*FP7-COOPERATION*-THEME 4

*NANOSCIENCES, NANOTECHNOLOGIES, MATERIALS AND*

*NEW PRODUCTION TECHNOLOGIES -NMP*

**New sustainable, functionalized and competitive PHB material based in fruit by-products getting advanced solutions for packaging and non-packaging applications**

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# PROJECT FINAL REPORT

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## Executive summary

PHBOTTLE project was a four-year project funded under the European Commission's Seventh Framework Programme (FP7), specifically under the programme "NMP – Nanosciences, Nanotechnologies, Materials and New Production Technologies". It started on May 2012 and finished on April 2016. The project consortium was formed by eleven partners from Europe and Latin-America: AINIA (Project coordinator, Spain), AIMPLAS (Spain), TNO (The Netherlands), INTI (Argentina), Logoplaste Innovation Lab (Portugal), Citresa (Spain), MEGAEMPACK (Mexico), Logoplaste do Brasil (Brazil), OMNIFORM (Belgium), Sivel (Bulgaria) and AIJN (Belgium).

PHBOTTLE project aimed to develop a new bottle (body, cap and sleeve) from a biodegradable polymeric material, PHB (polyhydroxybutyrate) which was obtained by fermentation of wastewater produced by the fruit juice industries (renewable biogenic resource). The properties of the PHB were improved by the incorporation of cellulose fibres (obtained from natural sources) and encapsulated ingredients with antioxidant properties. The aim was to develop an active packaging that increased the shelf-life of the packaged food. The new bottle was used to package the juice manufactured by the wastewater generator. Thus, the loop was closed: the wastes generator became the beneficiary of the new polymeric bottle. Besides, other applications were also studied such as non-food packaging and non-packaging (pieces for the automotive sector).

The main results achieved in the project were:

- Identification and selection of the wastewater stream from the fruit juice industry (Citresa) to be used as raw material.
- Selection of the PHB producing microorganisms and the creation of a culture collection.
- Development of the PHB bioproduction and recovery processes at different scales (lab and pilot plant); optimization of both bioproduction and recovery processes.
- Development of microfibrillated cellulose fibres for PHB compounds: selection of the cellulose source, isolation of microfibrillated cellulose from treated fibres and fibre surface modification for compatibilization of PHB.
- Development of microcapsules with natural antioxidant (limonene) to incorporate into PHB for obtaining an active packaging.
- Modification of the PHB polymer. Development of active packaging: development of different PHB compounds, containing cellulose fibres, antioxidant capsules and other additives and materials, for different package manufacturing processes and applications.
- Package manufacturing and validation: design of the PHBOTTLE, processing of the developed PHB compounds at pilot plant scale by different technologies (extrusion-blow moulding, injection-blow moulding, monolayer blow moulding…), validation of the processing of the PHB compounds at industrial scale (manufacturing of bottles made by extrusion-blow moulding and closures by injection moulding); packaged of orange juice in the PHB bottles and study of the physical-chemical and microbiological parameters.
- Validation of the PHB compounds for other applications: manufacturing of trays with a thin wall made by injection moulding and thermoforming; manufacturing of caps for car batteries made by injection technology.
- Eco-efficiency performance of PHBOTTLE: characterisation of the potential biodegradability of the PHB compounds, study of the environmental (LCA) and economic (LCC) assessments.

## Summary description of project context and objectives

In 2014, around 9,702 million litres of juice and nectars were consumed in the European Union. 60% of total volume consumed was packaged in carton, 25% in PET and the rest was packaged in glass and other formats. Environmental sustainability concerns are rising on the public agenda becoming more relevant to the consumers. The EU juice and nectars industry is aware of its important role and responsibility, making use of best practice which involves full Life Cycle Assessment (LCA) to reduce both carbon and water footprint.

As other food sectors, fruit and nectar industries are seeking more environmentally friendly packaging for their products. Meanwhile, packaging innovations is getting ahead of developments in the use of alternative materials and recycling. Therefore, there is a need to join efforts between packaging companies, recycling operators and governments to maximise the latest technology.

The use of conventional polymeric materials, based on oil, in packaging represents an important environmental impact and waste generation due to their non-biodegradability. Over 67 million tonnes of packaging waste is generated annually in the European Union, which correspond to around one-third of all municipal solid waste. In developed countries, food packaging represents 60% of all packaging. Food packaging has become the most obvious source of waste generated by the public.

Some alternative materials obtained from renewable resources can be found in the market. These materials are usually produced from renewable sources such as sugar cane or starch, using the products of the crops fields like raw materials instead of food for humanity. Therefore, it is necessary to search new sustainable raw materials for the production of this kind of alternative plastic materials.

On one hand, the application of biodegradable food packaging material can reduce energy use and carbon dioxide emissions as well as it will reduce waste treatment cost. On the other hand, food industry wastewater treatment is costly due to energy needs in the aeration basin and the costs of waste sludge disposal. Thus, recovering valuable materials from raw wastewaters will lead to a reduction in treatment and disposal costs. Both aspects fulfil the Europe 2020 strategy: both address the priorities of the strategy, especially smart growth and sustainable growth. And both particularly deal with innovation union and resource efficient Europe flagship initiative.

Joining the two previous concepts, PHBOTTLE project aimed to develop a biodegradable material, concretely PHB (polyhydroxybutyrate) by fermentation of food industry by-products such as juice processing wastewater. The main target has been for applications in the food packaging sector, mainly juice industry, one of the widest markets in Europe. From this material (PHB) a new package has been developed: bottle and cap. However, its use in other applications such as non-food packaging and non-packaging (automotive sector) were also tested.

The selection of the wastewater produced by the fruit juice industries is due to the fact that it contains high amounts of organic pollution like free carbohydrates, mainly fermentable sugars such as glucose, fructose or maltose. The concentration of fermentable sugars in juice processing wastewater can reach up to 70% of the total organic load, containing almost 20 g/l of fermentable sugars. Therefore, this kind of wastewater was considered a good candidate as cheap feedstock for PHB bioproduction.

Also, the management of the wastewaters in the juice industry is quite important due to their environmental impact. Taking advantage of the wastewaters capacity to be used as culture medium for PHB bioproduction, the environmental impact of these wastes can be reduced. The wastewaters can be considered as a valuable raw material instead of wastes.

The research has been focused on PHB bioproduction alternatives using juice industry by-products as culture medium, optimising energy and eco-efficiency. Besides, the packaging material properties were improved through the use of functional materials such as cellulose microfibers and encapsulated antioxidants.

The focus was placed on PHB because of its special properties and market potential. It has useful properties, similar to the petrol-based plastic polypropylene (PP): moisture resistance, lower water vapour permeability, water insolubility, optical purity and good oxygen barrier.

To achieve the main objective of PHBOTTLE projects, that was to develop a new sustainable, functionalized and competitive PHB material based on fruit by-products getting advanced solutions for packaging and non-packaging applications, several specific objectives were established:

1. To obtain raw materials and products with a competitive price compared to existing solutions: to decrease bioplastic processing cost by using industrial by-products as carbon sources for producing PHB. To get it, the project managed the following aspects:

- To use a cheap culture medium (wastewater).

