PROJECT FINAL REPORT

Grant Agreement number: FP7 – 265669

Project acronym: ECOBIOCAP

Project title: Ecoefficient Biodegradable Composite Advanced Packaging

Funding Scheme: Collaborative Project

Period covered: From 1 March 2011 to 28 February 2015

Project co-ordinator name, title and organisation:

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Figures, Tables and References

Part - 1.2 Summary description of project context and objectives

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Figure 1

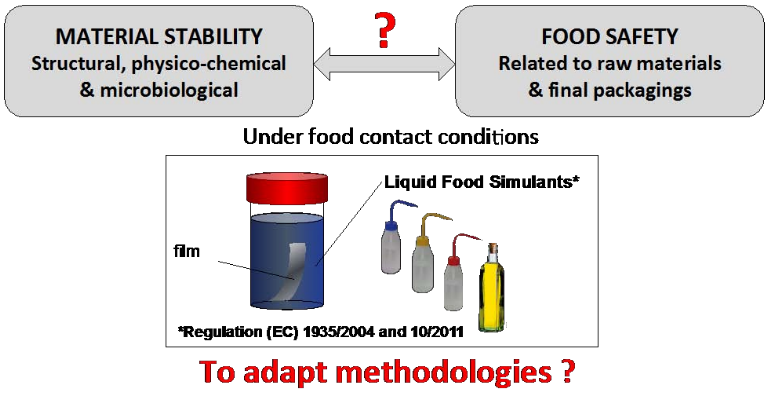

Figure 1 : General objectives of WP4.

Part - 1.3 Description of the main S&T results/foreground

Key result n°1:

Figure 2

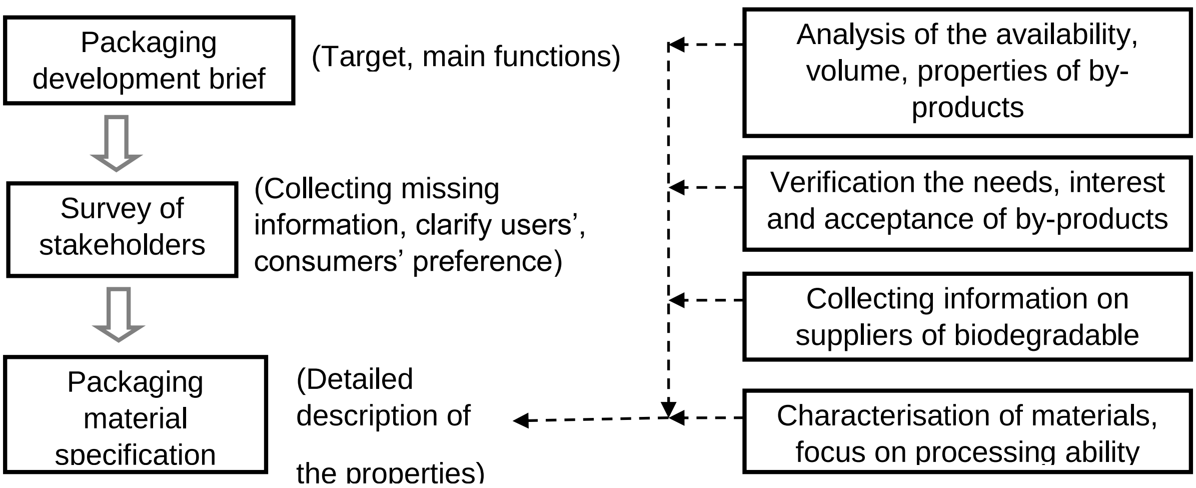

Figure 2**:** The schematic way for preparation of the packaging specifications

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Key results n°3:

Figure 3

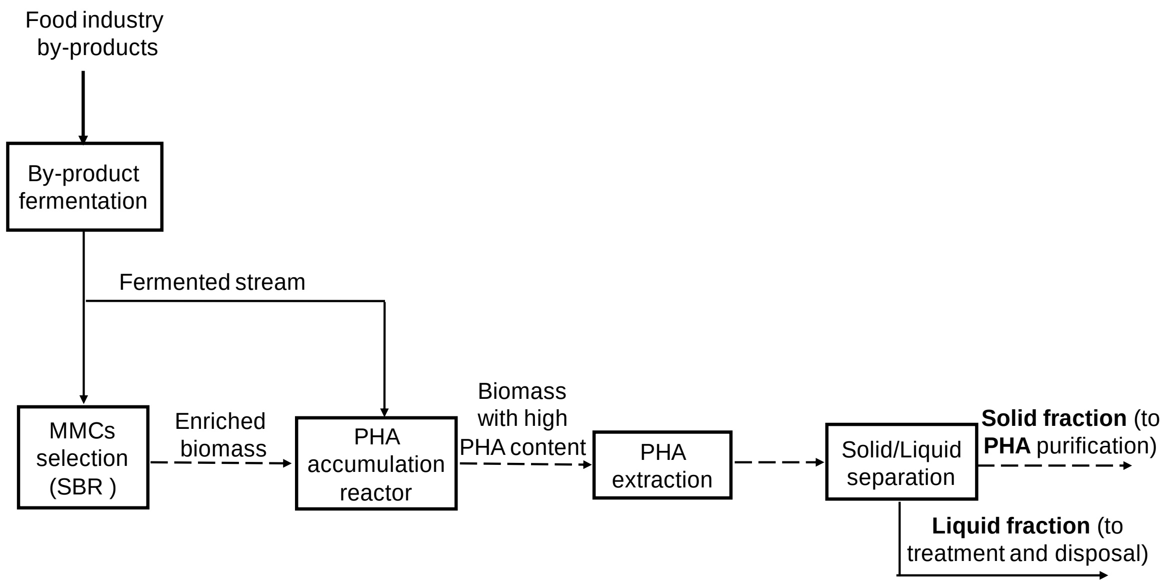

Figure 3: General scheme of the PHA production process with MMC from food industry by-products.

Figure 4

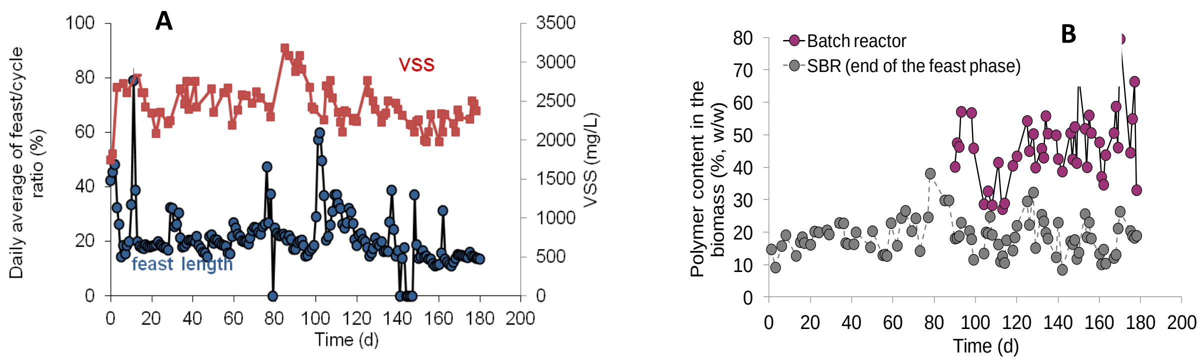

Figure 4. Trend of the length of the feast phase and VSS concentration in the SBR reactor used for the selection of PHA-storing mixed microbial cultures (A). Trend of polymer content in the biomass in both SBR and batch PHA accumulation reactor (B).

