

1 Evaluation of physiochemical/microbial properties and life cycle assessment (LCA)
2 of PLA-based nanocomposite active packaging

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19 Keywords: Packaging, bioplastics, Polylactic acid (PLA), Life Cycle Assessment (LCA),
20 physicochemical and microbial properties

21 **Abstract**

22 To attend the growing consumer demand for novel ready-to-eat fresh cut fruits packaging polylactic acid
23 (PLA)-based active packaging was realized. The aim of these packaging is to provide an improved
24 protection and even to extend their shelf-life. PLA-based active packaging was prepared by adding
25 nanoclays and surfactants in its formulation. The evaluation of PLA-nanocomposite packaging was done
26 in comparison to pristine PLA and conventional plastic (polyethylene terephthalate, PET) using fresh-
27 cut melons. Physicochemical properties were investigated by the means of weight loss, visual
28 appearance, pH, colour, and firmness. In addition, microbial profile was tested via microbiological
29 assays. In order to evaluate the environmental impact of PLA-based active packaging compared to
30 commonly used PET, life cycle assessment (LCA) was conducted. In terms of physicochemical and
31 antimicrobial properties, the results clearly showed that the presence of nanoclays and surfactants in the
32 PLA formulations improved their performance, thus contributing to bring the characteristic and
33 behaviour of PLA packages close to those of PET. Furthermore, assessment of life cycle environmental
34 impacts indicated that PLA packaging with nanoclays had the highest environmental performance.

35

36 **1. Introduction**

37 Health and sustainability are today's megatrends affecting also the market of food products. Consumers
38 have acknowledged the connection between food choices and well-being (Goetzke et al. 2014) as well
39 as environmental impacts (Grunert et al. 2014). Convenience and ease-of-consumption are also common
40 traits in high-income countries, where saving time and effort are regarded essential (Brunner et al. 2010,
41 Buckley et al. 2007).

42 Fresh-cut fruits respond to the demand for such healthy and easy products. Procedures such as cutting
43 and peeling expose the surface of the fruit to air and contaminants while also causing mechanical damage
44 to the cells which make the fruit more perishable (Ramos et al. 2013). As consumers tend to select fruit
45 products primarily based on their colour and appearance, fresh-cut fruits must indeed look fresh in order
46 to attract the attention of consumers (Barrett et al. 2010). Such delicate products require much from the
47 packaging. Studies conducted by Grönman et al. (2013) and Silvenius et al. (2014) have revealed that
48 the environmental impacts of the food are of a higher concern when compared to the product-packaging
49 system. Governments and customers have put much focus on the environmental impacts of the packaging
50 (Williams et al. 2008).

51 Plastic production for packaging represents the largest application for plastic nowadays (Lagaron and
52 Lopez-Rubio 2010). In particular, petrochemical-based plastics have been increasingly used as packaging
53 materials because of their large availability at relatively low cost and because of their good mechanical
54 performance (Lagaron and Lopez-Rubio 2011). In terms of costs, the production costs of petrochemical-
55 based plastics such as polyethylene terephthalate (PET) tend to increase due the high oil prices. However,
56 the packaging waste and its environments impacts is the most concern. Petrochemical-based plastics are
57 not totally recyclable and/or biodegradable causing several environmental impacts. Furthermore, plastic
58 packaging materials are often contaminated by foodstuff and biological substances, so recycling these
59 material is impracticable and most of the times economically not convenient. As consequence, tons of
60 plastics materials are landfilled annually increasing the problem of municipal waste disposal (Sorrentino
61 et al. 2007).

62 The growing environmental awareness imposed to packaging materials and processes is reflected in the
63 ambition for improving plastics towards functional and eco-friendly properties as biodegradability.
64 Bioplastics have attracted attention as an alternative to substitute petrochemical-based plastics (Bishai et

al. 2014, Peelman et al. 2013, Reddy et al. 2013). Polylactic acid (PLA), an aliphatic polyester, has been found as the most promising material (Nampoothiri et al. 2010). In addition to use a renewable resource (corn) as raw material, it is a biodegradable and biocompatible polymer, which can be processed using conventional thermoplastic processing methods (Mahalik et al 2010). Such characteristics make it suitable for reducing the environmental burden of solid waste accumulation (Nampoothiri et al. 2010) as well as fossil resource depletion. However, PLA suffers from shortcomings which limit its commercial opportunities compared to conventional polymers. Such characteristics include poor moisture and gas barrier properties and thermal resistance, brittleness as well as high cost (Nampoothiri et al. 2010). In this scenario, additives have been found to be an invaluable solution to improve the performance of bioplastics such as PLA (Peelman et al 2013, de Azeredo 2009, Sanchez-Garcia et al. 2010, Abdulkhani et al. 2014, Jamshidian et al. 2012, Marcos et al. 2014, Nofar et al. 2013, Suttiruengwong et al. 2014).

In this work, PLA-based packaging with different additives was prepared and their performance was compared with PET and pristine PLA by means of physicochemical analyses, microbiological assays and life cycle assessment (LCA). LCA is a widely used and standardized method utilized to address the environmental impacts of products and services throughout their life cycles. Comparison between PLA and conventional plastics in its sustainability has been studied using LCA (Gironi et al. 2011, Madival et al. 2009, Papong et al. 2014). The developed PLA-based packaging aimed to: (1) meet the needs and demands of the consumers by providing convenience with a single-serve size as well as increased appeal with the packaging design, (2) provide fresh-cut fruits improved protection and (3) extend their shelf-life.

2. Materials and Methods

2.1 Materials

Polylactic acid (PLA, Hycail[®] HM 1011) pellets were obtained from Hycail Oy (Finland). PLA pellets were dried at 50 °C in a dehumidifying air hopper dryer with regenerative desiccant beds for 4 hours. In order to improve PLA melt strength, the commercial masterbatch OnCap[™] Bio (PolyOne) was used. The carrier in this commercial masterbatch is PLA and contains a 40% of an additive named Paraloid BPMS-260 (from DOW). This additive is an acrylic melt strength enhancer designed to increase the melt elasticity of the blend. PLA/nanoclay composites were prepared using two types of nanoclays (Na-cloisite 20 and Na-cloisite 30B) which were purchased from Byk (Altana Group). Cloisite 20 is a bis(hydrogenated tallow alkyl)dimethyl, salt with bentonite, and Cloisite 30B is an alkyl quaternary ammonium bentonite. Surfactant Triton X-100 was purchased from Sigma Aldrich. Poly-(ethylene terephthalate) (PET) were obtained from DUPONT and control PET packaging from PREGIS. Melons used were from the variety “Pele de sapo” (also known as Santa Claus or Christmas melon), brand “Bollo”, from Brazil, characterized by a green colour and purchased in the local market Froiz Portugal.

2.2 Preparation of PLA-based active packaging

The PLA-based packaging was designed for fresh-cut fruits which can be easily bought and consumed on the go as illustrated in Figure 1. The packaging consists in four parts: terrine, lid, mesh and fork. The mesh was placed inside the terrine to enable the extra juices from the fruits to flow to the bottom of the terrine and therefore prevent the fruits from becoming soggy. The packaging was produced using a twin screw extruder with gravimetric feeders. The temperature profile was set at 145°C in all 6 heat zones and 280 rpm (production rate 5Kg/h). All the PLA-based packaging contained 3.5% OnCap[™] Bio in its formulation.

