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9 **Comparative environmental Life Cycle Assessment of integral revalorization of vine shoots**  
10 **from a biorefinery perspective**

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12  
13 Patricia Gullón<sup>1</sup>, Beatriz Gullón<sup>2</sup>, Izaskun Dávila<sup>1</sup>, Jalel Labidi<sup>1</sup> and Sara Gonzalez-Garcia<sup>1\*</sup>

14 <sup>1</sup> Chemical and Environmental Engineering Department, University of the Basque Country, 20018 - San  
15 Sebastián, Spain

16 <sup>2</sup> Department of Chemical Engineering, School of Engineering, University of Santiago de Compostela. 15782-  
17 Santiago de Compostela, Spain.

18 \* Corresponding author: E-mail address: [sara.gonzalez@usc.es](mailto:sara.gonzalez@usc.es)

19  
20 **Abstract**

21 The use of vine shoots as feedstock in biorefining activities to obtain bioproducts under efficient and  
22 optimized conditions could be crucial to make future high added value compounds and processes  
23 more sustainable. In this study, five different potential valorization scenarios from vine shoots differing  
24 on diverse extraction and delignification steps were assessed from an environmental perspective  
25 using the Life Cycle Assessment methodology to identify the most sustainable biorefining route. The  
26 main findings from this study report that an increment on the number of valorization steps involves  
27 higher energy and chemical requirements deriving on worse environmental profiles. Scenarios  
28 incorporating fermentation of the glucose liquors or organosolv delignification perform the worst profiles.  
29 Autohydrolysis, concentration and freeze drying and enzymatic hydrolysis are the main responsible

30 stages of the environmental burdens. Further research should be focused on optimizing chemicals  
31 and electricity requirements to develop greener systems.

32

33 **Keywords:** biopolymers; environmental profile; ethanol; LCA; organosolv

34

## 35 **1. Introduction**

36 Nowadays, the society is facing important challenges mainly related with the substitution of petroleum  
37 based materials by renewable ones. In this way not only the the high dependence on finite-fossil fuels  
38 consumption could be reduced but also the unstable production of petroleum based materials and the  
39 derived environmental impacts, as the greenhouse gases (GHGs) emission (Spiridon et al., 2016).

40 Therefore, numerous initiatives are being developed to promote the production and use of  
41 bioresources-based alternatives instead of petrochemical ones (Cherubini and Ulgiati, 2010; Gullón et  
42 al., 2016). In this sense, biomass could be considered as a sustainable alternative raw material to  
43 produce biomaterials because of its abundant availability, diversity and recyclability (Spiridon et al.,  
44 2016).

45 The use of bioresources instead of petrochemicals for the production of biofuels (González-García et  
46 al., 2010) and biofoams (González-García et al., 2016a) has been demonstrated to cause lower GHGs  
47 emission. Nevertheless, there are concerns regarding their benefits in terms of other environmental  
48 impact categories, as the use of the land could change due to the cultivation activities related with the  
49 biomaterial-feedstock production, mainly in areas that have significant social value (Searchinger et al.,  
50 2008). Therefore, a sustainability study should be required so as to identify the situations in which the  
51 use of bioresources could be environmentally feasible over petrochemical alternatives.

52 The use of lignocellulosic biomass residues and wastes from agro-food-industrial, agricultural and  
53 forestry activities could be considered as a potential alternative for the manufacture of added value  
54 products instead of using dedicated crops. The employment of biomass residues could avoid the  
55 derived negative impact from the cultivation phase of crops and it could give a solution to residual  
56 streams which must be managed in a sustainable manner without entering in confrontation with food  
57 availability. In fact, all these residues, which are generated in billions of tons a year, represent a  
58 renewable source available to be used for further applications (Forster-Carneiro et al., 2013). The use  
59 of residues is probably the lowest cost form of available biomass, but the range of costs can  
60 considerably vary depending on the residue as they involved items such as harvest, salaries and

61 transportation (Gallagher et al., 2003). Furthermore, the removal of biomass residues from croplands  
62 could also derive on negative consequences closely related to the loss of soil organic carbon content  
63 as well as to the reduction of crops yields (Wilhelm et al., 2007).