- To select the optimal PHB producing microorganisms.

- To establish optimal fermentation strategies to enhance the PHB production and optimising the PHB bioproduction process at pilot plant scale.

- To develop more efficient PHB recovery process.

- To reduce management costs, both plastic waste management costs and waste management costs from the food processing sector by valuation of fermentable organic matter contained in food processing by-products.

2. To overcome some actual technical limitations through functionalisation: fruit juices have, as spoilage factors, oxidative reactions with vitamin losses and they need packages that withstand distribution loads and thermal treatments. The technical objectives to achieve this were:

- To reduce the oxidation reactions of the packaged fruit juice by means of the functionalisation of PHB material with encapsulated antioxidants. These microcapsules allow preserving thermo-sensitive ingredients (antioxidants) during polymer transformation processes (extrusion or injection) and controlling the release of the food ingredient during the shelf-life of the juice.

- To use surface modification of cellulosic fibres materials as a way to increase mechanical resistance. The enzymatic approach achieves novel and eco-friendly micro-sized materials from cellulosic biomass against traditional methods.

- To maintain processing windows compared to conventional polymers. The new PHBOTTLE material was developed to be processed by conventional equipments available in the industry. The use of cellulose fibres helped to overcome brittleness of pieces formed with PHB.

3. To improve packaging sustainability in terms of:

- To reduce environmental impact through the use of non-oil based materials, contributing to the reduction of greenhouse emissions.

- To improve packaging residues valorisation via reuse, recyclability, biodegradability and compostability.

- To give European consumers biodegradable packaging materials which guarantee stability, safety and quality of the food packaged product during their shelf-life.

- To reduce wastewaters treatment in terms of volume and/or loading, which have to be treated before their final discharge and/or to decrease solid waste disposal and management problems involved, reducing treatment costs.

4. To develop a useful material for applications beyond juice packaging: the development of the PHBOTTLE material was focused on to be suitable for other industries or markets apart from the food packaging sector (i.e. pharma, cosmetic, home care …). Besides, non-packaging applications such as pieces for automotive sector was also considered.

5. To promote an active participation of industrial partners as capital added value:

- To facilitate the collaboration between RTDs and industries reinforcing the packaging industry by means of new materials development.

- To facilitate the technology transfer of the developed materials for new uses and markets.

- To improve European competitiveness among biodegradable polymeric materials manufacturers.

- New opportunities of collaboration between centres and companies form Europe and Latin America countries.

## Description of the main S & T results/foreground

The execution of the planned activities of the project achieved the following main results:

- Selected culture medium (wastewater from the fruit juice industry) and microorganism to produce PHB.
- Produced and extracted PHB using as culture medium the selected wastewater.
- Microencapsulated antioxidants to be incorporated to PHB matrix in order to contribute to advance packaging material development, delivering antioxidants additives to the packaged juice.
- Modified cellulose fibres that were incorporated into PHB biopolymer and contributed to advanced packaging material development, improving the mechanical properties.
- PHB developed composite (master) which was the material to be used by the polymeric material transformers. Different PHB composites were obtained depending on the manufacturing processes for obtaining the final solutions.
- Design of the bottle concept based on the study of the mechanical properties of the PHB composite.
- Manufacturing of PHB bottles, by extrusion-blow moulding, and PHB caps by injection to be used in the packaging of the orange juice manufactured by the wastewater generator.
- Processing of the PHB compounds to manufacture different non-food packaging and non-packaging parts through different polymer processing technologies.
- Full eco-efficiency performance of the PHB bottle which determined the viability and reliability of the eco-efficient realization of the production chain of the PHBOTTLE and its substances.

To achieve all of them, different challenging activities were carried out following the planned schedule. The first target of the PHBOTTLE project was to select the suitable wastewater streams from the fruit juice processing industry to be used as raw material in the subsequent fermentation processes. After the identification of the wastewater streams generated by process operations in CITRESA (including processing, cleaning and disinfection), a physical-chemical characterization of those streams was done in order to know the organic matter concentration (measured as Chemical Oxygen Demand *COD*, Soluble Chemical Oxygen Demand *sCOD* and Biological Oxygen Demand $BOD_5$), sugar content (measured by Brix degrees and total sugars), nutrients concentrations like nitrogen and phosphorus and volatile fatty acids concentrations. Moreover, in this characterization several physical-chemical parameters were also included to identify possible inhibition and toxicity effects (pH, conductivity and suspended solids). The streams that showed higher organic matter and sugar concentrations were those from the CIPs (Cleaning in Place) points. CIP cleaning is utilized to clean interior surface of tanks and pipelines of liquid process equipment. Therefore, the pre-rinsing CIP discharges from lemon and orange juice were identified as possible substrate (carbon source) for the PHB producer microorganisms. Besides, these identified streams are the most important ones in terms of flow in the CITRESA Company as well as in the citrus juice sector. This characteristic offers benefits to industrial scale-up and technology transfer.

Once the suitable wastewater streams were identified, the next step was to identify and select the microorganisms able to accumulate PHB using these streams as source of carbon. The criteria of selection were based on: (i) referenced PHB producers; (ii) referenced PHB producers from wastes; (iii) referenced high yield and short time of cultivation; (iv) available in cell culture collection. Following these criteria, 9 microorganisms were selected. Besides, an experimental design for carrying out the screening of the microorganisms was set up. This experimental design was divided into three steps: 1st, preparation of the inoculum in the basic media (nutritive media) at the optima temperature for each microorganism during 72 hours; 2nd, seeding the inoculum in the PHB producing media in a 96-multiwell plate; 3rd, reading the accumulated PHB in the microorganisms after the incubation at the optima temperature. An indirect method based on dye with Nile Red was used. The cellular growth was analysed by absorbance reading at 595 nm. The screening was developed based on the use of wastewater as carbon source and then two options were analyzed. One option was based on the preparation of mixtures of citrus wastewater and the synthetic media selected previously (different percentages from 0% to 100% citrus wastewater). And the another option was focused on the use of the citrus wastewater as solvent of the culture media and then wastewater was supplemented with nutrients (nitrogen source and minerals in the same concentration that synthetic media defined). The use of citrus wastewater as solvent of culture media showed better results than the method based on mixtures, so the mixtures method was discarded. One possible reason could be that wastewater was not diluted and then its nutrients were not in low concentration. In the screening based on wastewater as solvent, the effect of glucose supplementation from 1 to 10 g/l in the PHB production was analysed. It was observed that all tested strains were positive in orange wastewater even without supplementation: *Ralstonia eutropha* DSM531, *Bacillus megaterium, Ralstonia eutropha* DSM428, *Azotobacter vinelandii, Burkholderia sacchari* and *Bacillus licheniformis*. Regarding lemon wastewater, only two strains presented positive results: *Bacillus licheniformis* and *Bacillus megaterium*. The screening with the mixture orange and lemon wastewater (50%:50%) showed positive results in the microorganisms *Ralstonia eutropha* DSM531, *Bacillus megaterium, Ralstonia eutropha* DSM 428, *Burkholderia sacchari* and *Bacillus licheniformis*. Due to PHB accumulation in orange wastewater was higher than in citrus wastewater, the further research was done only with orange wastewater.