In addition to two PET packages used as reference, four different PLA-based packaging were prepared as described in Table 1. For physicochemical evaluation and microbiological assays, the packages were sealed by thermally applying compatible PET (thickness 27 µm) and PLA (thickness 40µm) films.

110 2.3 *Fresh-cut fruits preparation*

111 Whole unpeeled melons were sanitized by immersion in a 200 ppm sodium hypochlorite aqueous
112 solution at room temperature, during approximately 5 min. The melons were allowed to dry at room
113 temperature prior to further application. Trapezoid-shape pieces of melon were obtained by manual
114 cutting with a knife previously cleaned with ethanol 96 % (v/v). The edges of the peeled melon slices
115 were discarded – thus avoiding the extreme green or mature parts and increasing homogeneity. Nine
116 pieces of fresh-cut melon were randomly selected to each packaging and immediately sealed. The
117 packaging weight was measured before and after adding the fruits. The fresh-cut melons packaging were
118 stored in a climatic chamber (Binder, Tuttlingen, Germany) during 2, 5 and 7 days under the follow
119 conditions: 10 °C, 80 % relative humidity (RH), and 25 % of fan's intensity. Analyses were also
120 performed at day 0. In this case, the melons did not require the packaging procedure and were used as
121 reference to all the packaging conditions.

122 2.4 *Physicochemical analyses*

123 For each period, a total of 18 pieces of fresh-cut melon was subjected to several physicochemical and
124 textural analyses. The parameters assessed were weight loss, visual appearance, pH, colour (colorimeter),
125 and firmness (texturometer). For weight loss, each remain packaging weight was measured daily. Colour
126 determination was made by the means of a colorimeter (Minolta, CR-3200, Chiyoda TKY, Japan). The
127 CIE 1976 L*a*b* colour scale was used obtaining the parameters L* (lightness), a*[chromaticity (red-
128 green)] and b* [chromaticity (yellow-blue)], after calibrating against standard white plate. This colour
129 system was developed so that the degree of difference in measured values can closely match the degree
130 of perceived colour difference. Based on these observations, ΔE (total colour difference) was calculated
131 according to the following expression:

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (I)$$

Where:

$$\Delta L = L - L_{\text{standard}} \quad (II)$$

$$\Delta a^* = a^* - a^*_{\text{standard}} \quad (III)$$

$$\Delta b^* = b^* - b^*_{\text{standard}} \quad (IV)$$

And where: $L_{\text{standard}} = 97,10$, $a^*_{\text{standard}} = 0,05$ and $b^*_{\text{standard}} = 1,76$.

The textural measurements were performed using a TA-XT2 texture analyser (Stable micro systems, Surrey, United Kingdom). The texturometer was controlled using the software Texture analyser XT-RA Dimension, v 3.7G. A 5 kg load cell and a cylindrical probe were used, and the test speed was set to 0.5 mm/s. After textural analysis, the 18 damaged pieces of melon from the same type of package (2 packages total) were combined and minced with a hand blender (MR 400, Braun Minipimer, Havant, UK). The resulted minced mass was subjected to pH evaluation. The pH was determined with a potentiometer (Model 320, ATI Orion, Boston MA, USA) after calibration with buffers pH 4 and 7. Four measurements were made to each type of sample.

2.5 Microbiological assays

The key groups of microorganisms studied were: total mesophilic vegetative viable counts; total psychrotrophic vegetative viable counts; total coliforms and detection of *Escherichia coli*; *Enterobacteriaceae*; yeasts and moulds; Lactic Acid Bacteria (LAB); *Enterococcus*; *Pseudomonas* species and detection of *Pseudomonas aeruginosa*. The microbial enumeration was made resorting to microbial kits and not to conventional microbial enumeration. Total viable counts, *Enterobacteriaceae*,

152 yeasts and moulds, and total coliforms and *Escherichia coli* were enumerated with colour indicator
153 devices SimPlate® from BioControl Systems, Inc. (Bellevue WA, USA). *E. coli* presence was detected
154 with in a fluorescence chamber with a 366 nm lamp (Merck, Darmstadt, Germany). Furthermore, LAB
155 and *Enterococcus* were enumerated and detected using the compact dry TC and ETC medium (Nissui
156 Pharmaceutical Co. Ltd, Tokyo, Japan), respectively. Compact dry kits are ready to use chromogenic
157 micro-plates. Finally, *Pseudomonas* and *Pseudomonas aeruginosa* were enumerated and detected using
158 the chromogenic micro-plates from Microkit [Laboratorios Microkit, Valdemorillo (Madrid), Spain].
159 LAB, *Enterococcus* and *Pseudomonas* were inoculated via the spread plate method and total viable
160 counts determined by the technique of surface viable counts. Instructions from manufacturers were fully
161 followed during inoculation, incubation and enumeration – which were based on ISO standard official
162 methods.

163 Microbiological assays were performed under aseptic conditions. Solutions were autoclaved prior to use.
164 On day 0, only 1 sample was analysed, whereas on days 2, 5 and 7 an aliquot of the packaged cut-melon
165 pieces was used from each of the 6 types of package. During microbiological assays (days 0, 2, 5 and 7),
166 an aliquot of 25 g of cut-melon was weighed to a 400 mL blender sterile bag (VWR, Leuven, Belgium),
167 slightly minced by hand, and suspended in 225 mL of sterile alkaline saline peptone water solution
168 (Scharlau, Scharlab, S.L., Sentmenat, Barcelona, Spain), composed by 4 % peptone and 4 % sodium
169 chloride. The 1:10 mother mixture was aseptically homogenized for 15 min under gentle to middle orbital
170 agitation (182 rpm). Serial decimal dilutions (*i.e.* dilution to $1:10^i$, $2 \leq i \leq 10$) were then made on sterile
171 tubes with 9 mL sterile alkaline saline peptone water solution. Suspensions (original and following
172 dilutions) were kept without agitation at room temperature until analyses were in order. The appropriate
173 dilutions were used to inoculate each culture media kit, and an inoculation volume of 1000 µL was used.
174 The appropriate dilutions were initially estimated based on the literature (Aguayo et al. 2008, Fernandez

175 et al. 2010, Manzocco et al. 2011, Martinon et al. 2014, Rojas-Grau et al. 2007, Santos et al. 2010, Seow
176 et al. 2012, Silveira et al. 2013, Sipahi et al. 2013, Soliva-Fortuny et al. 2003) on previous results. In the
177 second replicate of experiment, these estimations were based in the previous one. All media were
178 inoculated in duplicate, thus 2 measurements were obtained for each sample and incubation conditions.
179 The results were expressed as log of colony-forming units (CFU) per gram of sample; the logarithmic
180 transformation was necessary for stabilization of variance and normalization of residuals.