Figure 5

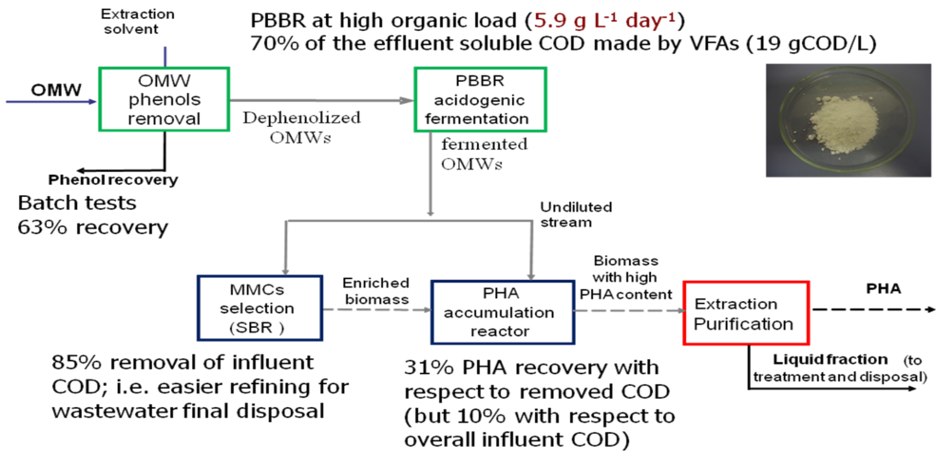

Figure 5. Flow sheet of a multi-stage process for PHA production with mixed microbial cultures (MMC) by using olive oil mill wastewater (OMW) as feedstock

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Figure 6

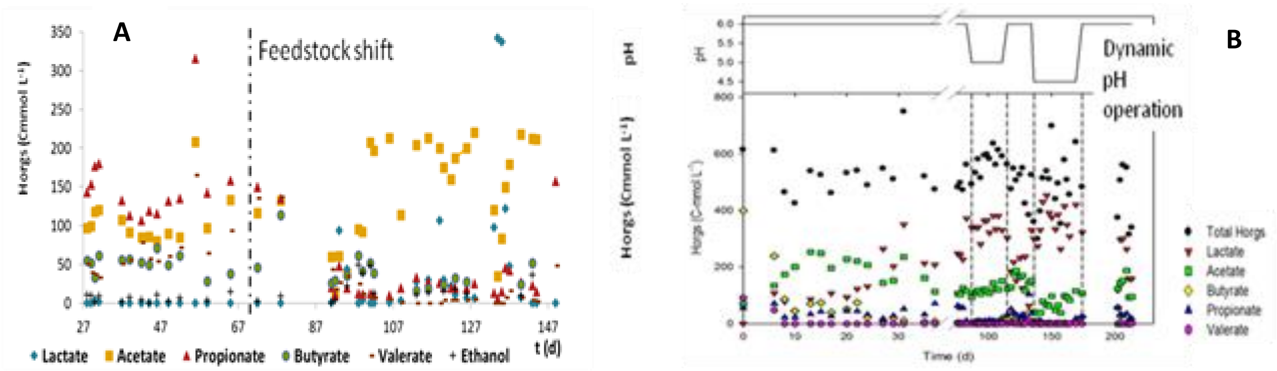

Figure 6. VFA profile in the acidogenic reactor during feedstock shift (A) and dynamic pH operation (B)

References for Key result n°3

Campanari S., Silva F. A., Bertin L., Villano M., Majone M. Effect of the organic loading rate on the production of polyhydroxyalkanoates in a multi-stage process aimed at the valorization of olive oil mill wastewater. International Journal of Biological Macromolecules **2014**, 71: 34-41

Duque A.F., Oliveira C.S.S., Carmo I.T.D., Gouveira A.R., Pardelha F., Ramos A.M., Reis A.M.A. Response of a three-stage process for PHA production by mixed microbial cultures to feedstock shift: impact on polymer composition. New Biotechnology **2014**, 31: 276-288

Keshavarz T. and Roy I. Polyhydroxyalkanoates: bioplastic with a green agenda. Current opinion in Microbiology **2010**, 13: 321-326

Martínez-Sanz M., Villano M., Oliveira C., Albuquerque M.G.E., Majone M., Reis M., Lopez-Rubio A., Lagaron J.M. Characterization of polyhydroxyalkanoates synthesized from microbial mixed cultures and of their nanobiocomposites with bacterial cellulose nanowhiskers. New Biotechnology **2014**, 31: 364-376

Ntaikou I., Kourmentza C., Koutrouli E.C., Stamatelatou K., Zampraka A., Kornaros M., Lyberatos G. Exploitation of olive oil mill wastewater for combined biohydrogen and biopolymers production. Bioresource Technology **2009**, 100: 3724–3730

Reis M., Albuquerque M., Villano M., Majone M. Mixed culture processes for polyhydroxyalkanoate production from agro-industrial surplus/wastes as feedstocks. In Comprehensive Biotechnology 2nd edition volume 6. Moo-Young M., Ed.; Elsevier, Amsterdam, **2011**: 669-683

Villano M., Valentino F., Barbetta A., Martino L., Scandola M., Majone M. Polyhydroxyalkanoates production with mixed microbial cultures: from culture selection to polymer recovery in a high-rate continuous process. New Biotechnology **2014**, 31: 289-296

Key result n°5:

Figure 7

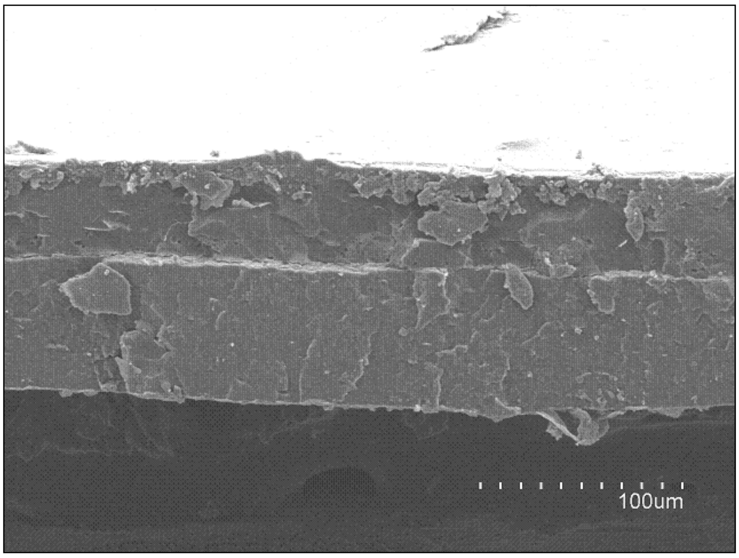

Figure 7: SEM images of the cross-sections from the EcoBioCAP PHBV3 multilayer system prepared with blends of zein and pullulan nanofibers (CSIC)

Figure 8

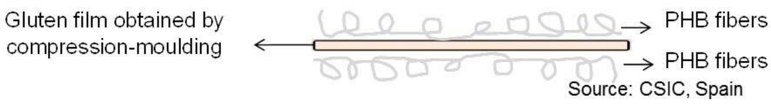

Figure 8: Multilayer of gluten with electrospun PHB fibres on the front and back side

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Table 1

Table 1: Properties (water vapour permeability (WVP), O_2 permeability (O_2) and CO_2 permeability (CO_2)) of films blown using the indicated ranges of BUR and TUR, and with PBAT as external layer.

