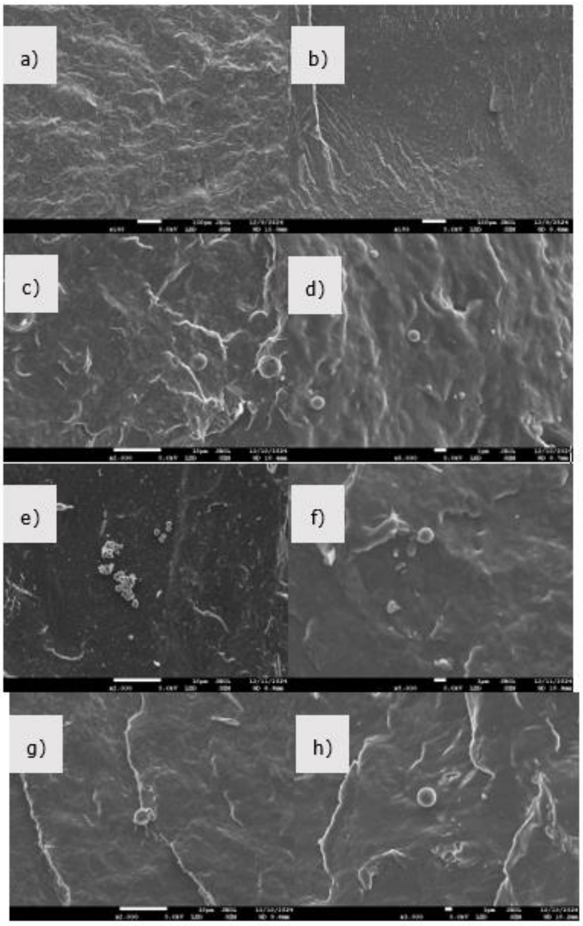


Figure 14 Cryogenic fracture SEM images of injected probes made from a) & b) PHA.B.2.2.0.4; c) & d) PHA.B.2.2.0.4 + 2 wt.% PHB MP; e) & f) PHA.B.2.2.0.4 + 2 wt.% PHB MP loaded with Curcumin; g) & h) PHA.B.2.2.0.4 + 2 wt.% PHB MP loaded with Quercetin.

PHB MPs can be seen in the fracture images of injection probes so we can conclude that the processing does not affect to the integrity of MPs in the PHA.B.2.2.0.4 matrix. Dispersion is not complete as we can see some images with agglomerations. In general, MPs melting does not occur, non-degradation and neither breakage. So that, it is expected the MPs survive to the injection processing and the active substances release occurs correctly in the rigid plastic prototype obtained. To check that, **release analysis** of both active substances (curcumin and quercetin) from the MPs to the matrix and from the matrix to an outer media were performed.

Active substance release from rigid plastics

Release analysis of both active substances (curcumin and quercetin) from the MPs to the matrix were first analysed in WP1 (see deliverable 1.2) where curcumin and quercetin were the active substances selected to continue the work (Figure 15). In WP3 & WP4 was performed the analysis of quercetin and curcumin analysis from the matrix to an outer media. These analyses were performed in the following liquid media: diethyl ether (DE), PBS (pH 7.4), 100% ethanol (E100) and 35% aqueous ethanol (E35) and alkaline sweat media (pH 8.0).

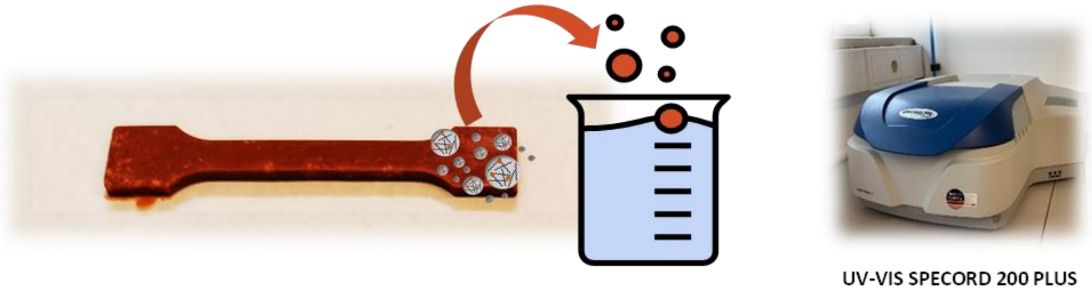


Figure 15 Release analysis performed in rigid plastics from injection specimens by using a UV-VIS spectrophotometer.