181 *2.6 Statistical analysis*

182 Each type of package described in Table 1 was tested within three replicates. For each replicate, 9 units
183 of the same packaging were filled with 9 pieces of fresh-cut melon as described in section 2.3. After each
184 storage period (2, 5 and 7 days), a total of 9 packages/type (i.e. 3 packages per each replicate) was
185 removed from storage conditions for physicochemical analysis and microbiological assays. For weight
186 loss, the average and standard deviation were calculated of 9-27 packages according to the remained
187 packages in each storage period. Colour and firmness average values were determined based on 4
188 packages for each package formulation. For each package, colour and firmness values were calculated
189 as the average of the measurements in 9 pieces of melon. pH measurements were repeated 4 times per
190 package type and the result presented as mean \pm standard deviation. For the microbiological assays, 2
191 measurements were performed for each replicate (i.e. a total of 6 measurements per package type).
192 Statistics were performed using Statistica version 8.0 (StatSoft, Inc, Tulsa, USA). One-way analysis of
193 variance (ANOVA) was performed after 5 days of storage for all physicochemical analyses and for total
194 mesophilic vegetative viable counts. Variances were tested for homogeneity and statistical significant
195 differences were analysed a posteriori with the Tukey test. The significance level was defined as $p \leq 0.05$
196 for all tests.

197 2.7 Life cycle assessment (LCA)

198 LCA is an environmental tool which evaluates a product taking in account the various stages through
199 their life cycle. A cradle to grave LCA evaluates the product from the extraction of raw materials until
200 the disposal of the product (landfill, incineration or recycle). However, the LCA evaluation can focus in
201 certain stages according to the defined goal, scope and system boundaries. LCA concerning the PLA-
202 based packaging was conducted from cradle to grave and followed the EN ISO standards 14040 and
203 14044. SimaPro 8™ software from PRe® consultant (The Netherlands) was used in defining the life
204 cycles according to the inventory data and calculating the impacts. Data, which could not be provided by
205 the project, was retrieved from the Ecoinvent 3 database. Impact method 'IMPACT 2002+' version 2.11
206 was utilized in conducting the impact assessment phase. The method was complemented with an
207 additional midpoint category – bulk waste – which was imported from EDIP 2003.

208 3. Results and Discussion

209 3.1 Physicochemical characteristics

210 3.1.1 Weight loss and pH

211 The weight losses and pH values for each studied period are shown in Figure 2. The weight losses for
212 PLA-based packaging were consistently higher than for PET (Figure 2A). This result was expected since
213 water vapour permeabilities for PLA films are approximately ten times higher than those from PET films
214 (ca. $1.4 \times 10^{-12} \text{ g m}^{-1} \text{ s}^{-1} \text{ Pa}^{-1}$). However, no significant weight losses (~1.7%) were observed for PLA-
215 based packaging after the total storage period (7 days) suggesting that the all PLA packages present water
216 permeability small enough for the storage of fresh-cut fruits. Among PLA-based packages, and unlike
217 the surfactant, the presence of nanoclays may be beneficial in reducing the water transfer phenomenon.

218 A significant drop of pH was evident after 2 days of storage for all tested packages (Figure 2B). Changes
219 in pH are often closely related to the microbial growth, particularly with those that produces acids during
220 fermentation. Nevertheless, the occurrence of a significant microbial activity does not imply necessarily
221 a decrease of pH but depends yet on the existing microflora and the predominant species. The
222 microbiological profile (section 3.2) for the fresh-cut melons indicate that the prevalent microorganisms
223 were lactic acid bacteria (LAB) which ferment sugars to acid lactic, causing the significant pH decrease.
224 In fact, for the fifth day, the lowest pH occurred for the package with the highest LAB content (P2); the
225 highest pH occurred for the package with the lowest LAB content.

226 *3.1.2 Colour and firmness*

227 As a result of the chemical and microbiological phenomena occurring during the storage period, changes
228 in colour of the fresh-cut melon was visually observed for all tested packages. The total colour difference
229 (ΔE) values are shown in Figure 3A. It is clear that ΔE increases for all packages along the storage period.
230 Pristine PLA (P2) showed to be less attractive than PLA with nanoclays (P3, P4, P5). In addition, PLA
231 formulations with nanoclays (P3, P4 and P5) demonstrated to prevent colour change effects similarly to
232 PET packages (Table 2).

233 Measurements along time of the maximum resistance exerted by the fresh-cut melon samples when
234 employing a force by a cylindrical probe in the texturometer is an effective way to elucidate the firmness
235 – and thus the level of degradation of the food – during shelf-life. For short storage periods (2 days), the
236 obtained results showed a clear benefit of using nanoclays in order to improve pristine PLA (Figure 3B).
237 For longer storage periods, a decrease in firmness is observed for all the tested packages. However, the
238 formulations including nanoclays still present a better performance than PLA pristine (P2) and
239 comparable performance with PET (P1 and P6).

240 3.2 Microbiological profile

241 In order to provide, a better picture of the microbiological profile along the storage period, a great average
242 was defined as the average of the microbial counts obtained from all packages in the same day (Figure
243 4). This result revealed that melons present naturally an important diversity of microorganisms. The
244 viable microbial counts found in the initial matrixes were in the safe range of 3.3-3.6 log CFU g⁻¹ for
245 total mesophilic, total coliforms, LAB and yeast and moulds. *Enterobacteriaceae* and *Pseudomonas* were
246 found at significant levels as well, viz. 2.8 and 2.5 log CFU g⁻¹, respectively. Psychrotrophic and
247 *Enterococcus* were found at lower levels. It is expected that the microbiological profile of fresh cut-fruit
248 develops during storage periods. As a result of competitive and synergetic interactions, total mesophilic,
249 total coliforms, *Enterobacteriaceae* and LAB became predominant after 2 days of storage – thus
250 contributing to a large extent to the general viable counts (Figure 4). *Pseudomonas aeruginosa* and
251 *Escherichia coli* were not observed during the entire period.

252 The viable yeasts and moulds reached values generally lower than those found in the dominant
253 microflora, viz. total coliforms, *Enterobacteriaceae* and LAB, but higher than the significant number of
254 *Pseudomonas*. In fact, yeasts and moulds were the second most abundant group of microorganisms
255 encountered in this study. Due to the high water activity, yeasts were not expected to be a dominant group
256 of microorganisms; however, they were found at very significant levels. Other factors may also be behind
257 this behaviour, such as the low temperatures employed during storage, the pH range (at a minor extent)
258 and the occurrence of competitive *Lactobacillus* and other LAB – coupled with the possible inexistence
259 of effective synergic interactions among the existing strains.

260 Except by the LAB group, the highest viable counts were reached at day 5 and minor changes occurred
261 from this point until total period of storage (7 days). Thus, under the extreme conditions used during

262 storage in the current study (10 °C and 80 % RH), the samples became generally deteriorated and
263 seriously unsuitable for consumption at day 5. These conditions were used to accelerate the fruit
264 deterioration and, thus they are expected to have a longer shelf life under the conditions commercially
265 used (around 0-4 °C). The significant decrease in the pH may also be influencing the rate of growth of
266 the more pH sensitive microorganisms thus stabilizing or decreasing their viable counts from day 5 to
267 day 7.

268 The viable microbial counts obtained for each tested packaging after 5 days of storage is showed in Table
269 3. For total mesophilics, pristine PLA (P2) presented the worse performance from all tested packaging,
270 proving a beneficial effect of the nanoclays in the PLA package formulations. This is also in accordance
271 to the physicochemical analyses. The growth of psychotropic microorganisms showed a relative extended
272 lag phase which is beneficial from a food safety standpoint.