BUR	TUR	Turbidity (%)	Tianan layer (micron)	Tear resistance (N)	WVP (g•100µm•m^{-2}•d^{-1}•bar^{-1})	O_2 10^3 (cm^3.100µm) bar^{-1}.d^{-1}.m^{-2})	CO_2 10^3 (cm^3.100µm) bar^{-1}.d^{-1}.m^{-2})
4.1	2.3	82.3	33 ± 10	8.7 ± 0.8	1805.76 ± 0.41	59.76 ± 18.08	14.69 ± 3.23
4.1	2.9	85.1	30 ± 10	7.4 ± 0.3	984.96 ± 0.18	35.97 ± 10.32	17.78 ± 7.38
3.9	3.6	81.8	20 ± 10	8.4 ± 0.8	976.32 ± 0.05	22.14 ± 9.60	20.43 ± 4.10
2.7	3.1	88.1	57 ± 13	11.6 ± 0.7	1978.56 ± 0.20	13.87 ± 7.84	9.79 ± 4.01
2.7	3.6	83.0	47 ± 13	10.9 ± 0.9	1442.88 ± 0.29	17.07 ± 7.39	18.60 ± 8.29
2.7	4.9	80.3	40 ± 20	10.4 ± 0.6	1762.00 ± 0.13	44.85 ± 15.76	15.84 ± 8.96

Figure 9

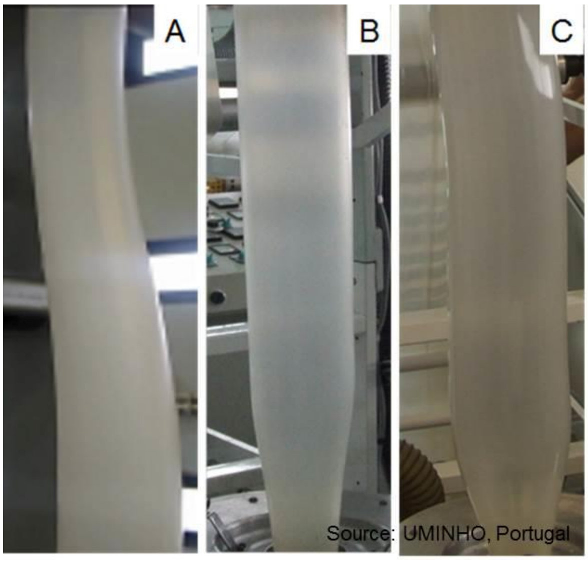

Figure 9: Bubbles blown with Tianan (A), with Tianan as external layer and Ecoflex as internal layer (B) and with Ecoflex as external layer and Tianan as internal layer (C)

Key result n°6

Figure 10

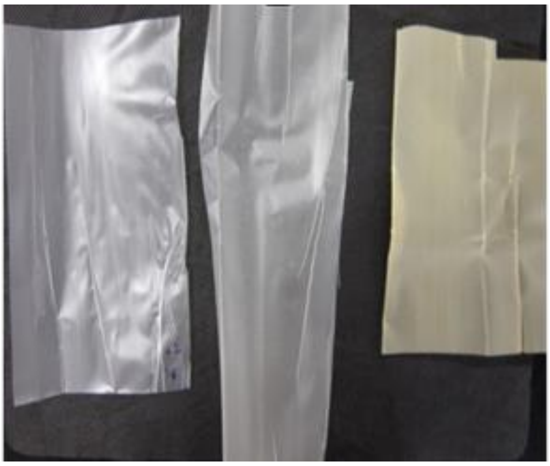

Figure 10: from left to right: PHA, PHA with 2 wt% BSGF and PHA with 5 wt% BSGF (UMINHO)

Figure 11

Figure 11: Cast film extrusion of PHBV

Key result n°7

Figure 12

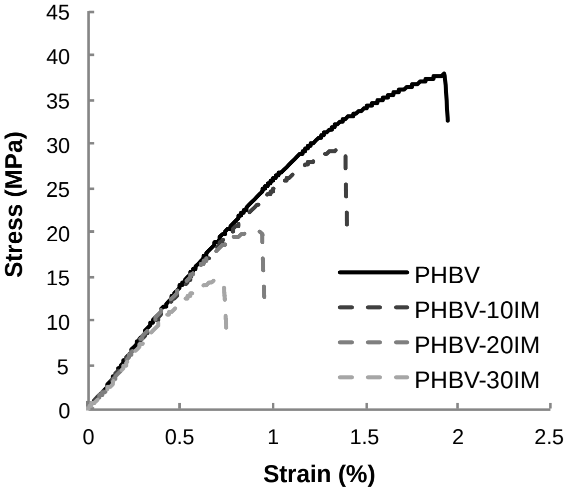

Figure 12. Typical curves of the stress vs. the strain for the neat PHBV matrix and PHBV-based composites filled with 10, 20 and 30wt% of impact milled wheat straw fibres (100-150 µm), noted PHBV-10IM, PHBV-20IM and PHBV-30IM respectively. From Berthet et al. (2015)[1]

Figure 13

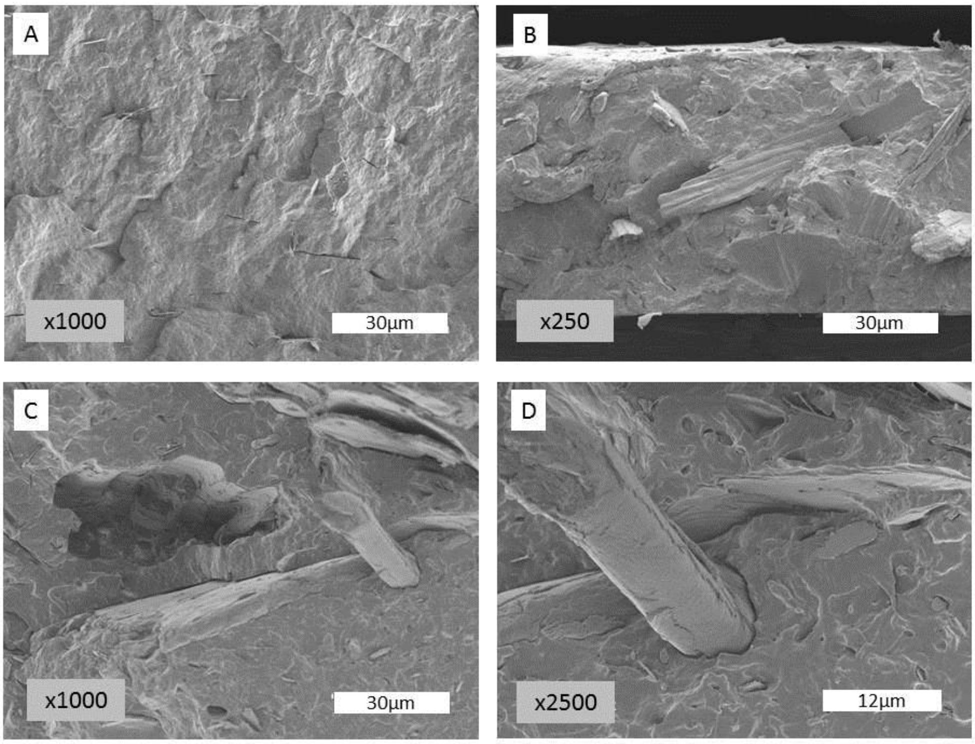

Figure13. SEM pictures of cryo-fractured surfaces of EcoBioCAP PHBV-based materials: A) PHBV (matrix), B, C & D) PHBV-20WSF.

[1] Berthet M.A., Angellier-Coussy H., Chea V., Guillard V., Gastaldi E., Gontard N. "Sustainable food packaging: Valorising wheat straw fibres for tuning PHBV-based composites properties", *Composites Part A: Applied Science and Manufacturing*, 2015, 72, 139-147.