The results for the first four media were widely described on Deliverable 3.4. (see *page 22-Release profile of biomaterials prepared with different substances loaded on PHB MPs*). The results for the last media (alkaline sweat) are shown in Figure 16.

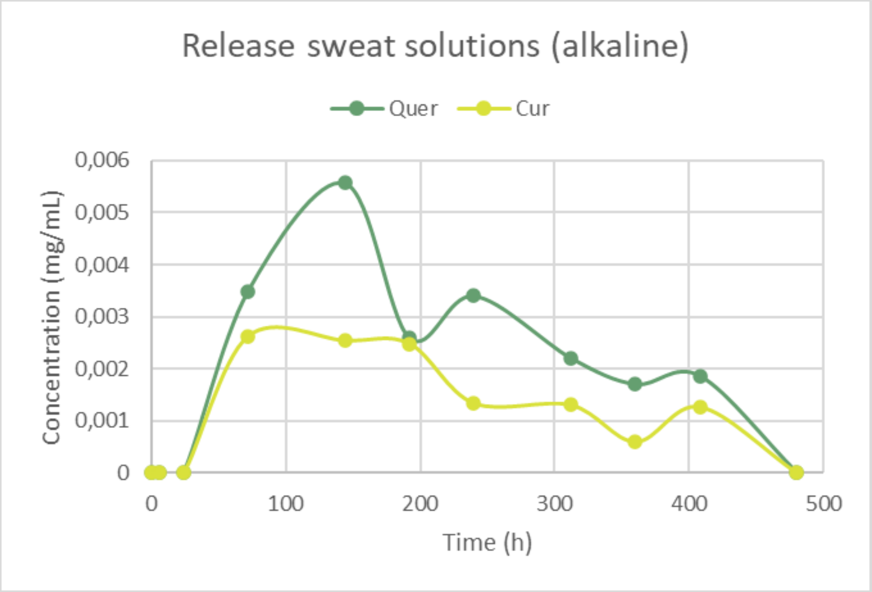


Figure 16 Release of substances in alkaline sweat media (pH 8.0).

The conclusions achieved in release analysis performed to rigid plastics filled with loaded MPs were the following:

1. The release activity depends directly on the media used. In Table 8 is reported data about release complete release time for each active substance in the different media used. Release of active substances from MPs to the outer media can occur by different mechanisms: dissolution/diffusion, osmotic pressure, and erosion. These 3 methods coexist at the same time but usually there is one of them that prevails depending on the chemical affinity/solubility of active substances as well as the physical interactions between active substances and PHB/PHA matrixes and the particle integrity over time.
2. In all the media analysed, the concentration detected of quercetin was always higher than in case of curcumin, probably due to the differences on the solubility of both substances.
3. Regarding to the cumulative concentration of each active substance, it means the final concentration of active substances which reaches the outer media at the end of the release, this is approximately 2 times higher for quercetin than for curcumin in all media.

Table 8 Complete release times for each active substance in different media tested

Active substance	Solubility in water	DE (time, h)	PBS (time, h)	E100 (time, h)	E35 (time, h)	Alkaline sweat (time, h)
Curcumin	insoluble	696	280	720	768	480
Quercetin	1 mg/mL	696	240	1000	1000	480

Antioxidant activity of rigid plastics

Antioxidant activity was first analysed in active substances loaded MPs (see deliverable 1.2, page 28) by two colourimetric methods (ABTS and DPPH). The antioxidant activity was expressed as Trolox equivalents per gram of sample. When the loaded MPs were analysed directly, the antioxidant activity of Quercetin was always higher (3.58 g Trolox/100g MP (ABTS method); 2.26 g Trolox/100g MP (DPPH method)) than the observed for Curcumin (0.78 g Trolox/100g MP (ABTS method); 0.13 g Trolox/100g MP (DPPH method)).

In a second attempt, films made by compression moulding from the disk form specimens (mini-injection) of PHA.B.2.2.0.4 neat and filled with both, quercetin and curcumin loaded MPs, were tested following the ABTS colourimetric method procedure. A similar antioxidant activity for both, Quercetin (0.027 g Trolox/100g composite) than for Curcumin (0.032 g Trolox/100g composite) was observed, (see deliverable 3.4, page 24) but lower than for the measured directly from the MPs as expected.