273 The packages tested can act as a physical barrier to microorganisms, but no differences in the microbial properties
274 were expected for the different formulations as no antimicrobial agent was added to the package formulation. The
275 differences in microbial growth are due to the differences in the barrier properties of the different package
276 formulations. In fact, the package can interfere with the internal environment of the package, particularly with the
277 CO₂, O₂, water vapour and others gases concentrations, which are major factors for microbial growth control. The
278 formulation of the package can lead to significant differences in the barrier properties to gases, thus changing the
279 conditions for microbial growth.

280 3.3 LCA

281 3.3.1 Goal, scope and functional unit of the assessment

282 The first phase of an LCA is goal and scope definition. In this phase the initial choices for conducting
283 the entire LCA – extent, boundaries and the overall level of sophistication – are determined. (Guinée

284 2004). In this work, the goal of the LCA was to provide information about the environmental profile of
285 the novel material and to evaluate its benefits and disadvantages compared to conventional plastic
286 materials. PLA-based packaging and PET were studied throughout their life cycles from cradle to grave;
287 i.e. the study started from the extraction of raw materials and ends with the disposal of the packaging.

288 For the PLA packaging, in addition to PLA/nanoclay and PLA/nanoclay/surfactant a third formulation
289 of PLA/nanowhiskers/surfactant was also included in the LCA. PLA and PET packages were assumed
290 to be similar; i.e. take-away packages of the same shape and size with fresh-cut fruit. Due to materials
291 properties, a pristine PLA packaging was measured to weight 8.28 g, while a PET packaging was
292 measured to be slightly heavier with 8.34 g. Considering the physicochemical and microbiological
293 results, the shelf-life of the food product of PLA/nanoclay/surfactant was expected to be longer compared
294 to that of the PET packaging (15 days).

295 An important aspect of scope definition is the functional unit. Functional unit can be seen as a reference
296 unit which represents the level of performance of the product system (UNEP/SETAC Life Cycle
297 Initiative 2013). The functional unit was chosen to be ‘providing customers with 100 000 kg of fresh
298 fruits during one year’, which enables the comparison between shelf-lives. In addition, the functional
299 unit was realized through different scenarios: the PLA-based packaging will keep the product fresh (i) 0
300 days longer, (ii) 1 day longer, (iii) 2 days longer etc.

301 3.3.2 System boundaries

302 Figure 5 shows the life cycle of the PLA-based and PET packages as well as the system boundary studied
303 in the LCA. The system boundary included raw material acquisition, production of the materials,
304 manufacturing of the packaging, filling of the packaging and the end-of-life solutions. The assessment

305 excluded the selling and using of the packages as they were assumed to be the same in all different
306 scenarios tested.

307 *3.3.3 Data sources*

308 Inventory data was collected mainly from the co-authors in this work by sending data collection sheets.
309 Most of the provided data was estimated based on laboratory tests (production of additives and novel
310 material) or small-scale production (manufacturing and filling of packaging). The data for raw material
311 acquisition, production of pristine PLA and PET pellets as well as end-of-life treatment was taken from
312 the Ecoinvent 3 database. Materials not be found from the database were selected as suitable substitutive
313 materials.

314 *3.3.4 End of Life*

315 The end-of-life (EOL) phase was modelled based on the current plastic packages treatment scenario in
316 Europe, as well as based on the desired scenario for the future.

317 Current waste treatment scenario:

- 318 - PET: 60 % incinerated, 40 % landfilled,
- 319 - PLA: 20 % composted, 40 % incinerated, 40 % landfilled.

320

321 Desired waste treatment scenario:

- 322 - PET: 100 % incinerated,
- 323 - PLA: 100 % composted.

324 Data regarding the incineration and landfilling of PET was available on Ecoinvent 3 database. Similar
325 information for PLA-based materials was not available. In the case of incineration the process itself was

326 modelled by selecting the incineration of plastic waste mixture as the 'base'. In order to portray the
327 different qualities of the materials the avoided heat and electricity produced by the incineration were
328 added as avoided products to this 'base'. The avoided products for PET were obtained through Ecoinvent
329 3 database (Table 4). For PLA, the avoided products produced were calculated utilizing its net calorific
330 value and the information that approximately 11 % of the net calorific value is transformed into electric
331 energy and 23 % to thermal energy (Table 4).

332 In the case of landfilling no avoided products were included as both materials were assumed to be inert
333 and therefore they do not produce any landfill gas which could be collected and utilized. Therefore, both
334 materials was modelled as plastic waste mixture. Composting of the PLA-based packaging material was
335 modelled as the composting of common bio-waste and entering nutrients (0,7 w% N, 0,4 w% P_2O_5 and
336 0,6 w% K_2O) as avoided products (data obtained from Ecoinvent 3 database).

337 *3.3.5 Assumptions and limitations*

338 Assumptions were necessary in order to be able to portray the life cycle of the studied PLA-based
339 packaging as well as the literature concerning such innovative solution is still limited. This resulted in
340 some limitations in the assessment, which need to be considered. The assumptions and limitations include
341 the following:

- 342 - The novel packaging weights slightly less; the weight of the packaging was, however, measured
343 without the additive alternatives which might increase the weight slightly.
- 344 - Information concerning the used PLA (Hycail HM 1011) could not be obtained from manufacturer,
345 but was modelled using the database.

- 346 - The manufacturing of the packaging was modelled similarly for both materials based on the the
347 production of 320 000 PLA packages. The possible difference in electricity consumption between the
348 materials was therefore excluded.
- 349 - Only limited data was obtained for the filling process and therefore its contribution to the life cycle
350 could not be fully determined. The obtained data was assumed to be the same for both packages.
- 351 - All additives were assumed to extend the fruits' lifetime equally long which is confirmed for
352 PLA/nanoclays/surfactant packaging by the presented physicochemical and microbiological results.
- 353 - The projected extended shelf-life of the PLA-based packaging was assumed to decrease the food
354 wastage caused by consumer behaviour as people tend to purchase fresh produce instead of the ones
355 close to the 'best before' or 'use by' dates. The reduced wastage was modelled by assuming that
356 people hesitate (50 % probability) to purchase products which have two days left before going bad
357 (for example the wastage of a packaging with 15 day shelf-life would be $(2/15) \times 50 \% = 6,7 \%$). Such
358 percentage is similar to the values found from literature for the wastage of fruits and vegetables in
359 Swedish and Norwegian stores (Koivuouri et al. 2016). The more wastage is created, the more fruits
360 and packages as well as more frequent transportation are required in order to satisfy the consumers'
361 demand of 100 000 kg of fruits per year.

362 3.3.6 Impact assessment

363 The normalized results for each impact category are presented in Figure 6. Normalized results are
364 obtained by dividing the impact with the corresponding normalization factor. This factor is calculated by
365 dividing the total impact of the examined category with the total European population (Sebastien et al.
366 2002).