Figure 14

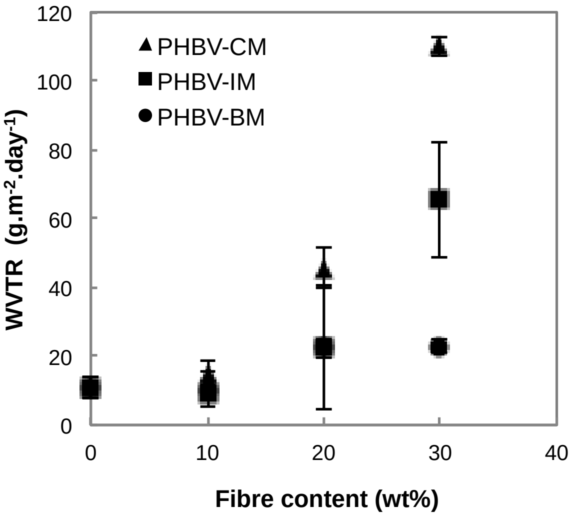

Figure 14. Evolution of the water vapour permeability as a function of wheat straw fibre size & content (standard deviation bars are not visible when they are lower than the symbol size). Figure 7. Water vapour sorption isotherms of the neat PHBV (♦) and impact milled wheat straw fibres (■). Standard deviation bars are not visible when they are lower than the symbol size. From Berthet et al. (2015)[2]

Table 2

Table 2: Results of the tensile tests for the multilayer setups in machine direction

Sample	Setup	Young's Modulus [N/mm²]	Tensile strength [N/mm²]	Elongation at break [%]
0	PHBV	4350 ± 249	36,2 ± 2,7	1,0 ± 0,1
1	Ecoflex/ PHBV/ Ecoflex	1960 ± 346	17,1 ± 1,9	4,0 ± 3,1
2	Ecoflex/ PHBV + WSF + Ecoflex/ Ecoflex	1400 ± 129	11,6 ± 0,5	3,6 ± 1,1

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[2] Berthet M.A., Angellier-Coussy H., Chea V., Guillard V., Gastaldi E., Gontard N. "Sustainable food packaging: Valorising wheat straw fibres for tuning PHBV-based composites properties", *Composites Part A: Applied Science and Manufacturing*, 2015, 72, 139-147.

Table 3

Table 3: Results of the permeability measurements for the multilayer setups

Sample	Setup	Overall thickness	Layer thickness distribution	WVTR 23/85 [g m^{-2} d^{-1}] Q (Q for 100µm)	OTR 23/50 [cm^3 (STP) m^{-2} d^{-1} bar^{-1}] Q (Q for 100µm)
0	PHBV (Tianan)	82		1,84 ± 0,1 (1,6 ± 0,1)	10 ± 1 (8 ± 0,8)
1	Ecoflex/ PHBV/ Ecoflex	300	72 / 159 / 69	2,8 ± 0,1 (9,6 ± 0,4)	10 ± 5 (51 ± 24)
2	Ecoflex/ PHBV+ WSF + Ecoflex/ Ecoflex	362	88 / 181 / 93	3,6 ± 0,2 (17,1 ± 0,8)	16 ± 3 (87 ± 15)

Figure 15

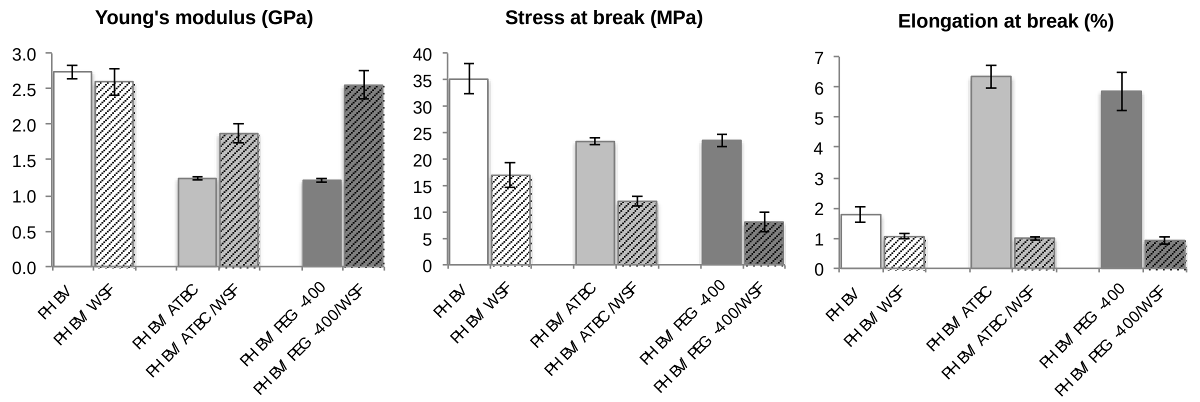

Figure 15. Mechanical properties of composite PHBV-based films filled with 20% (w/w) of wheat straw fibres (WSF). For comparison purposes, the corresponding PHBV-based matrix is also reported. The error bars represent the standard deviations. From Martino et al. (2015)[3]

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[3] Martino L., Berthet M.A., Angellier-Coussy H., Gontard N. "Understanding external plasticization of melt extruded PHBV-wheat straw fibres biodegradable composites for food packaging applications", *Journal of Applied Polymer Science*, 2015, 132, 41611.

Table 4

Table 4 : Results of the tensile tests for the blended samples in machine direction

Sample	Amount of blend partner [%]	Young's Modulus [N/mm²]	Tensile strength [N/mm²]	Elongation at break [%]
PHBV		4350 ± 249	36 ± 2.	1.0 ± 0.5
PHBV + MaterBi	20	3360 ± 278	31 ± 2.6	1.1 ± 0.1
PHBV + Ecoflex	20	2990 ± 273	31 ± 2.6	1.7 ± 0.2
PHBV + Ecovio	20	3420 ± 213	33 ± 2.7	1.1 ± 0.1
PHBV + PCL	20	3310 ± 234	37 ± 1.4	1.8 ± 0.2
PHBV + EVA	20	2640 ± 83	33 ± 1.5	1.8 ± 0.2

Table 5

Table 1 : Results of the permeability measurements for the blended samples

Sample	Amount of blend partner [%]	Layer thickness [µm]	WVP 23/85 [g 100µm m^{-2} d^{-1} bar]	OP 23/50 [cm^3 100µm (STP) m^{-2} d^{-1} bar^{-1}]
PHBV		82	67 ± 4.2	8 ± 1.1
PHBV + MaterBi	20	79	284 ± 16	36 ± 1.2
PHBV + Ecoflex	20	79	276 ± 13	34 ± 2.9
PHBV + Ecovio	20	90	268 ± 13	35 ± 2.3
PHBV + PCL	20	93	176 ± 8.4	29 ± 1.3
PHBV + EVA	20	76	364 ± 0	47 ± 3.5

Figure 16

Figure 16: EcoBioCAP injection moulded trays of PHBV

Figure 17

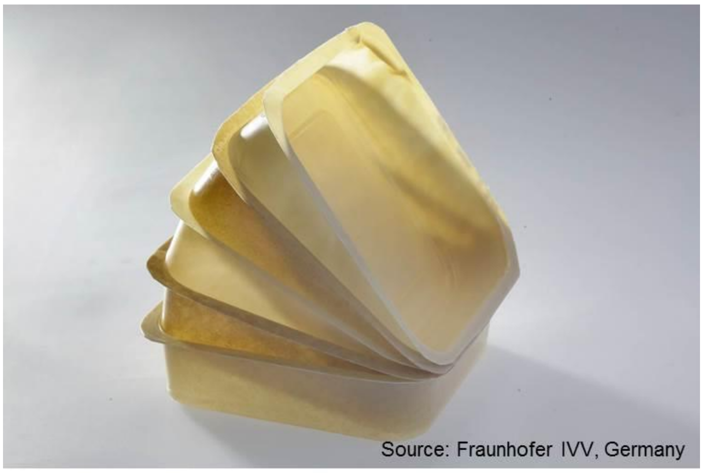

Figure 17: EcoBioCAP thermoformed trays of PHBV

Key results N°8

Figure 18

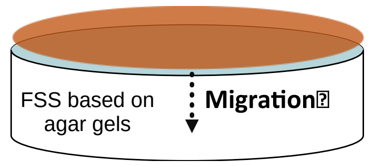

Figure 18 : EcoBioCAP experimental setup for the evaluation of the stability and the migration in Food Simulating Solids.

Reference 1

Mauricio-Iglesias M. et al. (2011) Raman depth-profiling characterisation of a migrant diffusion in a polymer. *Journal of Membrane Science*, 375, 2, 165-171. Martinez-Lopez B. et al. (2014) Determination of Mass Transport Properties in Food/Packaging Systems by Local Measurement with Raman Microspectroscopy. *Journal of Applied Polymer Science*, 131, 21.