The antioxidant activity diminishes when it is tested in the bone-shaped specimens obtained by the pre-industrial scale injected specimens as can be seen in Figure 17 for both active substances probably due to the higher thickness of injected probes respect to the films. Moreover, the dilution of active substances on the matrix and an irregular dispersion of MPs on the matrix can also contribute to this diminution. Antioxidant activity of Quercetin in the injected specimens is minor at longer times than for Curcumin, probably due to the interaction of Quercetin, through its catechol groups, with the biomaterial, delaying the mobility towards the release medium.

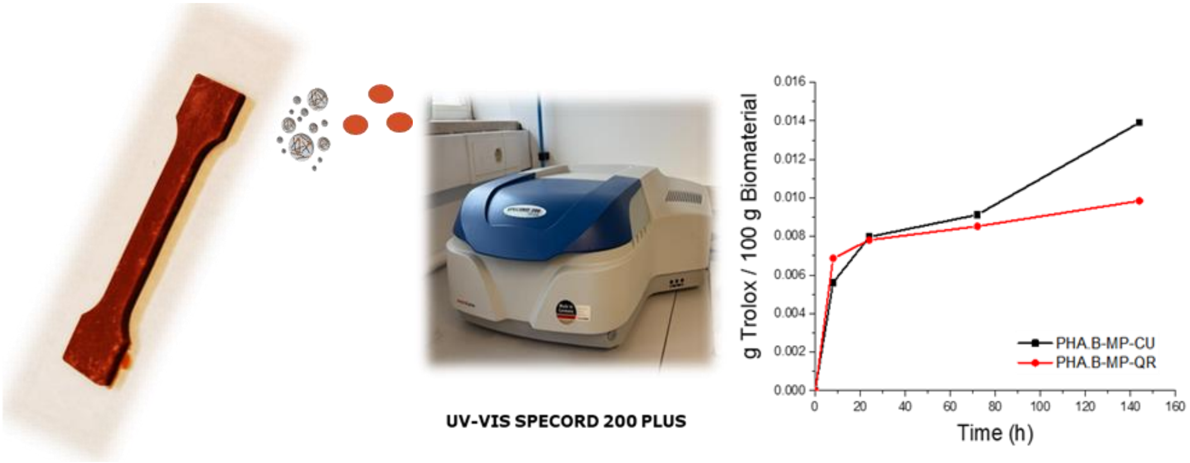


Figure 17 Antioxidant activity analysis performed on bone shaped specimens of rigid plastic.

Antimicrobial activity of rigid plastics

Antimicrobial analysis on rigid specimens made from PHA.B.2.2.0.4 and filled with active substances loaded MPs is still being assessed, so its results will be show at the final project presentation and report.

Mechanical properties

Mechanical Testing was made in an Instron 566 Universal Testing Machine for rigid materials (neat and modified with 2 wt.% of Quercetin and Curcumin loaded MPs). Commercial PHBV rigid plastics were tested also as aa reference to compare the results from **W2BC** rigid plastics obtained. Results were already reported in deliverable 3.4. Here is presented a summary of the results.

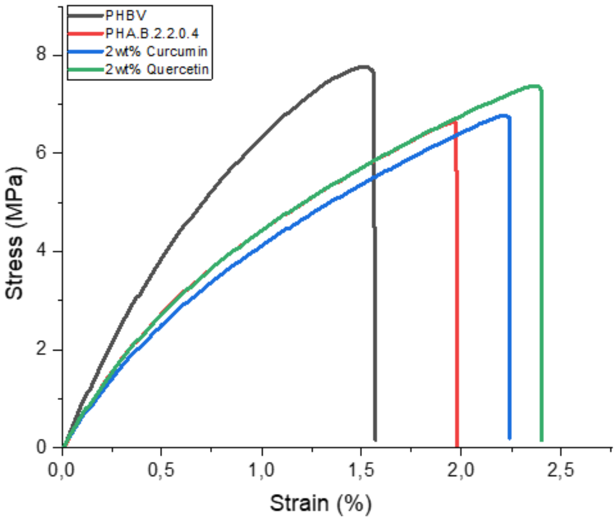


Figure 18 Instron 5566 Universal testing machine and stress-strain graph obtained for PHA.B.2.2.0.4 rigid plastics.