367 The obtained results (Figure 6) showed that PET presents lower impact in most of the categories which
368 can be explained by the fact that the PLA-based packaging has a higher energy demand due to the
369 manufacturing and mixing of the additives (Figure 7). Even though the bio-based material resulted to be
370 more environmentally benign (Figure 7), the energy consumption proved to weigh more, hence providing
371 results which favour PET. The PLA-based packaging with nanowhiskers additives production requires
372 more energy and materials and, as consequence, presented the largest impacts in all categories compared
373 to the other PLA based solutions. However, the additives had only a minor influence on the materials
374 compared to pristine PLA and PET pellets. The influence of transportation resulted to be exceptionally
375 high due to the diesel used for internal transportation during packaging manufacturing (see damage
376 category 'Resources'), while the external transportation was found to play relatively small role. The
377 waste scenarios (avoided products not shown) had only a minor influence on the overall environmental
378 impact. The units (Pt, point) in Figure 7 represent the average impact of a category caused by one
379 person/on a person during one year in Europe (Sebastien et al. 2016).

380 Of all the life cycle phases – material production, packaging manufacturing, filling of the packaging,
381 waste scenario and transportation – the packaging manufacturing process resulted to be the largest
382 contributor to the environmental impacts (Figure 8). This is mainly due to the high energy consumption
383 but, to some extent, also to the emissions caused by the process. Due to the packaging manufacturing
384 being modelled similarly for both materials as a small-scale process, the energy consumption and
385 emissions were excessive in both cases and can thus explain such high impact. Material production and
386 transportation resulted to have the second highest influence while the waste scenario had only a minor
387 impact.

388 The largest difference between the two the life cycle phases can be seen during the production of the
389 material (Figure 8). While the PLA-based material requires more energy for its production compared to

PET, PET material is less environmentally friendly compared to the novel material. However, as the energy plays a more important role (Figure 7), the production of the novel material results in a higher overall value. Due to the higher energy consumption of the PLA-based material, its influence on climate change is higher than PET's influence. The bio-based nature of the novel material results in lower values for the impacts on human health (e.g. carcinogens and non-carcinogens), but higher values for ecosystem quality (e.g. aquatic eutrophication and land occupation) compared to PET.

3.3.7 Influence of extended shelf-life

The influence of the extended shelf-life was studied utilizing the single score results (Table 5). The aim was to see when the single score results of the PLA-based packaging go below the results of the PET packaging. It should be remembered that single score results provide only a one-sided view of the results and, therefore, they should not be used alone as presenting the preference of one solution over the other.

The results indicate that, if the shelf-life of the PLA-based packaging can be extended by approximately 30 %, the novel packaging can be regarded as equally or more environmentally friendly than the PET packaging. This is due to the decreased wastage in stores and thus fewer packages as well as fewer trips to the store. From the three alternative solutions, both the one with nanoclay and nanoclay with surfactant would be competitive with PET when considering the environmental impacts.

3.3.8 Waste treatment scenarios

The study revealed that, according to the Ecoinvent database, the order of preference for end-of-life treatment would be landfilling or composting while incineration is the least preferable option. This is not in line with current waste legislation (Council TEPaot 2013), where it is clearly stated that landfilling is to be avoided in all cases. Thus the results concerning the waste treatment was examined with caution (Figure 9).

412 The current waste scenario provides a slight advantage to PET because, while composting is less of a
413 burden, the avoided energy produced by incineration compensates the adverse impacts of waste
414 incineration. As the incineration of PET provides more heat and electricity than the incineration of PLA
415 and more of PET goes to incineration than the novel material, the scenario for PET has a lower overall
416 environmental impact.

417 The results further indicated that the desired waste scenario where the novel packaging is 100 %
418 composted is more environmentally friendly than the current waste scenario (Figure 9). The high values
419 for the current scenario for climate change are caused by incineration, while composting does not have a
420 notable influence on climate change and only a slight influence on the rest of the damage categories. In
421 the case of the PET packaging, the current waste scenario resulted in a lower overall environmental
422 impact compared to the desired scenario. This is due to the database considering landfilling to be more
423 environmentally friendly than incineration. The results would suggest that the desired waste treatment
424 scenario benefits the novel packaging significantly over the one of PET packaging.

425 **4. Conclusions**

426 PLA-based packaging with nanoclay and surfactant were tested by physicochemical and microbiological
427 assays in comparison with conventional plastic packaging (PET). The presence of nanoclay and
428 surfactant in PLA-based packaging conferred properties that approaches to the performance of PET and
429 improved the PLA pristine as well. Moreover, PLA-based packaging with nanoclays and surfactant
430 revealed to be the best approach to prevent microbial growth. The type of nanoclay (20 and 30B) did not
431 show significant distinction among PLA packages. Furthermore, the PLA-based packaging was
432 evaluated for their environmental sustainability in comparison with PET using life cycle assessment.
433 Although PLA-based packaging with additives require more energy for their production, LCA results

434 clear showed that PLA-based packaging with nanoclay and surfactant is very competitive with PET for
435 environmental impact. PLA-based packaging revealed to be more environmental friendly as well as
436 causes lower impact in human health. In addition, the results indicated that PLA-based packaging with
437 additives can reduce food loss through extending the shelf-life of the produce. These results encourage
438 further development PLA-nanoclay composites packaging as a great potential in the application fresh
439 food packaging.

440 **Acknowledgements**

441 This research was conducted within the Smart and sustainable food packaging utilizing flexible printed
442 intelligence and materials technologies (SusFoFlex, grant agreement no. 289829) project, which was
443 funded by the Seventh Framework Programme. The authors would like to thank all the project partners
444 in SusFoFlex contributing to the data collection. LAQV received financial support from FCT/MEC
445 through national funds and FEDER, under the Partnership Agreement PT2020 (reference UID/
446 QUI/50006/2013 - POCI/01/0145/FEDER/007265).

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560 **Tables and Figures**

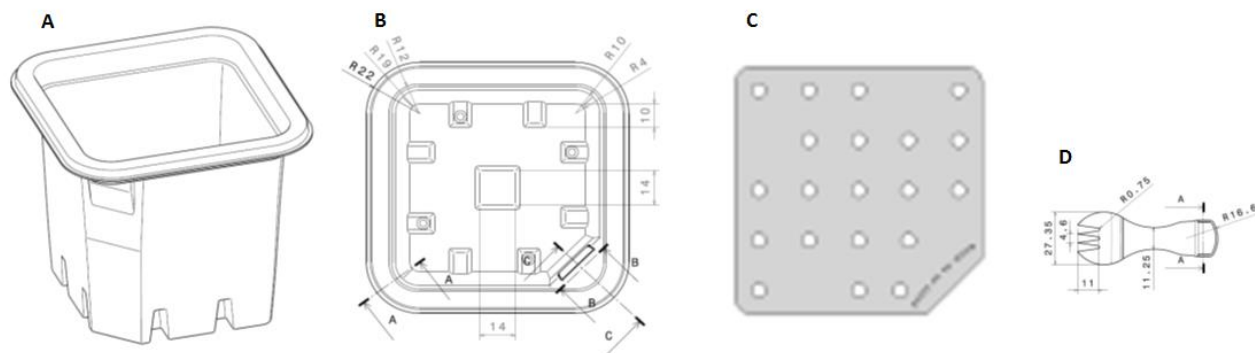


Figure 1 PLA packaging design for fresh-cut fruits. (A) Overall view, (B) Top view, (C) Mesh, (D) Fork. The PLA-based packaging was developed and designed under the SusFoFlex consortium (grant agreement no. 289829).