Figure 19

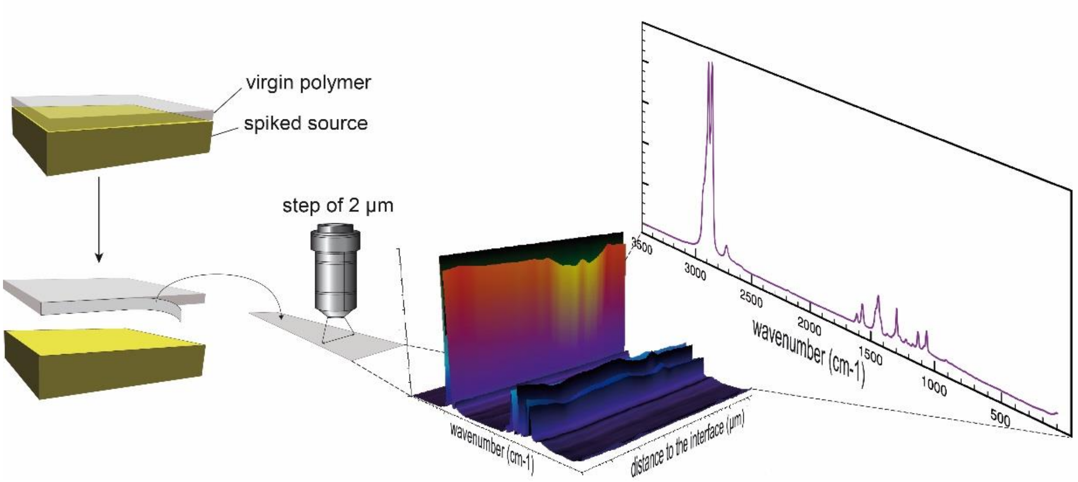

Figure 19. Experimental setup for the characterisation of concentration profile in surrogate using Raman micro-spectroscopy.

Reference 2

Kristo E. et al. (2008).Thermal, mechanical and water vapor barrier properties of sodium caseinate films containing antimicrobials and their inhibitory action on Listeria monocytogenes. Food Hydrocolloids, 22, 373–386.

Figure 20

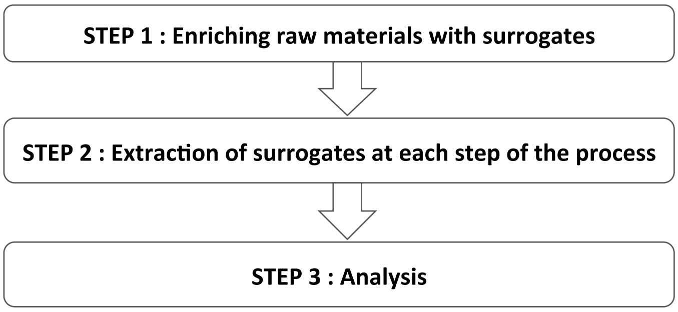

Figure 20. General successive steps for EcoBioCAP challenge tests.

Reference 3

Thompson T.S. et al. (2011) *Food Chemistry* 127, 321.

Reference 4

Zhan J. et al. (2012) Generic and rapid determination of veterinary drug residues and other contaminants in raw milk by ultra performance liquid chromatography–tandem mass spectrometry. *Journal of Chromatography B*, 906, 48-57.

Reference 5

Luzardo O.P. et al. (2012) Polychlorobiphenyls and organochlorine pesticides in conventional and organic brands of milk: Occurrence and dietary intake in the population of the Canary Islands (Spain). *Chemosphere* 88, 307-315.

Figure 21

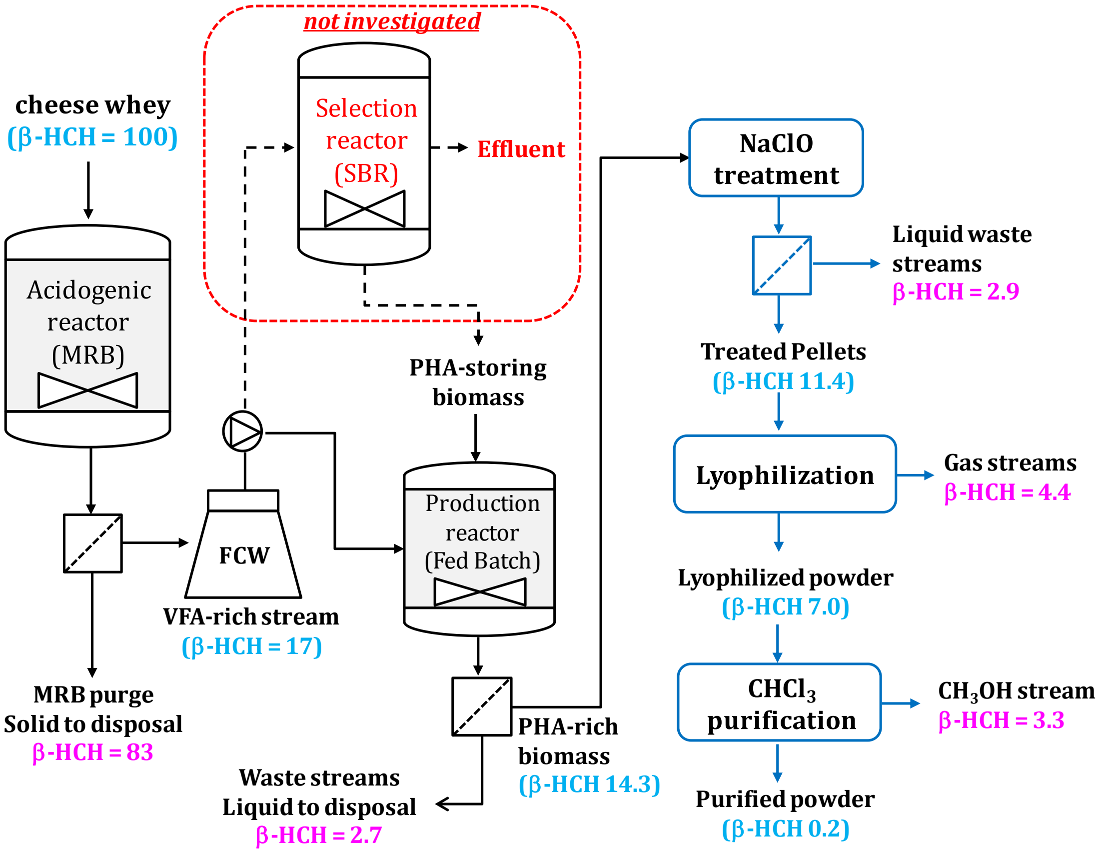

Figure 21: Mass balance of HCH during the EcoBioCAP PHA production process

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Figure 22

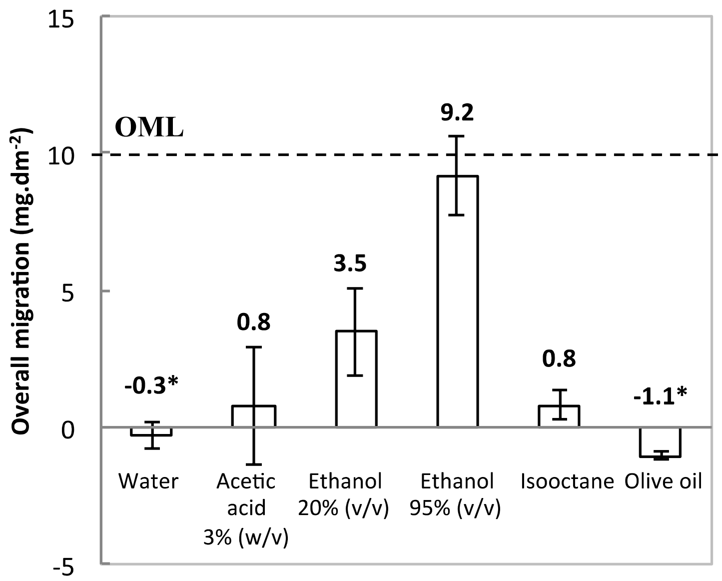

Figure 22. Overall migration of PHBV in food simulant liquids (water, acetic acid 3% (w/v), ethanol 20% (v/v), ethanol 95% (v/v), iso-octane and olive oil). OML is the overall migration limit (10 mg.dm-2 of film surface area) set out by the Commission Regulation (EU) N° 10/2011. * The negative value is within the analytical tolerance of the method. No overall migration was detectable.