After that, an optimization of the use of citrus wastewater as carbon source was carried out. For it, an initial selection of strains as PHB producers from wastewater was done. The selection criterion was the ability to accumulate PHB and then higher PHB producer were selected, measured as high levels of fluorescence units. The selected strains were *Ralstonia eutropha* DSM531, *Burkholderia sacchari* and *Bacillus licheniformis.* For each strain, the growth conditions were characterized such as glucose concentration, time and temperature of incubation. Then different orange wastewater culture media were evaluated: (i) without supplementation, (ii) supplemented with nitrogen source and minerals and (iii) supplemented with nitrogen source, minerals and different glucose concentrations. The results showed that the optimum conditions to PHB accumulation depends on the strain. The scale up from microlitres to millilitres confirmed the results of previous experiences: *Burkholderia sacchari* and *Ralstonia eutropha* DSM531 were able to use orange wastewater as carbon source to accumulate PHB. However, *Bacillus licheniformis* reached more PHB accumulation (higher fluorescence units) than *Burkholderia sacchari*. Therefore, the strain *Ralstonia eutropha* DSM531 was selected to study the PHB production at higher scale.

The next step was to study the intensive variables that control the fermentation process for obtaining PHB (time, temperature and substrate additions) and the synthesis of intracellular PHB particles at laboratory scale. The variables studied were initial inoculum, time of process, temperature, addition of substrates and composition of the culture medium. These studies were carried out with two different strains: *Ralstonia eutropha* and *Bacillus megaterium*. The first strain was selected previously as the best producer of PHB in the wastewaters and the second one was considered as a back-up. Therefore, all the trials were performed with *Ralstonia eutropha* strain. The study of optimum temperature confirmed that a higher value of fluorescence units (related to the accumulation of PHB) was obtained at higher temperature than the optimal growing temperature of the strain (30º C). The results also confirmed the statement of the reduction of intracellular PHB after 72 hours of incubation. The study of the optimum process time concluded that maximum accumulation of PHB would be between 48 and 72 hours; after that, the intracellular PHB decreased. Regarding addition of substrates, any enhancement in the intracellular PHB of the cultures was not revealed when substrates (glucose and nitrogen nutrient) were added at 48 hours. Although in this particular scale the addition of glucose and nitrogen nutrient did not have significant effect in the intracellular PHB accumulation, in bigger scales fed batch fermentation strategies were considered.

The optimization of the fermentation conditions for the production of PHB was proved to be successful when the productivity of biomass was enhanced and the accumulation of PHB inside the cells was as high as possible. It was taking into account that PHB synthesis was favoured by environmental stresses conditions such as nitrogen or oxygen limitation. That is why the accumulation stage was studied isolatedly; a series of shake flask two-stage (growing stage and accumulation stage) experiments were performed. Although a high level of viable cells reached the 48 hours of incubation, the cultures with a lack of oxygen showed a decrease in the number of viable cells. This may confirm the need of oxygen for maximize the biomass present in the culture. Regarding the PHB accumulation, the maximum value of fluorescence was reached after 24 hours of incubation in the medium with lack of nitrogen source. It may seem that the samples with excess of oxygen got best results. At the end of the process, the accumulated PHB obtained in the trial with lack of nitrogen was only 25% that indicated the PHB was consumed, possibly due to a lack of carbon source at the end of the process.

Once the intensive variables were studied, the next step was the study of the extensive variables that control the fermentation process and the synthesis of intracellular PHB particles at pilot scale. This study was carried out in a 300 litres bioreactor located at AINIA. The studied variables were aeration and stirring. In addition, different systems for the production of intracellular PHB were considered like (i) batch production, (ii) 2-batch production and (iii) fed-batch production. After the study of the batch production, where the influence of aeration and stirring in the production of PHB was also studied, it was concluded that a high concentration of dissolved oxygen was needed for the enhancement of the biomass weight whereas a low concentration of dissolved oxygen was needed for the enhancement of the intracellular PHB. Thus, a two stages fermentation process may seem the best option.

Taking into account the results of the assays performed regarding the composition of the medium and the fermentation conditions, the best results for the production of intracellular PHB were achieved when the conditions for the production changed depending on the stage that were the culture, that meant the culture being on growth stage or PHB accumulation stage. The fed-batch production system was considered the best strategy for changing these conditions during the process due to the feeding of the culture with a “feeding solution” that changed the composition of the culture media during the process and allowed that the concentration of some critical substrates remained above a critical concentration. Regarding the feeding of the system, two different strategies were tested: (i) continuous feeding where the feeding solution was added continuously into the bioreactor and (ii) regulation by pH where the pH of the process was set at 7.0 and controlled automatically by the system adding feeding solution when it was needed. The results of the performed assays showed that the best strategy for PHB production was the fed-batch with feeding regulated by pH.

Apart from the study of the PHB production, the recovery process was also studied. This study was one of the most challenging activities because it was noticed that the final quality of the produced

PHB depended strongly on the different chemical reactions occurred during the recovery process. Three different extraction alternatives were considered: enzymatic extraction, chemical extraction and organic solvent extraction. The starting biomass was the obtained previously at pilot plant scale with *Ralstonia eutropha* as PHB producer microorganism. Though the enzymatic extraction may seem the most attractive alternative due to environmental issues, enzymes had not achieved to solubilise completely all the biomass. The chemical extraction strategy, based on the use of NaOH and other different surfactants, showed good results in terms of high PHB recovered yields but the characterisation of the extracted PHB showed that it did not have an adequate quality probably to the fact that the basic hydrolysis may break down the polymer chains. Besides, the presence of additional substances such as rests of biomass and/or growing media may influence the quality of the polymer. PHB compounding tasks carried out afterwards with the extracted PHB confirmed that this biopolymer was not able to process because its rheological feature was not enough. Besides, the degradation temperature was lower than the PHB commercial grades.

The PHB recovery process based on solvent extraction followed by a precipitation in cold methanol was studied. The chosen solvent was dichloromethane because it showed a similar yield in comparison to chloroform. This selection was done taking into account that several industrial batches of PHB production and recovery processes were done with the aim of obtaining certain amount of PHB for carrying out the below project tasks. The obtained PHB samples showed a high degradation temperature, similar to PHB commercial grades. The performed physical-chemical characterization showed that the obtained PHB was a homopolymer, without the presence of the copolymer PHB-V, and it was similar to commercial grades.

At this point and taking into account that the PHB production yield was not higher than it was expected, it was decided to scale-up the integrated process (bioproduction and recovery) to industrial scale with the aim to get the maximum amount of PHB in every batch. The industrial scale-up was done comprising two stages: production of the PHB rich biomass in a 3,000 litres bioreactor and recovery the PHB in a 500 litres extractor. For it, two Spanish companies were subcontracted for carrying out both processes. Three batches were done but any of them resulted in the expected amount of pure PHB due to several mechanical and contamination problems. Even though the contamination problems occurred during two of the bioproduction stages, it was decided to continue with the process and sending the final biomass to the recovery company. The colour of the final PHB obtained was brown, quite different from the white one of the pure PHB commercial grades. The characterization of the biopolymer showed that its viscosity was not as high as required for an adequate extrusion process and the mechanical resistance of the plastic prepared probes was low in comparison to the PHB commercial grade used as standard. After analysing the characterization results and the yields obtained in both bioproduction and recovery processes, it was concluded that every industrial batch meant an important effort regarding logistic (the facilities were located in different geographic areas of Spain), resources (reagents, microorganisms, etc…) and a high economic expense. The industrial scaling up of the integrated process showed many risks and its adjustment in subcontracted industrial facilities needed its own development, meaning by itself an individual project.