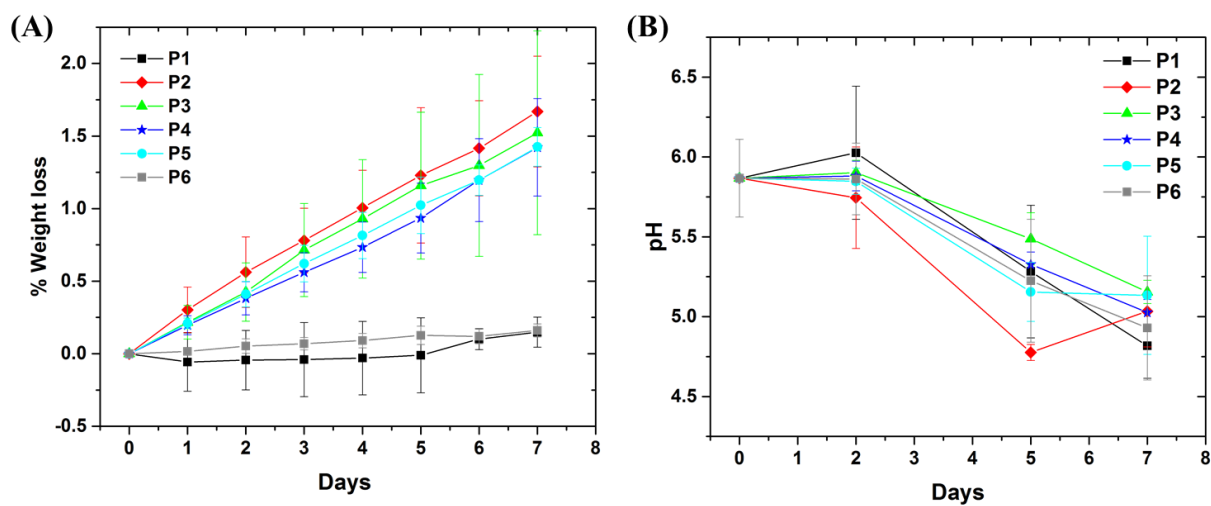


Figure 2 Weight losses (A) and pH (B) average values for each correspondent packaging formulation (Pi, i = 1-6, identified in Table 1).

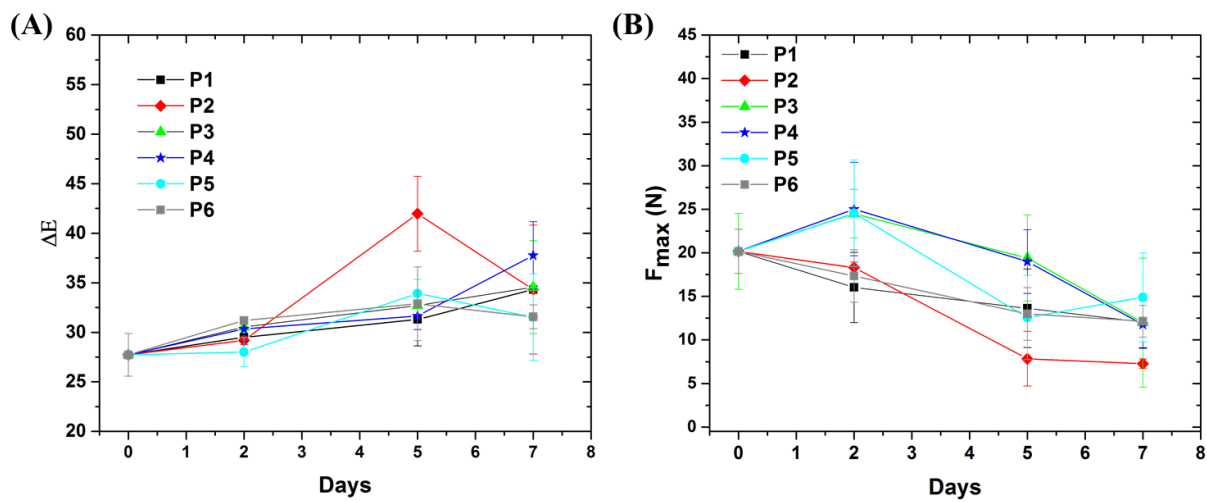


Figure 3 Colour and firmness of cut-fresh melon pieces from different package formulations (Pi, i = 1-6, identified in Table 1) throughout shelf-life: (A) ΔE values based on the CIE 1976 $L^*a^*b^*$ colour difference (expression I) and (B) maximum resistance force exerted by the fresh-cut melon.

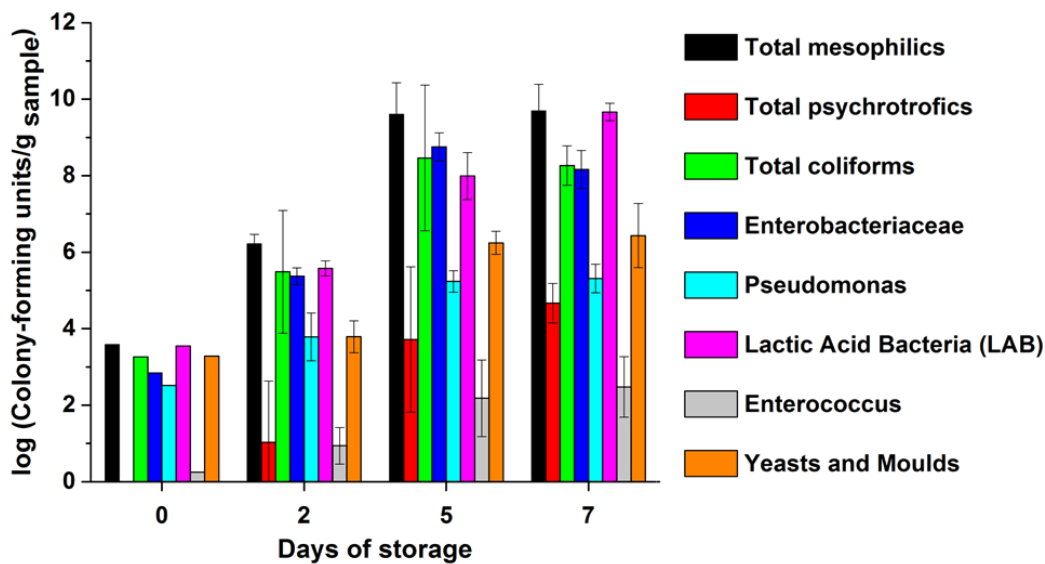
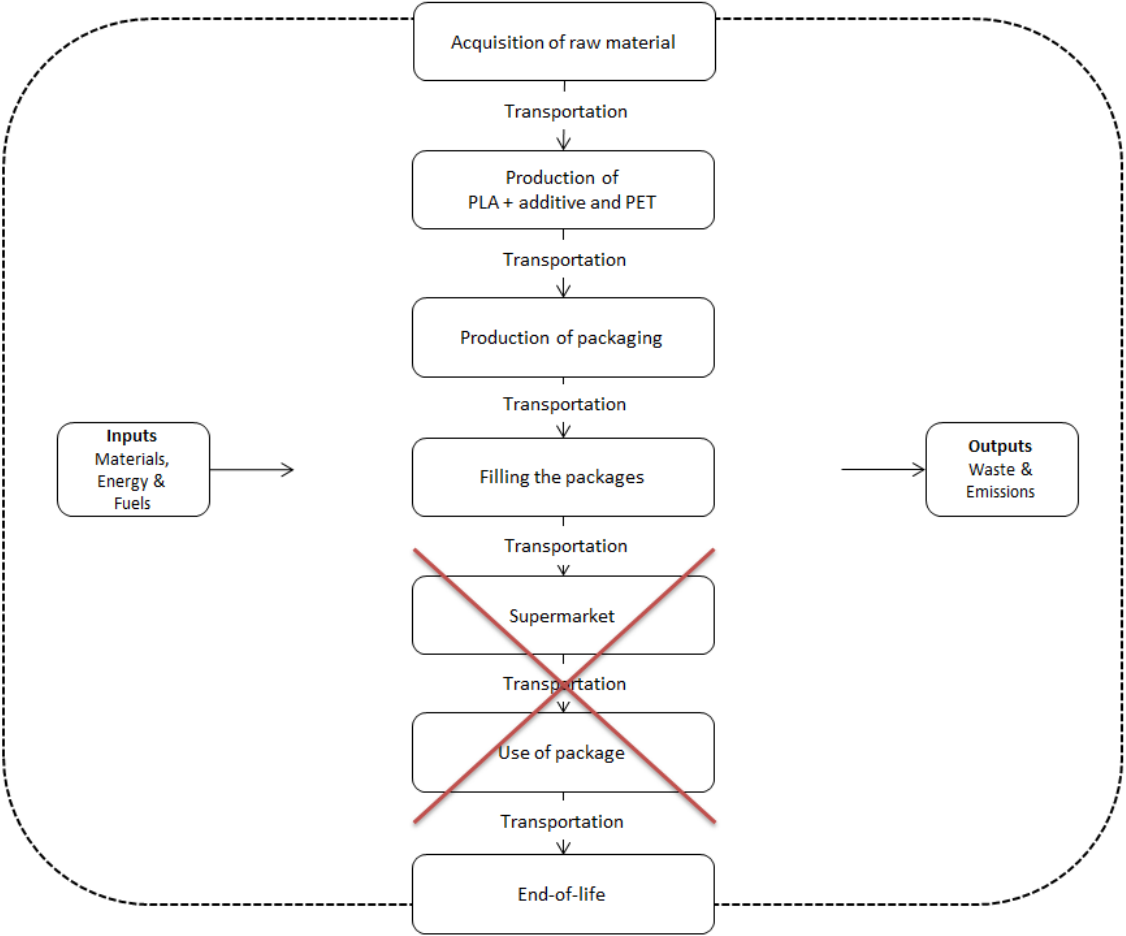