Table 9 Tensile test results for injected materials

Sample	Young´s Module (MPa)	Strain at yield point (%)	Tensile strength (MPa)	Elongation at break (%)	Stress at break (MPa)
PHBV	926.4±82.0	8.5±1.4	1.7±0.5	8.5±1.4	1.7±0.5
PHA.B.2.2.0.4	645.7±34.3	1.9±0.3	6.6±0.6	2.0±0.3	6.7±0.6
PHA.B.2.2.0.4 + 2 wt.% PHB MPs loaded with Quercetin	627.0±24.2	2.0±0.3	6.8±0.7	2.0±0.3	6.8±0.7
PHA.B.2.2.0.4 + 2 wt.% PHB MPs loaded with Curcumin	605.7±29.0	2.2±0.3	7.0±0.6	2.2±0.3	7.0±0.6

In general, according to results obtained (Figure 18 and Table 9) we've found that the material PHA.B.2.2.0.4 is perfectly injectable. The processing window should be carefully controlled as it is smaller than usual but, it can be controlled. The rigid plastic materials obtained have minor modulus than the commercial PHBV so, they have minor stiffness, but an improvement of flexibility and strength is observed also with MPs addition

Fracture Testing of Charpy Impact test specimens obtained by injection were made in a Pendulum impact tester.

Table 10 Charpy impact tests results for injected materials

Sample	Charpy fracture toughness acU (J)
PHBV	6.2±0.5
PHA.B.2.2.0.4	19.6±3.5
PHA.B.2.2.0.4 + 2 wt.% PHB MPs unloaded	14.0±3.8
PHA.B.2.2.0.4 + 2 wt.% PHB MPs loaded with Quercetin	17.5±3.2
PHA.B.2.2.0.4 + 2 wt.% PHB MPs loaded with Curcumin	19.3±4.0

Higher toughness and ductility were observed in injection specimens obtained from PHA.B.2.2.0.4 in the above-mentioned processing conditions than commercial PHBV according to data reported in Table 10. Moreover, MPs addition hardly affects to energy absorption capacity of these specimens.

Barrier properties

Permeability testing of films made by compression moulding from disc form probes obtained by injection in the mini-injection machine were performed against both, water vapour and oxygen gases in the following equipment:

- OXYGEN_OX-TRAN, model 1/50 G under ASTM D-3985 specifications
- WATER VAPOUR, Permatran-W, model 1/50 G under ASTM E-398 specifications

The results are described on Table 11. The results are compared with the obtained for a commercial PHBV used as reference. Regarding to oxygen, the **W2BC** PHA.B.2.2.0.4 has a higher oxygen transmission rate value and it increases when the particles are added to the matrix, especially when the MPs are loaded with the active agents. In case of water vapour, the transmission rate is always lower than the observed for PHBV matrix. In specimens filled with MPs the transmission also increases respect to the neat PHA.B.2.2.0.4, especially for loaded MPs. The increment in transmission rate when the MPs are added to the matrix can be due to an increase in the crystallinity of the material as well as to MPs agglomerations which increase the free volume on the matrix favouring the gas permeation.

Table 11 Barrier properties for films made from PHA.B.2.2.0.4. neat and filled with loaded MPs.

Sample	OTR (cc/m²/day)	WVTR (gm/m²/day)
Commercial PHBV	42 ± 4	26 ± 1
PHA.B.2.2.0.4 (from task 1.1)	73 ± 38	14 ± 6
PHA.B.2.2.0.4 + 2 wt.% PHB MPs unloaded	75 ± 5	13 ± 4
PHA.B.2.2.0.4 + 2 wt.% PHB MPs loaded with Quercetin	121 ± 5	17 ± 9
PHA.B.2.2.0.4 + 2 wt.% PHB MPs loaded with Curcumin	124 ± 15	22 ± 6

PHA.B.2.2.0.4 Charpy Impact specimens were sent to IVW partners to perform **weathering tests** and mechanical analysis before and after weathering tests. The results are below.

Weathering and 3-Point Bending Test on rigid plastics

The aim of this investigation was to study the effect of weathering on the mechanical properties of the specimens. Weathering can significantly influence the durability and structural integrity of materials, making it a crucial factor for material selection in engineering applications.