Table 6

Table 6. Tensile properties and water vapour permeability (WVP) of PHBV films before (control) and after immersion in different food simulant liquids (FSL).

FSL	Elongation at break (%)	Stress at break (MPa)	Young's modulus (GPa)	WVP ($\times 10^{-13}$ mol.m.m^{-2}.s^{-1}.Pa^{-1})
Control	2.8 ± 0.3	29.0 ± 2.0	2.5 ± 0.2	5.8 ± 1.5
Water	2.3 ± 0.3	33.2 ± 3.5	2.5 ± 0.1	8.2 ± 2.2
Acetic acid 3% (w/v)	2.5 ± 0.2	27.6 ± 1.7	2.6 ± 0.1	-
Ethanol 20% (v/v)	2.8 ± 0.3	26.9 ± 1.9	2.4 ± 0.2	-
Ethanol 95% (v/v)	3.6 ± 0.1	22.6 ± 1.4	1.7 ± 0.1	9.9 ± 3.1
Isooctane	2.4 ± 0.1	28.4 ± 2.2	2.7 ± 0.0	-

Reference 6

Chea V. et al. (2015) Poly (3-hydroxybutyrate-co-3-hydroxyvalerate) films for food packaging: Physical-chemical and structural stability under food contact conditions, *Journal of Applied Polymer Science,* DOI: 10.1002/app.41850.

Figure 23

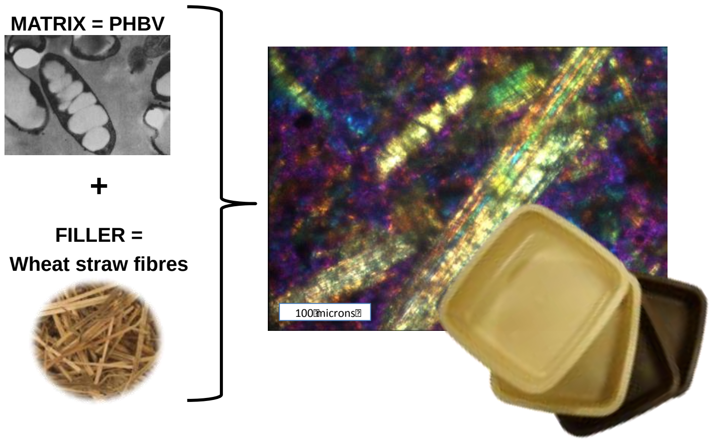

Figure 23. EcoBioCAP PHBV (Tianan grade)/wheat straw fibres biocomposites.

Figure 24

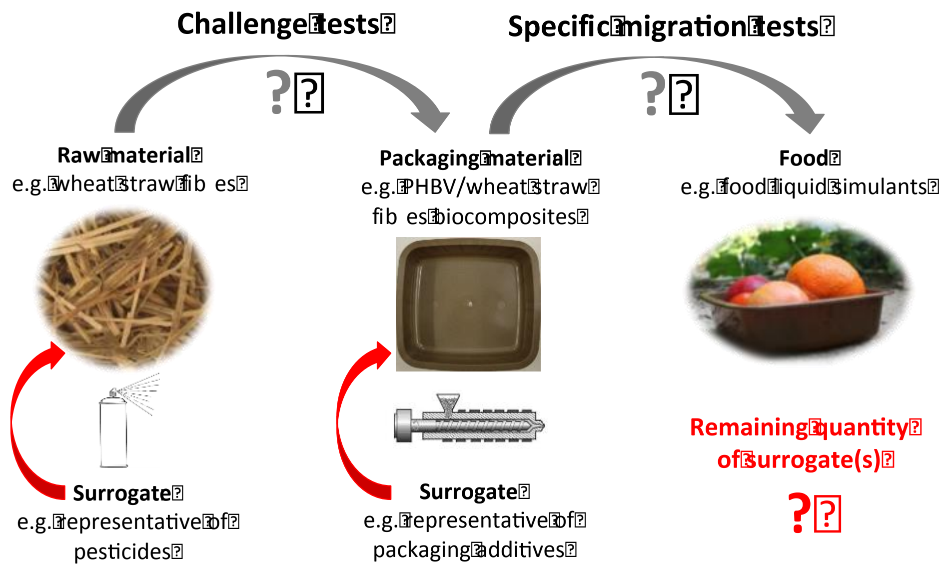

Figure 24. EcoBioCAP strategy to study the chemical safety by coupling challenge tests and specific migration tests.

Figure 25

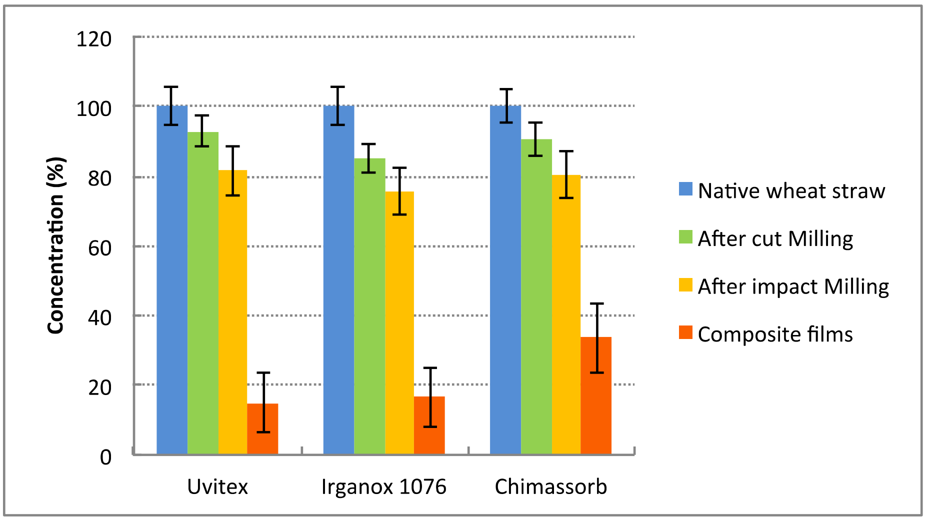

Figure 25. Decontamination efficiency of the EcoBioCAP processes used to produce composite films based on wheat straw fibres : Evolution of the concentration of surrogates in wheat straw fibres at each processing step.

Figure 26

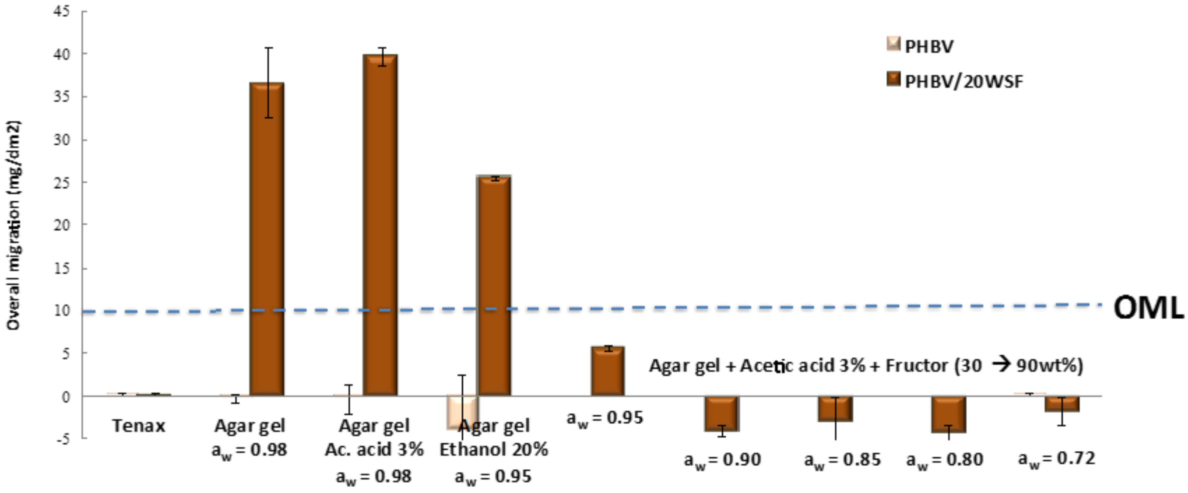

Figure 26. Overall migration results of PHBV and PHBV/WSF films (fibre content of 20 wt% - thickness of 250 µm) under contact with FSS.