Meanwhile, microfibrillated cellulose fibres from agricultural residues/natural fibres/by-products or residues from food processing and microencapsulated active compounds were obtained in order to develop composites and active materials from PHB for packaging applications. The production of microcellulose fibres and their application in polymeric composite materials has gained increasing attention due to their high strength and stiffness combined with low weight, biodegradability and renewability, together with their abundance. The use of surface cellulosic fibres has been a way to increase PHB material mechanical resistance. To obtain the microfibrillated cellulose fibres (MFC), several lignocellulosic sources were used such as phormium, colihue, cotton, sisal, oat hulls, banana rachis, malt dregs, peanut and rice hulls. The cellulose purification process employed to obtain purified cellulose from the different lignocellulosic sources had different steps: (a) griding; (b) defatted; (c) alkaline treatment; (d) oxidative treatment (bleaching); (e) acid treatment; (f) enzyme treatment. Natural fibres were chemically treated in order to remove lignin-containing materials such as pectin, waxy substances and natural oils covering the external surfaces of the fibre cell wall. After treatment, the fibrils were revealed and a rough surface topography of the fibre was obtained.

The chemical treatment had the effect of splitting the individual fibres from fibre bundle, increased the surface roughness and the amount of cellulose exposed on fibre surface. The cellulose yield after the chemical and enzymatic treatment procedure was high for all fibre sources studied (between 80-90%). Between bleached and acid treatment, no significant differences were observed for cellulose content; acid treatment produced more fibrillation of the cellulose sources which would influence the performance of the next microfibrillation process. After enzymatic treatment, no significant differences were observed in cellulose content compared to acid treatment, as function of fibre source. The most important mass lost for all fibre sources occurred between alkaline and bleaching treatment.

Once the purified fibres from different sources were obtained, they were dispersed in water and pass through different mechanical processes in order to produce fibre disruption (fibrillation). The processes were, first of all, mechanical homogenizer and/or microfluidizer in which large pressure drop facilitated microfibrillation; secondly, high power sonication where ultrasound energy was transferred to cellulose chains through a process called cavitation which refers to the formation, growth and violent collapse of cavities in water. Different mechanical processes for fibre disruption were evaluated in order to select the most suitable one to produce microfibrillated cellulose (MFC). The processes were: cryogenic grinding, high pressure mechanical homogenization and microfluidization.

It was succeeded in obtain microfibrillated cellulose from phormium, oat hulls and rice husks after chemical treatment. Microfibrillated cellulose was obtained using both high pressure mechanical homogenizer and microfluidizer processors. The microfibrillated cellulose obtained from several natural fibre sources seemed promising in terms of width reduction of the fibrous structures. After that, a pilot plant scaling up was done for cellulose purification and microfibrillation processes; the selected raw materials were oat hulls, rice husks and peanut shells. The higher cellulose yield obtained and the better mass recovery after purification processes corresponded to rice husks. Therefore, they were chosen as source for MFC.

Regarding microencapsulated antioxidants to incorporate into PHB matrix, the challenge was to find a functional material which was able to support the high temperatures required for the compounding processes, preventing the degradation of the active agent. This fact defined the wall material which determined the microencapsulation technique. Therefore, two different groups of active substances were selected as core materials for microencapsulation. First of all, pure active components were employed to ease the process development and characterization techniques selection. Then, to obtain a real applicable and affordable process, natural extracts with high antioxidant activity were chosen such as thymol, carvacrol, eugenol, sodium benzoate and limonene. Microcapsules containing sodium benzoate were prepared to compare the activity of a standard preservative with microcapsules of naturally derived compounds such as thymol and eugenol. Also, natural extracts as orange peel oil, clove oil, rosemary oil, thyme oil, origanum oil and orange/lemon oil (limonene) were selected because of high content of the same compounds. Wall materials were selected among the most commonly used ingredients for microencapsulation, considering physical properties to achieve protection against high temperatures, their delivery capabilities and their compatibility with the PHB matrix as well as their cost. The selected ones were arabic gum (AG) and β-cyclodextrin (CD). Microencapsulation methods were selected according to different criteria: the nature of the core and wall materials and the feasibility of scaling-up and the most common technologies available for food applications. Depending on the physical-chemical properties of wall materials, different microencapsulation techniques were selected for the experimental development. One of the most economical processes at industrial scale is spray drying. It is cheap, rapid and scalable. It has been studied for many years for different core and wall materials. Spray drying of aqueous feed was used for wall materials as celluloses, carbohydrates and proteins. Inclusion complex formation was proved using two different techniques: (i) inclusion complex formation in aqueous media and particles separation by spray drying; (ii) supercritical fluids technologies, taking the advantage of the high diffusion capacity of supercritical carbon dioxide (SC-$CO_2$).

After a deep study of the alternatives, the selected ones were: (1) microencapsulation of limonene in β-cyclodextrins using supercritical fluids; (2) microencapsulation of thymol/carvacrol (50%) in β-cyclodextrins using spray-drying and (3) microencapsulation of thymol-eugenol in arabic gum using spray-drying. Produced microcapsules in cyclodextrins with supercritical fluids and also with spray drying were about 10% of oil inclusion; a slow release kinetic of antioxidant activity was achieved. Antioxidant activity was also proved after a thermal treatment what it meant that microcapsules exhibited protection against tested temperature of 140ºC.

Both processes using cyclodextrins were transferred to larger scale, producing some kilograms of samples. The quality of these capsules was tested, resulting in similar properties to those obtained at smaller scale. Characterization results of produced samples in arabic gum using spray drying showed homogenous appearance microcapsules, wrinkled and some collapsed. All the batches produced homogenous powders, not agglomerated and well-dispersed. Active microcapsules showed thermal stability up to 260ºC (DSC result). Microencapsulation processes using spray drying were also transferred to larger scale. The obtained results, comparing both scales, were similar in terms of quality. Supercritical fluids production of microencapsulated particles at pilot plant were done using orange peels from CITRESA as limonene raw material, as an alternative method to produce limonene extraction from orange juice by-products. The characterization of these microcapsules showed the same proper results.

The next step of the project was to develop new thermoplastic compounds based on PHB which were modified with the cellulose fibres and the antioxidant microcapsules synthesized before. Different PHB compounds were developed taking into account the different current polymer processing processes (extrusion blow moulding, injection moulding, injection,…). In order to adjust the compounding parameters, commercial PHB was used as material to be mixed with the necessary commercial processing additives, cellulose microfibers and antioxidant microcapsules. Once the adequate formulation was determined for each application to be studied in the project, the compounding process was adapted to introduce the PHB synthesized using as raw material the wastewater of the juice industry.