In this report, the samples underwent an artificial weathering process to simulate long-term environmental exposure. The weathering conditions in the chamber were designed to resemble the European climate, including variations in temperature, humidity, and UV radiation. The aim of the three-point bending tests was to investigate the effect of 120-hour aging as well as the influence of bioactive additives (2 wt.% curcumin and 2 wt.% quercetin) on the mechanical properties of the biopolymer **W2BC** PHA.B.2.2.0.4 and **W2BC** PHA.B.2.2.0.4 The three- point-bending test provides valuable insights into the material's flexural strength and stiffness, both of which are essential properties for many structural applications. Following the method specified in Table 1, five specimens were subjected to a three-point bending test to assess the initial performance of the materials and the changes after the weathering.

This report presents the experimental setup, methodology, and results of the conducted tests. The findings were analysed to determine how weathering affects the bending properties of the samples, contributing to a better understanding of material performance under real-world conditions.

Experimental Setup und Methodology:

The samples used in this experiment were provided by UDC and consisted of three material compositions:

- 1.) PHA.B.2.2.0.4
- 2.) PHA.B.2.2.0.4 + 2 wt.% MP (curcumin)
- 3.) PHA.B.2.2.0.4 + 2 wt.% MP (quercetin)

The weather conditions in the weathering chamber followed a four-phase cycle, with each phase representing different seasonal conditions. This setup simulated both cold and warm periods. The conditions are listed in Table 12.

Table 12 Weathering conditions used on specimens from UDC

	Phase 1	Phase 2	Phase 3	Phase 4
Irradiance	60 W/m^2	60 W/m^2	60 W/m^2	60 W/m^2
Run	synchronization	synchronization	synchronization	synchronization
Sample chamber temperature	20 °C	20 °C	40 °C	40 °C
Rain	No	Yes	No	Yes

Colour Analysis: After every 24 hours in the weathering chamber, a reference sample was tested for colour change. For the least data point, the colour measurement was performed 44 hours after finishing the weathering.

Table 13-Table 15 show colorimetric measurements (L*, a*, b*, C*, h°, ΔE*) for different samples subjected to light, heat, and/or UV exposure over a period of up to 164 hours. The parameter ΔE* represents the colour difference compared to the initial (standard) sample. A higher ΔE* value indicates a stronger colour change. The other parameters are from the CIELAB colour space:

- L*: Lightness (0 = black, 100 = white)
- a*: Red-Green axis (positive = red, negative = green)
- b*: Yellow-Blue axis (positive = yellow, negative = blue)
- C*: Chroma (colour intensity/saturation)
- h°: Hue angle (colour tone)

Table 13 Results of colour measurement for PHA.B.2.2.0.4.

	L*	a*	b*	C*	h°	ΔE*
Standard	59.21	9.70	23.72	25.63	67.76	0.00
0h	59.14	9.54	23.51	25.37	67.91	0.27
24h	56.95	4.66	13.98	14.74	71.57	11.19
48h	55.75	3.45	11.45	11.96	73.23	14.20
72h	54.90	2.58	9.51	9.85	74.82	16.47
96h	53.98	2.14	8.33	8.60	75.59	17.93
120 + 44 h	63.51	7.71	21.34	22.69	70.14	5.30

Table 14 Results of colour measurement for PHA.B.2.2.0.4 + 2 wt.% MP (curcumin)

	L*	a*	b*	C*	h°	ΔE*
Standard	48.02	24.35	32.74	40.80	53.36	0.00
0 h	43.82	25.90	33.51	42.35	52.30	4.54
24 h	55.67	7.32	14.54	16.28	63.28	26.07
48 h	55.22	4.63	11.75	12.63	68.49	29.69
72 h	54.37	3.22	9.86	10.37	71.91	31.79
96 h	53.89	2.46	8.48	8.83	73.82	33.20
120 + 44 h	61.24	6.26	16.52	17.67	69.25	27.66

Table 15 Results of colour measurement for PHA.B.2.2.0.4 + 2 wt.% MP (quercetin)

	L*	a*	b*	C*	h°	ΔE*
Standard	42.64	20.67	28.72	35.38	54.26	0.00
0 h	42.77	20.57	28.67	35.29	54.34	0.17
24 h	47.40	16.93	20.71	26.75	50.73	10.04
48 h	47.66	15.96	18.93	24.76	49.87	11.96
72 h	47.69	14.32	17.64	22.72	50.93	13.73
96 h	47.16	13.08	15.98	20.65	50.70	15.50
120 + 44 h	48.56	17.01	18.04	24.79	46.68	12.75

Three-Point Bending Test: The three-point bending test was carried out in accordance with the DIN EN ISO 178 standard. The equipment used was universal testing device Type 1445 Retroline by Zwick Roell.Table 16, Table 17 and Table 18 show flexural strength (σfM) and maximum strain (εM) for the different samples before (0 h) and after weathering (120 h).