Reference 7

Berthet M.A. et al. (2015) Sustainable food packaging: Valorising wheat straw fibres for tuning PHBV-based composites properties", *Composites Part A: Applied Science and Manufacturing*, 10.1016/j.compositesa.2015.02.006.

Figure 27

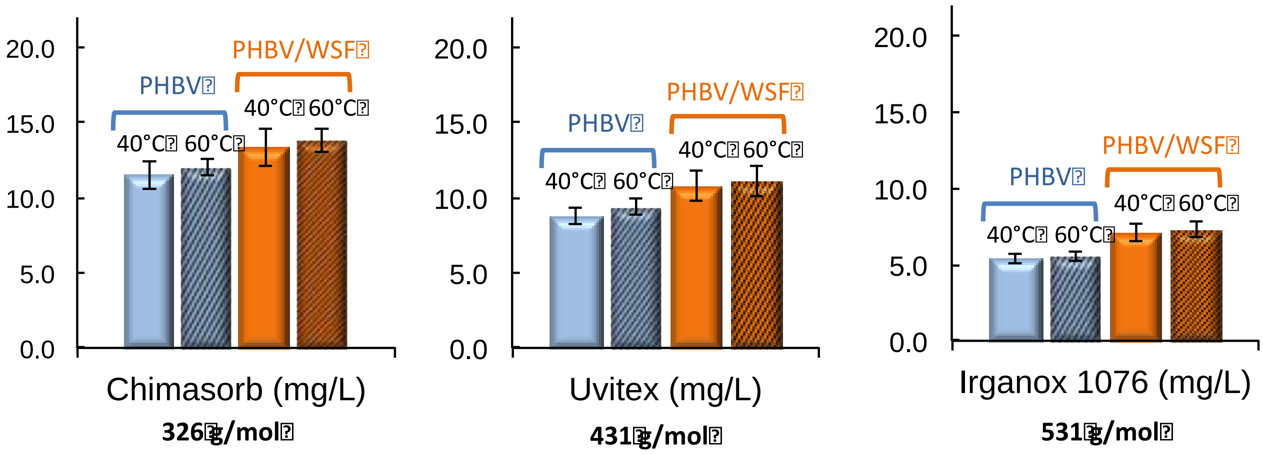

Figure 27. Specific migration levels of surrogates in ethanol 95% (v/v) at 40°C and 60°C of PHBV and PHBV/WSF films (fibre content of 20 wt% - thickness of 250 µm).

Key result n° 9:

Figure 28

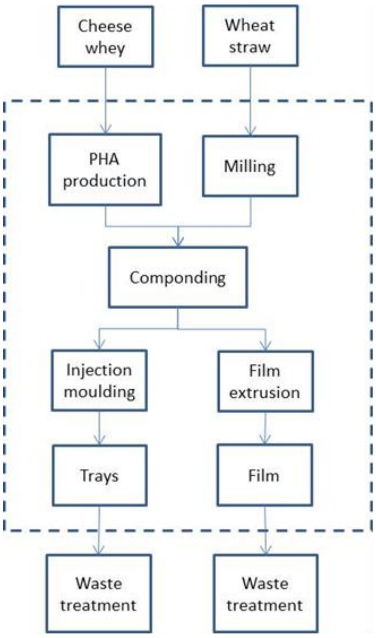

Figure 28. Flowchart of production of EcoBioCAP PHA/fibres materials

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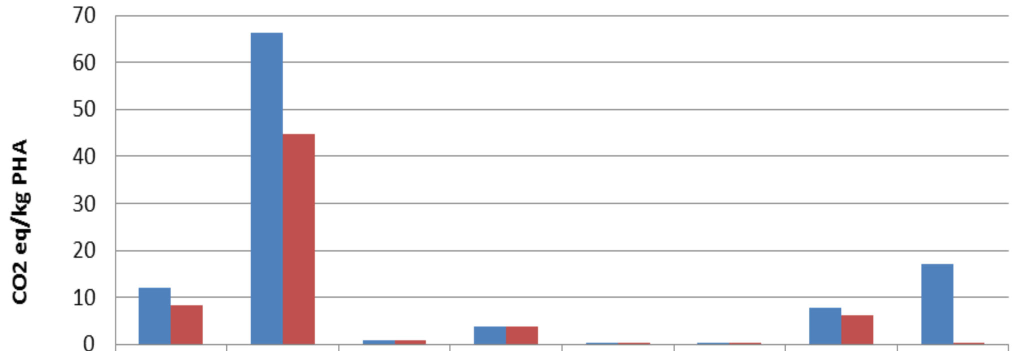
Figure 29



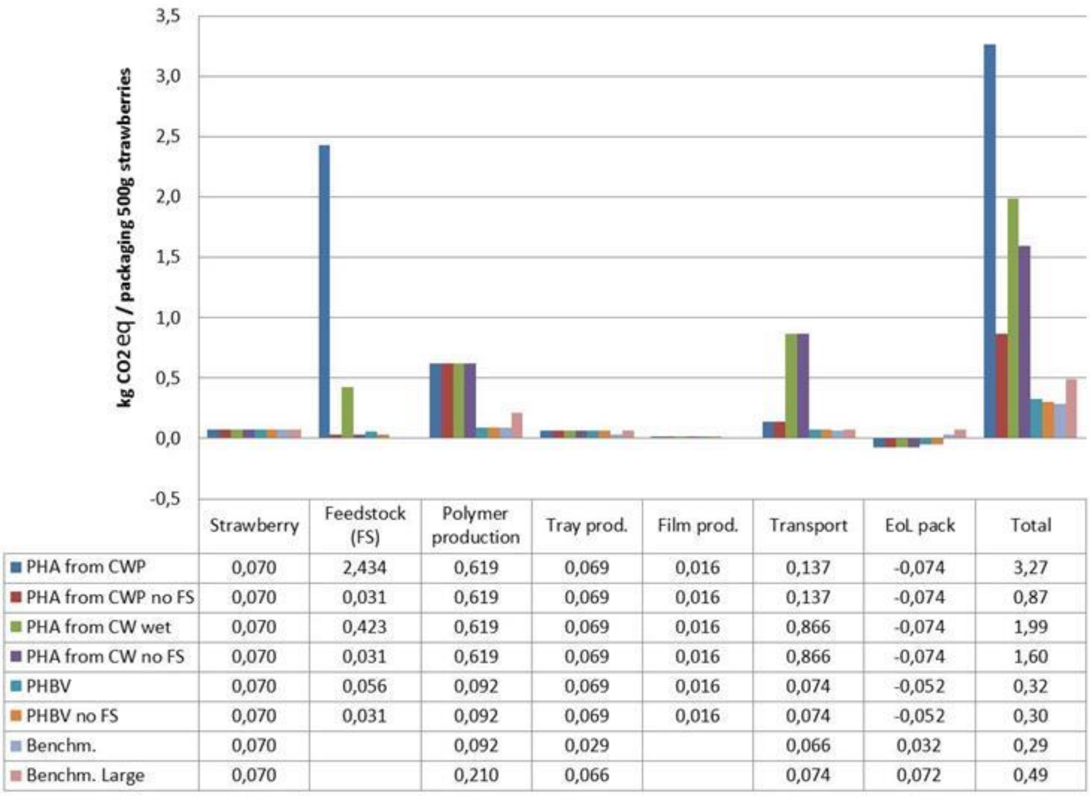
	whey	spray drying	tap water	chemicals	P nutrient	N nutrient	production energy	waste water treatment
Scen.1	12,2	66,3	1,0	3,7	0,2	0,4	7,7	17,1
Scen.2	8,2	44,8	0,8	3,7	0,2	0,4	6,3	0,5

Figure 29. The impact to climate change in CO2 equivalents from the production of 1 kg PHA polymer.