For developing the PHB compounds, it was taking into account that PHB materials have low melt strength and low melt stability, molecular weight (Mw) decreases during the processing and it is a brittle material. Moreover, PHB is sensitive to high processing temperature, leading to a decrease in molar mass as well as lower melt viscosity. One of the challenges of PHBOTTLE project was to improve the performance and to toughen the material taking into account the different requirements of each polymeric processing technology (extrusion blow moulding, injection moulding, extrusion blow film…). Thus, PHB was blended with other biopolyesters like PBS (poly(butylene succinate)), PBAT (poly(butylene adipate-co-terephatalate) and PLA (polylactic acid). The aim was to improve the processability of the PHB polymer by extrusion and injection process due to its poor stability during processing. After the first compounding trials it was concluded that the blending route for PHB could improve the compound stability during processing.

The melt flow index (MFI) results showed that the PHB formulated using plasticizers and the blends PHB/PBS with less content of PBS seemed more suitable for injection purposes where higher MFI values are required. In addition, a masterbatch of organic peroxide was added to the mentioned PHB/PBS blends. The aim was to induce branching/crosslink in the blend and to generate in situ copolymers of PHB/PBS that can be placed in the interface of both phases and act as a compatibilizer. The addition of the peroxide gave to the formulated blends lower MFI values which fitted better for extrusion processes (blow moulding or blow film extrusion). In the formulated blends using PLA, an increase in the tensile modulus values and tensile strength compared to the raw PHB was obtained. But a minor decrease in impact strength and evaluation values was achieved. The following steps took into account the formulation route to improve the elongation properties increasing the percentage of plasticizer on the blending.

According to the different blending strategies performed before and the results, different compounding routes were scaled up in order to obtain different PHB batches for each processing technology to be studied. In this sense, formulations using PHB in combination with PBS, P(3,4HB) (less than 50%) or PVAc (20%) made them suitable for blown moulding extrusion purposes due to their properties of viscosity and melt strength. Most of the developed formulations using PHB homopolymer and their blending with the PLA or PBS at low contents showed good processability and properties for injection applications. The improved elongation properties obtained in the compounds of PHB homopolymer with their blending using PVAc and P(3,4HB) were the best formulations candidates to be used in the injection stretch blow moulding. For the film extrusion processes, the route of compounds followed the formulations based on the use of blends of PHB homopolymer with less than 50% of P(3,4HB) and 30-40% PVAc.

On other hand, due to the chemical structures of natural fibres (cellulose fibres) and polymeric matrix are different, couplings between these two phases needed to be considered. The different chemical structures would cause ineffective stress transfer throughout the interface of the composites and thus lower composite mechanical properties. In the case of microfibrillated cellulose, the high number of hydroxyl groups produces strong hydrogen interactions. Another drawback is the high hydrophilicity which tends to form agglomerates. Therefore, it was necessary to improve the compatibility between the MFC and the PHB matrix in order to improve the performance and the properties of the resulting blend. Three different strategies of compatibilization were performed: water removal, surface modification and solvent exchange. Only the solvent exchange strategy showed the more promising results. Exchange-solvent required eliminating water from the system preserving the MFC structure. Water could be replaced with another compound which must keep the MFC structure and be compatible with PHB formulation and processing. PHB plasticizers appeared as a good option. Polyethylenglycol (PEG) and acetylbributyl citrate (ATBC) were tested as exchange solvent; good MFC-PEG gels were obtained, which maintained the microfibrillated structure. The composites films of PHB with MFC-PEG showed increase in Young modulus.

The incorporation of microcapsules in the PHB compound was carried out in two steps: in the first step, a premix of PHB, additives and microcapsules (MC) was carried out in a turbomixer with vacuum. In the second step, the premix was compounded in a twin-screw extruder. In a first set of tests, microcapsules of arabic gum containing thymol and eugenol were incorporated to the PHB matrix at three concentrations: 1, 3 and 5%. The compounding parameters, torque and SME (specific mechanical energy) were not significant; therefore the presence of MC at low content did not have influence in the performance of the compounding equipment. The results of the mechanical characterization of the PHB with MC compounds showed a reduction in the tensile and impact strength of PHB but had only a low impact in tensile modulus.

In a second set of experiments, different microcapsules were incorporated at 3 wt% in order to compare the performance of the different microcapsules. The microcapsules were: limonene in β-cyclodextrins by FSC, extracted orange peel in β-cyclodextrins by FSC and thymol-carvacrol in β-cyclodextrins by spray-drying. The extracted orange peel in β-cyclodextrins by FSC compound showed the best mechanical properties and the best impact and tensile strength. Besides, the PHB blend containing the MC (5 wt%) was injected into test specimen and an antioxidant release study was carried out. The objective was to analyze the release kinetics of antioxidant activity from the polymer to a food simulant. The release of antioxidant compounds for each kind of microcapsules was slow; this effect was higher in the limonene MC. Therefore, it was decided to use microcapsules of limonene in β-cyclodextrins by FSC at 5 wt% in the final formula of the PHB compound.

Taking into account the results obtained in the incorporation of both limonene microcapsules and cellulose microfibers to the PHB formula, the synergic effect of the combination of both was evaluated only in the case of blends used for injection applications. This was due to the fact that the incorporation of MFC had a strong influence in the elongation properties, an essential property for extrusion blow moulding and injection stretch blow moulding, both bottle manufacturing processes. Therefore, it was decided not to include them in the PHB blend to be used for bottle manufacturing. However, the limonene microcapsules were also added to the PHB blend to be used in the extrusion blow moulding technology, for obtaining the final bottles.

Finally, once the first trials of the processability of the developed PHB blends were done and the mechanical parameters characterized, a tuning of the blends was necessary. New PHB blends were developed depending on the processing technology to study. The final PHB blends were:

(i) PHB/PBS/PVAc, at different concentrations of PHB and PBS, and PHB/PLA/PVAc for extrusion blow moulding process.

(ii) PHB/PBS (low % of PBS) with both MFC and MC for injection moulding process.

(iii) PHB/PLA at different PHB and PLA contents for injection stretch blow moulding.

(iv) PHB/PBS/PVAc for film extrusion.

It is well-known that the processing parameters have a high influence over the morphology of the biopolymer and, in consequence, over the end properties of the processed plastic piece. Taking into account that the main drawbacks of the current market available PHB grades is their narrow processing window, one of the project challenges was to develop a suitable manufacturing process for the production of plastic packages using the PHB blends developed. This work was very important because these studies represented the base knowledge to predict the behaviour of the material in a specific design. Therefore, different design experiments were done to achieve the optimal parameters of each PHB compound developed in order to prevent premature mechanical, thermal or chemical failures. The packaging design was developed taking into consideration the different requirements in term of functionality and to be safe to human health and for the environment. Each processing technology used in bottle manufacturing has their own characteristics in terms of process conditions and can limit the packaging geometry and design. CAE (Computer Aided Engineering) simulations were carried out in order to identify the behaviour of the different PHB materials in terms of processing and required mechanical properties, depending on the technology.

Different bottle concepts (different geometries) were designed to be studied in Finite Element Analysis (FEA) based on the results of the study of mechanical properties of the PHB blends for extrusion blow moulding and injection stretch blow moulding. After analysing and comparing the different results regarding load at maximum tensile stress, top load and side load resistance, the final design of the PHB bottle to be processed by extrusion blow moulding and injection stretch blow moulding was the honeycomb model.