Figure 4 Great average of logarithm of total viable counts obtained in different inoculation and incubation conditions. The great average was calculated defined as the average of the microbial counts obtained from all packages in the same day.

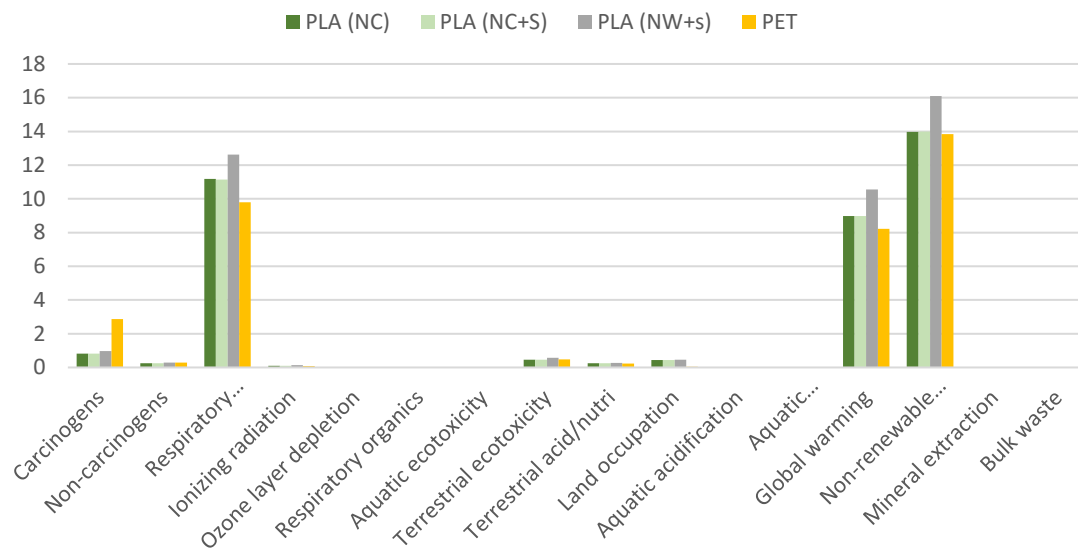
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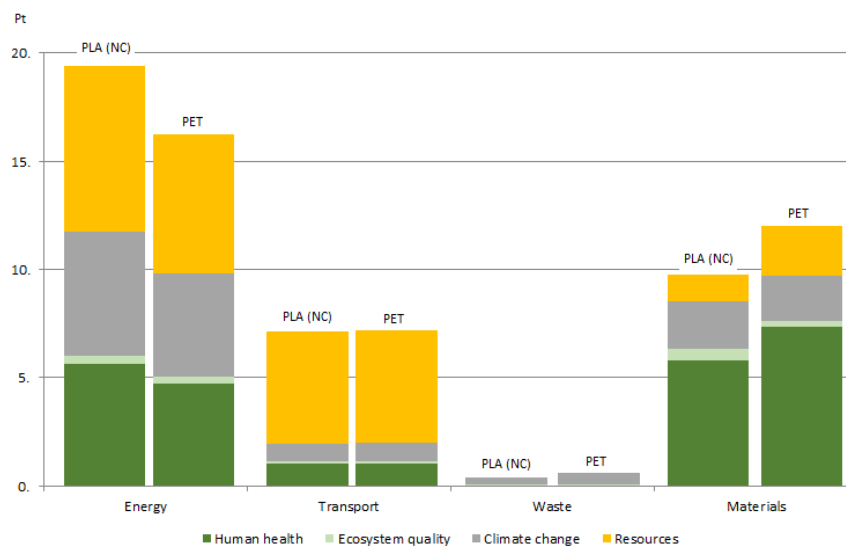
577 **Figure 5.** System boundary of the life cycle assessment for PLA-based and PTE packages from cradle to grave. The
578 assessment excluded the selling and using of the packages as well as the transportation between these two phases.

579



580

581 **Figure 6** Normalized results for the life cycles of three novel PLA based packages and a PET package. All modelled with the
 582 shelf-life of 15 days and current waste scenario. (NC = Nanoclay, S = Surfactant, NW = Nanowhiskers)



583

584 **Figure 7** Results for contribution of energy, transport, waste and materials to the environmental performance of novel
 585 packaging with nanoclay and PET packaging during their life cycles. Both modelled with the shelf of 15 days and current
 586 waste scenario.