Figure 30

Figure 30. The packed strawberries in EcoBioCap packaging, left, and the selected benchmark packaging, right.

Figure 31



	Strawberry	Feedstock (FS)	Polymer production	Tray prod.	Film prod.	Transport	EoL pack	Total
PHA from CWP	0,070	2,434	0,619	0,069	0,016	0,137	-0,074	3,27
PHA from CWP no FS	0,070	0,031	0,619	0,069	0,016	0,137	-0,074	0,87
PHA from CW wet	0,070	0,423	0,619	0,069	0,016	0,866	-0,074	1,99
PHA from CW no FS	0,070	0,031	0,619	0,069	0,016	0,866	-0,074	1,60
PHBV	0,070	0,056	0,092	0,069	0,016	0,074	-0,052	0,32
PHBV no FS	0,070	0,031	0,092	0,069	0,016	0,074	-0,052	0,30
Benchm.	0,070		0,092	0,029		0,066	0,032	0,29
Benchm. Large	0,070		0,210	0,066		0,074	0,072	0,49

Figure 31. The climate impact from the different life cycle stages for the different packaging scenarios, expressed as kgCO2eq/FU

Key result 10

Figure 32

Sample of **PHBV** after 22 days of test

Sample of **PHBV+20WSF** after 22 days of test

Sample of **PHBV** after 70 days of test

Sample of **PHBV+20WSF** after 70 days of test

Figure 32. Pictures documenting the disintegration process on provided EcoBioCAP materials

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Table 7

Table 7. Results after 78 days of biodegradability test

Test Material	% Biodegradation	% Relative Bio.
PHBV Enmat Y1000P	93,6	98
PHBV + 20% WSF	97,6	>100
Cellulose (Positive control)	95,4	100

Table 8

Table 8. Results after 55 days of biodegradability test

Test Material	% Biodegradation	% Relative Bio.
PHBV Enmat Y1000P	77,6 (±13,5)	78
Cellulose (Positive control)	98,8 (±6,9)	100

Table 9

Table 9. Combustion enthalpies of the EcoBioCAP PHBV materials

Sample	Calorific combustion enthalpy (MJ/Kg)
PHBV Control	23.0
PHBV AUATBC	23.4
PHBV 10ATBC	22.5
PHBV 20WSF	22.3
Cellophane	46.3
EcoFlex	25.0

Table 10

Table 10. Characteristics of the material tested

Acronym	Provenience	Main Features		
		Organic source	Composition/ Additives	Thickness
PHBV	INRA / Tianan		3% HV 1 % nucleating agent (boron nitride) 0,5% antioxidant (Irganox 1010?)	149 +/-21 µm
composite	INRA	Wheat bran	(any pesticide?)	194 +/- 29 µm
PHBV3+3%BCNW FD	CSIC / Tianan		3% bacterial cellulose nanowhiskers	100-150 µm
multilayer WG+PHBV3	CSIC / Tianan		outer layers: Tianan films Inner layers: wheat gluten	100-150 µm
PHB12	CSIC / Biomer		12% HV Plastification: 10% citric ester	100-150 µm
PHB12+10% wt PCW	CSIC / Biomer		Additive: nanokeratin	100-150 µm

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Key result 12

Figure 33

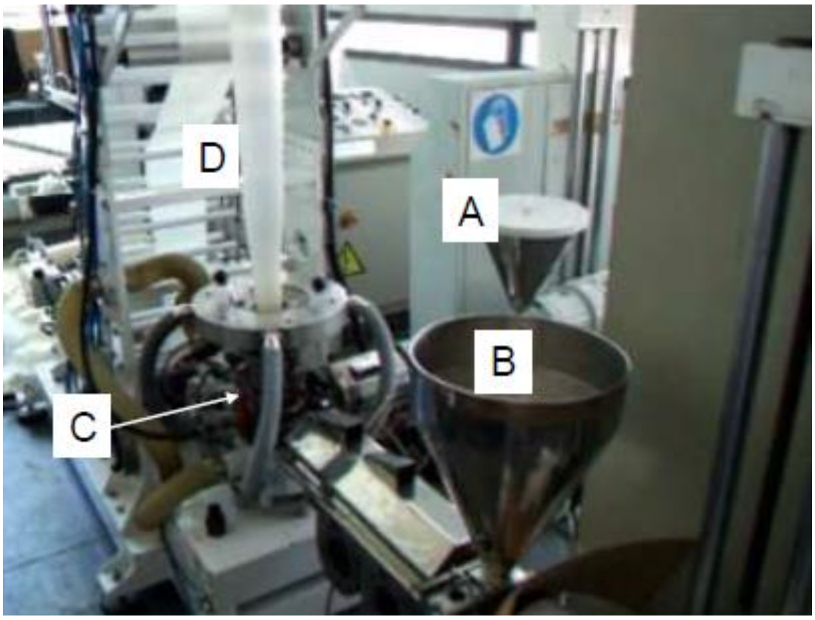

Figure 33. The small scale film blowing production line for bi-layer films. The small scale film blowing production line for bi-layer films consists in one moveable single screw extruder (D=25 mm, L/D=20) for the external layer (A) with temperature profile 150 ºC-155 ºC-155ºC and a second identical extruder (B) for the internal layer set with identical temperature profile. The two extruders are connected to a co-extrusion tubular die (C) with temperature profile 155 ºC - 155 ºC - 160 ºC, diameter of 50 mm, and die gap of 1.5 mm

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Key result 14

Figure 34

Figure 34- Large pilot-scale production of EcoBioCAP bilayer film.

Figure 35

Figure 35- Large pilot-scale production of EcoBioCAP composite tray.

Figure 36

Figure 36- Strawberries packaged in EcoBioCAP trays and flexible film ready for shipping

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Figure 37

Figure 37- Strawberries for acceptance test in biodegradable and in benchmark

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Reference 8

So far, the only alternative tests that could be recommended for such materials should be derived from either paper testing methods or active packaging. Paper and board materials are among the oldest biodegradable packaging materials but are not yet regulated by a specific Directive. The only alternative test recommended to determine migration from paper containing potential organic migrants considers the use of Tenax ™ [modified poly(phenylene oxide); Varian, Houten, the Netherlands] according to the conditions set in the European Committee for Standardization norm CEN/TC 172, as successfully demonstrated by Nerin and Asensio (2007). However, such a limited approach fails to reproduce the variety of foodstuff packed in these materials and only imitates dry fatty products. Furthermore, the use such an approach of rises up the problem of contact at the interface with the materials since Tenax™ is in the form of powder. In the case of active packaging such as oxygen scavenger, a dedicated test was developed to perform an overall migration with filter paper soaked with simulant (Dainelli et al., 2010). This method showed much lower and more realistic overall migration than with the conventional test according to Directives 82/711/EEC and 85/572/EEC. However, this method still uses FSL and is not really representative of solid products.

Nerin, C. Asensio, E. 2007. Migration of organic compounds from a multilayer plastic– paper material intended for food packaging. *Analitical and Bioanalitical Chemistry* 389, 589-596.

Dainelli D., Gontard N. et al. (2008) “Active and intelligent food packaging: legal aspects and safety concerns”, Trends Food Sci. Technol., 19, 99-108. In this test, samples are wrapped with the filter paper, which is saturated with simulant and placed between glass plates. To simulate the possible weight of food a total weight of 70g (including the glass plate of 20g) is placed on top of the package. The whole package including weight is placed in the oven and stored for the correct time/temperature conditions. After the storage period the filter paper is extracted with the food simulant to determine the overall or specific migration.