The processability study of the PHB compound by injection moulding (PHB/PLA) resulted that it was not possible to obtain a good preform. Different defects appeared such as black specks and contamination, bubbles, perform buckling and/or resin streaks. Due to this, it was decided discard this technology and obtaining the bottles by extrusion blow moulding. The processability studies of the PHB blends (PHB/PBS/PVAc and PHB/PLA/PVAc) in standard monolayer blow-moulding equipment concluded that the better processability and comprise of process conditions were found with the PHB compounds with the lower amount of PHB. The bottles produced with this compound performed well in the cutting of the ping-off. However, it showed stick behaviour on the mould creating issues on the de-moulding. To reduce this effect, it was necessary to use a lubricating spray on the mould. Taking into account these results, PHB/PBS/PVAc blends (with low PHB content) containing limonene microcapsules were processed for manufacturing PHB bottles at different weights which were used to package the juice and carrying out the shelf-life studies afterwards.

An initial characterisation of the different PHB bottle, with and without microcapsules, parts was done. The gas barrier (oxygen and water vapour transmission rates) and migration (overall) properties were determined together with the drop resistance. PET bottles were also characterised to be used as reference. The results showed that both PHB (with and without microcapsules) and PET bottles had the same oxygen and water vapour transmission rates, no significant differences were observed between the PHB bottle and the reference. Besides, the both PHB bottles fulfilled the overall migration limits stated in the European Regulation 10/2011, for contact with all aqueous and acidic foods and alcoholic foods up to a content of 20%, for all storage times at refrigerated and frozen conditions. Comparing the mechanical behaviour of the PET bottles (produced with the same geometry, the honeycomb) with the PHB bottles, it was observed that, for equal weight, the top load resistance of the PHB bottle was similar to the reference PET bottle.

PHB bottles obtained by extrusion blow moulding from the PHB/PBS blend and containing microcapsules were used to carry out the juice packaging; PET bottles were used again as reference. The used orange juice came from the wastewater generator. Once the juice was packaged, the evolution of physical-chemical (pH, Vitamin C and Brix degrees) and microbiological parameters of the juice were monitored. After one month, it was observed that there was not difference between the parameters of the juice packaged in both PHB and PET bottles. This meant that PHB could replace PET as packaging material in the long term, assuring the quality of the orange juice.

Regarding film extrusion technology, the PHB blend that showed a better processability was PHB/PBS/PVAc, with high PBS content. It was possible to downgauge to 100 μm and the obtained film was more flexible. The blend showed good stretchability. The use of this blend was for obtaining the sleeves of the final PHB bottles. Due to some technical issues of the partner in charge of this task, it was not possible to manufacture the final sleeves of the bottles.

The processability of the PHB blends by injection moulding technology was also studied, employing a simple injection mould that produces a package tray. The blend PHB/PBS, with high PHB content and containing limonene microcapsules and cellulose microfibers, showed the best mechanical properties and the best processing behaviour. Hence, it was used for obtaining PHB trays by injection moulding. The use of this blend was for obtaining the caps of the final PHB bottles.

The processing behaviour of PHB blends for both extrusion blow moulding and injection moulding technologies were validated at industrial scale. For it, a standard extrusion blow moulding machine and injection moulding machine were used in order to manufacture 950 ml bottles that currently produced in HDPE with 35 g. The PHB closures were produced using a modified 6 cavities production tool. The PHB blends used for the bottles were based on PHB homopolymer, PLA and PBAT. The results of the processability studies at this point showed that PHB homopolymer could be used for bottle extrusion blow moulding in stable conditions under contents below 40% and using high melt strength PLA as main component. Drop test performance was an issue in bottle performance but it seemed to be solved by modifying the formulation with a toughening additive. The injection moulding trials were performed in a Netstal synergie 1750 machine, with 45 mm screw and barrel capacity of 220 gr (about 8 shots with the tool used). It was used a 6 cavities tool used for caps injections moulding used on the bottles blown on EBM trials. The main constraints in injection moulding were the crystallization kinetics; it should be fast enough to allow good demoulding with shorter cooling times, avoiding high residence times on the injection barrel. Long residence times on barrel would increase significantly the material thermal degradation.

In order to know if the PHBOTTLE material was suitable for non-food packaging and non-packaging applications, samples were obtained at industrial scale. In both cases, the PHB blend used was PHB/PBS, with high PHB content, which was developed for injection processes. In the case of a non-food packaging, injected trays were produced; in the case of non-packaging applications, battery caps, thick discs, pole hoops and spools were obtained.

Finally, it was determined the viability and reliability of the eco-efficient realization of the production chain of the PHBOTTLE and its substances such as biocomposites. The eco-efficiency performance was compared with the performance of the already existing alternative bottles for the same function such as PET. First of all, the potential biodegradability of the prepared materials was characterized. For it, it was necessary to do an inventory of the potential end-of-life routes of the PHB material. Two end-of-life routes were selected for PHB as a packaging material: (i) PHB in a biodegradable application where the packaging would be collected with other biowastes; (ii) PHB in a bio-stable application where the packaging would be collected separately (with or without other packaging materials) or remain in the rest fraction of municipal solid waste (for post-collection separation). In the first end-of-life route, the thermophilic fermentation would be the selection treatment because it provided better practical results than meshophilic fermentation and showed equal performance as composting, as well as producing renewable energy in the form of biogas. In the second end-of-life route, the mechanical recycling would be the selected treatment because this option is practically feasible and in line with the current processes of other plastic packaging materials (PET or PLA).

Simulations of both end-of-life selected routes were done. The aerobic and anaerobic biodegradation tests concluded that after 18 weeks incubation in a composting environment, it appeared that 1 $cm^2$

pieces of the PHB package with and without cellulose microfibers were degraded for about 60%. From the recycling experiments carried out with commercial available PHB products, it was concluded that PHB seemed to be instable to some extent, with suitable stabilization of the virgin material as well as during recycling; it should be possible to recycle the PHB material.

The eco-efficiency performance is the combination of the environmental impact and the economic impact. The results of the Life Cycle Assessment (LCA) and the Economic Assessment Study (LCC or TCA) were combined and the balance between external, society costs and internal packaging chain costs were taking into account. Results from the LCA indicated that PHB could compete with fossil alternatives such as PET, PP or HDPE in several packaging markets. But the results of the laboratory scale calculations showed that optimization activities were needed to improve the integral environmental impact of the PHB bottle and to approach the competitive ones. With the selected Life Cycle Costing (LCC) assessment methodology, CAPEX (Capital Expenditures) and OPEX (Operating Expenditures) contributions and figures were calculated for the economic profiles of PHB bottles. The first indications was that PHB obtained in the project could compete with commercial available PHB, and probably later on with fossil alternatives such as PET, PP and HDPE in several packaging markets, when some improvements were realized successfully. Moreover, the overall main conclusion of the LCA and LCC studies was that they were relevant supporting tools for the direction of improvements in process design as well as improvements in product design.