Table 16 Three-Point Bending Results for ***W2BC*** *PHA.B.2.2.0.4.*

Sample	0 h		120 h	
	σfM in MPa	**εM in %**	**σfM in MPa**	**εM in %**
1	22.7	3.8	16.4	2.0
2	22.4	3.0	18.2	1.7
3	21.0	3.9	16.9	1.8
4	19.2	2.6	15.6	1.9
5	18.4	2.9	18.7	2.4
Media	20.7	3.2	17.2	2.0
Standard deviation	1.9	0.6	1.3	0.3

Table 17 Three-Point Bending Results for PHA.B.2.2.0.4 + 2 wt.% MP (curcumin)

Sample	0 h		120 h	
	σfM in MPa	**εM in %**	**σfM in MPa**	**εM in %**
1	23.7	3.5	18.6	1.9
2	23.8	3.6	18.5	2.0
3	24.2	3.6	18.5	1.9
4	24.5	3.8	18.0	1.9
5	23.3	3.5	19.4	2.2
Media	23.9	3.6	18.6	2.0
Standard deviation	0.5	0.1	0.5	0.1

Table 18 Three-Point Bending Results for PHA.B.2.2.0.4 + 2 wt.% MP (quercetin)

Sample	0 h		120 h	
	σfM in MPa	εM in %	σfM in MPa	εM in %
1	23.1	3.5	18.1	2.0
2	23.6	3.8	17.8	1.9
3	23.9	3.6	17.5	1.8
4	21.7	3.3	18.9	2.2
5	21.9	3.3	18.3	2.0
Media	22.8	3.5	18.1	2.0
Standard deviation	1.0	0.2	0.6	0.1

Colour Measurement of PHA.B.2.2.0.4 (No Additives)

The sample without any additives shows a progressive increase in the colour difference (ΔE*) over the first 96 hours, reaching a value of 17.93, which indicates a substantial change in colour due to environmental exposure. Both, the a* and b* values, decrease over time, suggesting a loss of red and yellow hues, which corresponds to a general fading of the colour. Interestingly, after 120 + 44 hours, the ΔE* decreases to 5.3, while the L* value increases sharply, indicating a significant lightening of the sample. This may be attributed to surface oxidation, matrix rearrangement, or other photochemical processes that partially restore or alter the visual appearance of the material.

Colour Measurement of PHA.B.2.2.0.4 + 2 wt.% MP (curcumin)

The Curcumin-containing sample exhibits the highest colour deviation of all tested materials, with ΔE* values reaching 33.2 after 96 hours. The rapid increase in L* and decrease in both a* and b* values indicate a strong fading of colour and desaturation, likely due to the photodegradation of curcumin, which is known to be unstable under UV/light exposure. Although there is a partial recovery in chromatic values at 120 + 44 hours, the colour remains significantly altered compared to the standard. This behaviour suggests that Curcumin is not a suitable additive for long-term colour stability in this polymer matrix under light or UV exposure.

Colour Measurement of PHA.B.2.2.0.4 + 2 wt.% MP (quercetin)

The sample with Quercetin shows a more moderate colour change compared to Curcumin, with ΔE* values peaking at 15.5 after 96 hours and slightly decreasing to 12.75 at the final time point. The L* value rises only slightly, indicating limited lightening, while the a* and b* values decline steadily, implying a gradual loss of red and yellow tones. These results suggest that Quercetin undergoes some degree of photodegradation but maintains better colour stability than Curcumin under similar conditions. Therefore, Quercetin appears to be a more UV-stable additive for maintaining colour in PHA-based biopolymer systems.

Three-Point Bending Test

The aim of the three-point bending tests was to investigate the effect of 120-hour aging as well as the influence of bioactive additives (2 wt.% curcumin and 2 wt.% quercetin) on the mechanical properties of the biopolymer **W2BC** PHA.B.2.2.0.4.