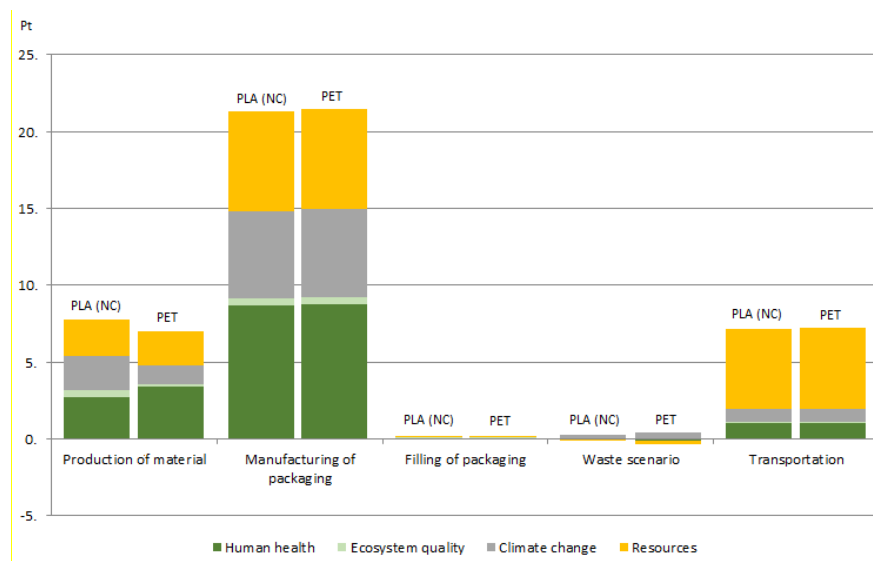


Figure 8 Results for the contribution of the life cycle phases to the environmental performance of the novel packaging with nanoclay and PET packaging. Both modelled with the shelf of 15 days and current waste scenario.

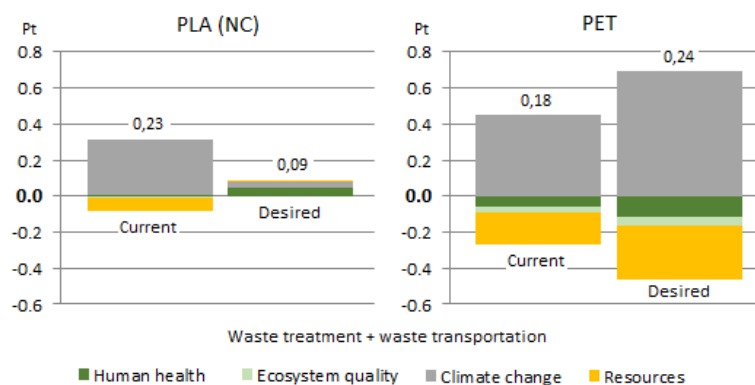


Figure 9 Comparison of current and desired waste scenarios of novel packaging with nanoclay and PET packaging. Current: PET 60 I / 40 L and PLA 20 C / 40 I / 40 L. Desired: PET 100 I and PLA 100 C. Values indicate the overall results which combine the burden of the process (positive values) and benefits of avoided products (negative values).

597 **Table 1.** Identification of the packages used

Package	Characteristics
identification	(Polymer, nanoclay, surfactant and plasticizer)
P1	PET – package commercial available (control)
P2	Pristine PLA formulation with 3,5% OnCap™ Bio (PolyOne)
P3	PLA; 1% Na-CLOISITE 20; TRITON; 3,5% OnCap™ Bio (PolyOne)
P4	PLA; 1%-Na CLOISITE 30B; 3,5% OnCap™ Bio (PolyOne)
P5	PLA; 1% Na-CLOISITE 30B; TRITON; 3,5% OnCap™ Bio (PolyOne)
P6	PET – package produced at Andaltec with same design used for PLA packages (control)

598

599 **Table 2.** Physicochemical properties (average ± standard deviation) for cut-fresh melon from different package formulations
600 (Pi, i = 1-6, identified in Table 1) after 5 days of storage.

	P1	P2	P3	P4	P5	P6
Weight loss (%)	0.0±0.3 ^a	1.2±0.5 ^b	1.2±0.5 ^b	0.9±0.2 ^b	1.0±0.2 ^b	0.1±0.1 ^a
pH	5.3±0.4 ^{a,b}	4.8±0.1 ^c	5.5±0.2 ^a	5.3±0.1 ^{a,b}	5.2±0.2 ^b	5.9±0.2 ^d
Colour, ΔE	31±3 ^a	42±4 ^b	33±1 ^a	32±1 ^a	34±1 ^a	33±4 ^a
Firmness, F_{máx} (N)	14±5 ^{a,b}	8±1 ^b	19±3 ^a	19±4 ^a	13±5 ^{a,b}	13±3 ^{a,b}

601 Different superscripts letters within same line indicate significant differences (p<0.05), according to the Tukey test.

602

603 **Table 3.** Logarithm of viable counts (average ± standard deviation, CFU/gsample) on different culture media for cut-fresh
604 melon from different package formulations (Pi, i = 1-6, identified in Table 1) after 5 days storage

	P1	P2	P3	P4	P5	P6
Total mesophilic	9.7±0.4 ^{a,b}	11±0 ^a	8.6±0.3 ^b	9.5±0.6 ^b	9.0±0.3 ^b	9.9±1.3 ^{a,b}
Total psychrotrophic	5.0±0	4.0±0.2	4.1±0.2	5.3±0.3	0±0	4.0±0.0
Total coliform	8.1±0.2	8.4±0.5	8.4±0.4	8.4±0.5	8.0±0.1	9.5±0.6
<i>Enterobacteriaceae</i>	8.5±0.2	9.0±0.3	8.5±0.1	8.6±0.1	8.4±0.4	9.4±0.7
Lactic acid bacteria	8.0±0.3	8.7±0.3	8.2±0.3	7.5±1.2	8.5±0.3	7.1±1.2
Yeast and moulds	6.2±1.5	6.4±1.1	6.0±0.6	6.0±1.6	6.1±0.9	6.8±0.9

<i>Pseudomonas</i>	5.7±0.5	5.3±0.31	5.2±0.2	5.2±0.21	4.8±0.7	5.2±0.2
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605 Different superscripts letters within same line indicate significant differences (p<0.05), according to the Tukey test.

606

607 **Table 4.** Electric and thermal energy of PET (data from Ecoinvent database) and PLA (calculated based on data for PET, PP
608 and PE). Net calorific value of PLA obtained from (Detzel 2006).

	Net calorific value	Electric energy	Thermal energy
	[MJ/kg]	[MJ/kg]	[MJ/kg]
PET	22.95	2.46	5.03
PLA	18.00	1.98	4.14

609

610 **Table 5** Single score results for three PLA-based packaging solutions and PET packaging; influence of extended shelf-life of
611 the novel packaging. Shelf-life of PET packaging is 15 days (used as baseline) and the waste scenario is ‘Current’.

Difference in shelf-lives [days]	PLA-based packaging with nanoclay [Pt]	PLA-based packaging with nanoclay and surfactant [Pt]	PLA-based packaging with nanowhiskers and surfactant [Pt]	PET packaging [Pt]
0	36.5	36.5	42.0	35.9
1	36.3	36.3	41.8	35.9
2	36.2	36.2	41.7	35.9
3	36.1	36.0	41.5	35.9
4	36.0	35.9	41.4	35.9
5	35.9	35.8	41.3	35.9

612