The results of the combination of both LCA and LCC showed that PHBOTTLE production at laboratory and industrial scale can not compete with the fossil references. The environmental performance was similar at both scales; however, the production costs of a bottle at laboratory scale are much higher due to economy of scale and the mismatch between the capacities of the various steps in the production chain.


**Figure 1. PHBOTTLE prototypes**

## Description of the potential impact and the main dissemination activities and exploitation of results

The results of the PHBOTTLE project have an important impact in four main aspects: (i) the reduction of plastic packaging wastes; (ii) reduction of the dependence on petroleum as based material; (iii) the management of the wastewaters generated in the food industries and (iv) the reduction of food wastes.

Nowadays authorities and consumers are demanding more varied foodstuffs, with high quality standards, health and safety but also meeting physiological and nutritional needs. Besides, they are asking for products that respect the environment and reduce the pollution. For this reason, companies know that they have to modify and increase their offer with new products that can adapt to the current market and the consumers' expectations. One of the ways to improve the quality and extend the shelf-life of foodstuffs is through improvement of their packaging. But the materials used by food industry are polymeric, based on oil; their use represents an important environmental impact and waste generation due to their non-biodegradability. Therefore, there is a need to use new plastic materials that satisfy the social demand of the consumers for environmental friendly packaging.

The biopolymer obtained in the PHBOTTLE project can contribute to reduce the environmental impact of the plastic packaging wastes generated. The oil-based materials used in the packaging sector are not biodegradable and their environmental impact is so high. They need more than one hundred years for being completely degraded. One solution to reduce this amount of wastes is the incineration but it produces large amount of carbon dioxide and creates global warming. The European society is aware of this problem because it is becoming more evident that ecosystem is considerably disturbed and damaged as a result of the exhaustive use of non-renewable resources like petrochemical based polymers. The environmental impact of long-lasting plastic wastes is increasing global concerns. For this reason, there is an urgent need to develop and use renewable source-based plastic materials, especially for short-term applications like packaging. In 2012, 156.8 kg of packaging waste was generated per inhabitant in the EU-28. 19% of that amount corresponds to plastic packaging wastes which resulted in a total volume of 15.1 million tonnes. The PHBOTTLE material is a biopolymer that is biodegradable and compostable; 60% of the PHB bottle obtained is degraded over a period of time of 9 weeks under the study conditions. Therefore, the PHBOTTLE material can contribute to reduce the environmental impact produced by the plastic packaging wastes. According to data published by the European Bioplastics Association, packaging sector remains the single largest field of application for bioplastics with almost 70% of the total bioplastic market. Due to the excellent fit of bioplastics in the packaging market, this number is expected to increase to more than 80% in 2019. Besides, PHBOTTLE biopolymeric material can be used in other applications where biopolymers have a place in like non-food packaging or automotive sector. These sectors also demand oil-based plastics and their problems related to the wastes disposal and environmental impact need to be taken into account.

On other hand, petroleum resources are finite and price changes are directly conditioning the price of conventional plastics, so an additional important impact associated with bio-based polymers is a reduction in economic risk associated with reliance on petroleum as a base material. One of the most important barriers to the general use of biopolymers is the price comparing with polymer derived from petroleum. Until now, higher prices and some limited features have conditioned the desired use of biopolymers. Although the price of PHB in general is much higher than starch-based polymers and other bio-based polyesters due to raw material and processing costs and small production volumes, PHBOTTLE material can help to solve this problem because it is obtained from wastewater food processing industries. The raw material is cheaper than the current ones. Besides, it saves costs in crop fields because the nature of the used raw material can help to reduce the price of the PHB comparing to the commercial ones.

Regarding the kind of raw material usually used in the production of PHB, today most of the bioplastics are made of carbohydrates from plants such as sugar cane or corn, the called food crops or first generation feedstock. This kind of raw material is currently the most efficient for the production of bioplastics as it produces the highest yields. Taking into account this, it can be thought that crops are used for the bioplastic industry instead of being used for feeding humans. Apart from this, the use of food crops as substrate for bio-based plastic production increases the carbon footprint and, therefore, emissions of greenhouse gases due to the use of fertilisers and other chemicals. The PHBOTTLE biopolymer helps to solve this problem because the needed sugars for the PHB fermentation process comes from the wastewaters of the food industry.

On other hand, for food industry the management of their wastewaters is an important issue. For this industry it is quite important to preserve the environment in which raw materials are grown because this sector depends on the quality of natural resources, especially land and water. The level of pollution in wastewater and the amount of waste produced by industry can represent a significant load. Most of the water which is not used as an ingredient ultimately appears in the wastewater stream. Typically, untreated wastewater is high in organic matter: levels can be 10-100 times higher than in domestic wastewater. PHBOTTLE has demonstrated that the use of the wastewater from the food industry as raw material in the PHB production can improve the quality of the wastewater to be finally recycled as well as to treat the wastewater as a by-product instead of a common waste to be disposed and pay for. In this sense, PHBOTTLE project reduces water and waste related costs (intake, treatment and reuse), increasing the ability to comply with environmental legislation and improving company marketing strategies.

In this point, it is quite important and necessary to note that PHBOTTLE is a clear example of a project based on the circular economy. In this kind of economy, the value of products, materials and resources is maintained in the economy for as long as possible and the generation of waste minimised. The efforts are focused on the development of a sustainable, low carbon, resource efficient and competitive economy. In 2015, the European Commission adopted an ambitious Circular Economy Package to stimulate Europe’s transition towards a circular economy which will boost global competitiveness, foster sustainable economic growth and generate new jobs. The actions proposed by the European Commission will contribute to “closing the loop” of product lifecycles through greater re-use and recycling, and bring benefits for both the environment and the economy. In this sense, PHBOTTLE project has taking the advantage of the residues generated by the fruit juice processing industry (waters in this case) for being use as raw material in the bioproduction of a biopolymer. This biopolymer has been used for manufacturing bottles for packaging the juice produced by the wastewater generator industry. The cycle has been closed: the waste generator has become the beneficiary of the new package, tailored to the need of its product.

The results of the PHBOTTLE project fit perfectly and contribute to fulfil the targets of the EU for reduction of waste and establishment an ambitious and credible long-term path for waste management and recycling. Under the Circular Economy Package, the EU asks to contribute to preserve the environment and our planet, bring a substantial cut in carbon emissions and preserve threatened resources. Also the EU is asking for taking measures which can reduce greenhouse gas emissions and increasing the competitiveness of key industry sectors including manufacturing, waste management and recycling as well as reducing the dependency of the EU on raw material imports.

Another important challenge for Europe, in particular, and the World, in general, is the reduction of the food wastes. Around 88 million tonnes of food are wasted annually in the EU, with associated costs estimated at 143 billion Euros. About a third of all food produced globally for human consumption is lost or wasted. Some factors that contribute to food waste are, among others, misunderstandings about the meaning of “best before” and “use by” date labels leading to edible foods being throw away; stock management issues for manufacturers and retailers; overproduction or lack of demand for certain products at certain times of the year, product and packaging damage or an inadequate storage or transport at all stages of the food chain. The developed material in the PHBOTTLE project has the property of being active due to the encapsulated antioxidants included into the biopolymeric matrix. These antioxidant capsules contribute to extend the shelf-life of the packaged juice and, therefore, the waste of food can be decreased. Hence, PHBOTTLE material has the potential to overcome one of the current greatest socio-economic challenges at global level. There are several factors that affect the quality and safety of the packaged food such as microbial spoilage or oxidation due to the oxygen action. Oxidation is one of the most usual mechanisms of food deterioration; therefore, if antioxidant agents are present in the food packaging material, they can release from the packaging to the food and delay the oxidation reactions.