1. Effect of Aging on the Unmodified Reference Material

Comparing the reference samples before and after 120 h of aging reveals a significant decrease in both flexural strength (σfM) and maximum strain (εM). The average flexural strength dropped from 20.74 MPa to 17.16 MPa, representing a decrease of approximately 17.3%. Meanwhile, the strain at break decreased substantially from 3.24% to 1.96% (a 39.5% reduction). These results indicate a pronounced embrittlement of the material likely due to thermal-oxidative or hydrolytic degradation during the aging period.

2. Influence of curcumin additive

The modification with 2 wt.% Curcumin resulted in a noticeably higher initial flexural strength (average σfM = 23.9 MPa) compared to the unmodified reference (Ø = 2.74 MPa). The strain at break also slightly increased to 3.6%, suggesting a potential reinforcing or plasticizing effect of curcumin on the polymer matrix.

After 120 hours of aging, the flexural strength decreased to 18.6 MPa, marking a 22.2% drop from the fresh state. While this decrease is slightly higher than that observed for the reference, the strain remained at 1.98%, which is comparable to the aged reference sample. Overall, curcumin appears to provide a mild improvement in aging resistance, particularly in terms of preserving mechanical integrity.

3. Influence of quercetin additive

The samples modified with 2 wt.% Quercetin also exhibited improved initial mechanical properties. The average initial flexural strength was 22.84 MPa, higher than the unmodified reference, but slightly lower than the curcumin modified variant. The initial strain was 3.50%, similar to curcumin.

After 120 hours of aging, the flexural strength dropped to 18.12 MPa, reflecting a 20.6% decrease, comparable to curcumin. The strain decreased to 1.98%, mirroring the aged values of both other sample types.

<u>Conclusion and Outlook on weathering test and three-point bending analysis</u>

The colour measurements reveal significant differences in photostability between the tested additives. Curcumin undergoes rapid and intense degradation. In contrast, Quercetin shows better resistance to colour change, while the unmodified sample exhibits moderate but partly reversible discolouration. The material exhibits partial colour restoration over time.

All materials exhibited a clear degradation of mechanical performance after 120 hours. However, both curcumin and quercetin additives led to higher initial strengths and a slightly improved retention of mechanical properties over time. Particularly in the curcumin-modified samples, the low standard deviation in fresh condition (σfM = 0.452 MPa) indicates a more homogeneous and stable material structure.

The results suggest that Curcumin and Quercetin are promising bioactive additives for improving the mechanical performance and aging stability of PHA-based biopolymer materials. While the absolute degradation after 120 hours could not be completely prevented, both additives contributed to higher initial flexural strength and reduced variability between samples. Further studies focusing on molecular interactions and long-term environmental durability would be valuable to assess the full potential of these additives.

3. Conclusions

Over 300 meters of continuously extruded tubular film for flexible packaging applications have been successfully produced in operational environment with a continuous industrial process using PROPAGROUP's existing blow extrusion line and PHA.K material developed in the project.

The thermal and mechanical analyses of this PHA/PLA film confirmed that the targeted material properties were successfully achieved. The film exhibited stable thermal behaviour, consistent structural integrity, and mechanical performance suitable for flexible and biodegradable packaging applications.

Moreover, PHA.B material from HSKL was successfully injected in a pre-industrial scale injection machine in UDC laboratory. This PHA was also used to obtain rigid plastic demonstrators, both with flat and curved edges, by compression moulding. The optimal processing conditions were defined for both processing methods.

In addition, microparticles made from commercial PHB were loaded with Quercetin and Curcumin and embedded into these PHA.B rigid plastic prototypes to confer them antimicrobial and antioxidant properties. MPs survived to the injection processing and the active substances release occurred correctly in the rigid plastic prototype obtained, maintaining their antioxidant effect.

According to mechanical tests, the PHA.B material resulted to be perfectly injectable and the rigid plastic demonstrators showed an improvement of flexibility and strength, also with MPs addition.

The non-toxicity of these materials is shown in deliverable D6.1; their biodegradability in D6.2; and their recyclability in D5.2.

waste2
biocomp

www.waste2biocomp.eu

Waste2biocomp

@waste2biocomp

Waste2biocomp Project

Funded by
the European Union