PHBOTTLE was a small or medium-scale focussed research project where a Specific International Cooperation Action (SICA) to promote the participation of emerging economies and developing countries was required. Research cooperation with international partners was an important objective of the Seventh Research Framework Programme. In the case of the call of PHBOTTLE project, the countries should be from Latin America. The Latin American partners included an Argentinean RTD centre (INTI), which was in charge of cellulose fibres purification and modification, and two industrial partners: one from Mexico (MEGAEMPACK) which was in charge of the sleeve production, and one from Brazil (Logoplaste Brazil) which validated at industrial scale the bottles and caps manufacturing. Involving Latin-American industrial and RTD companies enriched the project goals and the use of their results since they have different markets with different requirements of packages features and different Regulations. At the same time, transnational partnership between industry and academic institutions in Europe and Latin America was facilitated with balanced benefits between academic partners and the companies. The project provided new inputs to industrial and academic institutions and created economic and ecologic benefits by converting the juice industrial wastewater into value added.

Regarding the dissemination of the project, PHBOTTLE has had a great international impact due to the international nature of the consortium, composed by European and Latin-American partners. Along the whole project, more than 200 publications have been done in scientific journal, technical magazines, mass media and social networks. During the first weeks of the project (May-July 2012), around 30 publications in Spanish newspapers (El Pais, ABC) and radio (La Cope, La Ser), European technical magazines (Food and Beverage Packaging, Alimarket, World Packaging News, Food Production Daily..) were registered, as well as at the end of the project, when the results were presented in a workshop held in Brussels. In just one day (19th April 2016) more than 30 communications were done worldwide. Among others, it is remarkable that the public Spanish television (TVE) covered the news of the development of the bottle three times in its news broadcast at prime time.

Apart from the publications, PHBOTTLE has been disseminated in different scientific and technical forums such as the 10th International Conference Advances in Plastics Technology (APT’13), From Biobased Polymers to Bioplastics – An interface between R&D and applications, VIII Iberoamerican

Congress on Pulp and Paper Research (CIADICYP), 3rd International Symposium International Meeting on Packaging Material/Bioproduct Interactions (MATBIM 2015), 8th European Symposium on Biopolymers (ESBP 2015), Biorefineries – Science, Technology and Innovation for the Bioeconomy and Juice Summit 2015.

Two workshops were organized; the first one was held in Sao Paulo (Brazil) in October 2014 and the second one in Brussels (Belgium) in April 2016. The aim of both workshops was to disseminate information about the PHBOTTLE project activities to different stakeholders and to facilitate stakeholder inputs and opinions regarding these results. The title of both workshops was "Biopolymers: Present and Future Directions". The intention was to give a deep vision of biopolymers in different areas such as research, development, market and legislation in Europe and Latin America. In both workshops, apart from showing the different project results by each partner and the training sessions about Life Cycle Assessment (LCA), important biopolymer companies, such as Braskem and Avantium, and packaging company (Tetra Pak) participated as speakers showing their products and overviews of the current use of biopolymers in Europe and Latin America. In total, more than 70 people attended both workshops, coming from different areas: food packaging, petro chemistry, research institutes, etc.

Other dissemination materials produced during the project were two leaflets (at the beginning and at the end of the project), which were distributed by the project partners in different European trade fairs, a poster and a video. The video is uploaded onto the project website and the YouTube channel of AINIA (www.youtube.com/user/ainiatecnologia). The video was presented for the first time in the second PHBOTTLE workshop, held on 18 April 2016. During the next ten days, the video was seen 472 times. At the beginning of the project, a website was developed (www.phbottle.eu) which informs the public about the project and its objectives and results, the consortium as well as press releases launched during the project life. Moreover, the website contents a private area where the confidential information is available for the consortium. At the end of the project (30th April 2016), the webpage had around 7,100 visits from worldwide countries; it is remarkable that people from all the European and American (North and South America) countries had visited the PHBOTTLE webpage.

Regarding the exploitation of the project results, in the preparation stage the expected project results were defined in advance and a first draft of the Intellectual Property (IPR) ownership and user rights were established taking into account the use and protection of the background and foreground of each partner. The defined and achieved project results are:

R1. Culture medium (wastewater) and microorganisms to produce PHB: this result concerns to the selected wastewater from the fruit juice industry which has been used as raw material in the production of PHB. Besides, the choice of the best PHB-producer microorganism is part of this result.

R2. Produced and extracted PHB: this is one of the most important result of the project due to it is the polymeric material obtained from renewable resources such as the wastewater from the fruit juice industry.

R3. Microencapsulated antioxidants: these capsules have been incorporated to the PHB obtained in the project for contributing to advanced packaging material development. These capsules deliver natural antioxidant additives to the packaged juice for delaying the juice oxidation.

R4. Modified cellulose fibres: the fibres obtained from agricultural residues (rice hulls) have been also incorporated to the PHB matrix for making it higher strength and stiffness.

R5. PHB developed composite (master): the master is the processable biopolymeric material used by the transformers (packaging and non packaging) for obtaining different kind of samples (bottles, trays, caps, pool hoops, etc). The master is composed by the PHB and other additives such as the microcapsules and microfibers (depending on the material processing technology requirements) which are necessary for the improvement of the material processability.

R6. Preform designs: a specific perform was designed in the framework of the project for making PHB master processable and useful to manufacture the bottle.

R7. PHBOTTLE material processing: the processing conditions have been determined for each PHB master and each processing technology for manufacturing the different types of packages (for food and non food applications) and non-packaging applications.

R8. Full packaging system (PHBOTTLE): this is the most important project result due to PHBOTTLE package is a sample of a sustainable packaging solution (bottle and cap) produced from renewable resources such as by-products from the food production industries.

R9. PHBOTTLE eco-efficiency performance: this result is related to the viability and reliability of the eco-efficient realization of the production chain of the PHBOTTLE package and its substances.

During the project, an internal analysis related to the project results, their inter-relations and the links among the partners for achieving the results were performed in collaboration with the EU Commission's service of IPR and Business Development. A Project Risk Assessment (PRA) resulted which was focused on the risks associated to the exploitable results. Among others, regulations were identified as the most critical risk because the PHBOTTLE material packaging has to overcome the mandatory tests to obtain the approval of Food Safety Authorities if the packaging would want to put into the market. Moreover, once all the project objectives were achieved, the consortium identified the constraints of each project result and the further activities to be performed if they are wanted to put into the market. It was concluded that more activities related to PHB processes optimization, reduction of costs and amortization of the technologies are required before to jump to the market. It was highlighted that PHB production and extraction processes are relatively news and they need more research for achieving a final biopolymer with a high quality, the needed requirements and a competitive price comparing with other current biopolymers like PLA.

## The public website address

www.phbottle.eu