64 The composition of the lignocellulosic residues changes depending on the feedstock species and  
65 origin. However, in general lines, they are mainly constituted by cellulose, hemicellulose and lignin as  
66 well as by organic extractives and inorganic minerals (Dávila et al., 2016; Thakur et al., 2015) and they  
67 can be considered as attractive biorenewable polymers (from now, biopolymers) which could replace  
68 the traditional synthetic ones (Thakur et al., 2015). For that reason, biorefineries based on these  
69 lignocellulosic materials are drawing special attention (Dávila et al., 2016; González-García et al.,  
70 2017) for different applications from biomedical to automotive (Thakur et al., 2015). Examples of  
71 lignocellulosic biorefineries viable at industrial scale are Borregaard Company and Envergent  
72 Technologies. Borregaard Company, is a Norwegian company which in the 1930s started the  
73 production of bioethanol by the fermentation of the sugar obtained from spruce wood. It is organized  
74 into five production sections, such as: cellulose and bioethanol, lignin-based binding and dispersing  
75 agent, fine chemicals for the pharmaceutical sector, vanillin for the food sector and microfibrillated  
76 cellulose. Envergent Technologies is a corporate between Honeywell's UOP and Ensyn Corporation,  
77 established in 2008 in Des Plaines, Illinois. Ensyn is the proprietary of the Rapid Thermal Processing  
78 (RTP™) technology. Nowadays, there are seven commercial RTP plants in the United States and  
79 Canada. These biorefining industries are converting residual biomass (mainly wood and agricultural  
80 wastes) into more than 30 added value products encompassing green fuels, chemicals, food  
81 flavorings, adhesive resins for construction, etc. (Sillanpää and Ncibi, 2017).

82 Cellulose is the most abundantly available bioresource and its demand is continuously increasing due  
83 to its environmental friendly, biodegradable and biocompatible nature (Watkins et al., 2015).

84 Nowadays, cellulose finds several applications as building materials, paper, textiles, as well as  
85 clothing (Brichi et al., 2013). Another rising application of cellulose is as reinforcement in composite  
86 materials (Watkins et al., 2015). In the last two decades the employment of cellulose to obtain energy  
87 has been extensively exploited, as great efforts have been performed to improve the conversion of  
88 cellulose to ethanol, methane and in the most recent years to hydrogen (Menon et al., 2012).

89 After cellulose, the second most abundant renewable polysaccharide on the earth is the  
90 hemicellulose. (Gullón et al., 2016). Thus, it could offer a sustainable alternative for materials,  
91 chemicals and fuels production (Kemppainen et al., 2014). However, the use of this biopolymer is not  
92 so extended than other natural polysaccharides (e.g. chitosan, cellulose and starch) mainly because  
93 its heterogeneous composition makes its application difficult at industrial scale (Gullón et al., 2016).  
94 Nevertheless, in recent years, numerous research activities have been developed to integrate this  
95 attractive, renewable and cheap polysaccharide in the industry under a biorefinery approach  
96 specifically to produce prebiotic functional food ingredients (Nabais et al., 2010; Dávila et al., 2016;  
97 González-García et al., 2016b). The employment of hemicelluloses for the production of films  
98 (Hartman et al., 2006) and hydrogels (Gullón et al., 2016) as well as their use as food additives  
99 (González-García et al., 2017) are attracting special interest.

100 After cellulose lignin is the next most abundant natural polymer in the nature. Lignin possess chemical  
101 and physical properties that make it an excellent candidate to substitute any product obtained from  
102 petrochemical sources; moreover, it is a natural and biorenewable feedstock, obtainable at an  
103 affordable cost. Lignin can be used as emulsifiers, dyes, synthetic floorings, sequestering, binding,  
104 thermosets, dispersal agents, paints and fuels to treatmentsfor roadways (Watkims et al., 2015).

105 Within the different activities carried out in the agricultural sector, grape cultivation requires special  
106 interest since it is one of the most spread crops in the world (Nabais et al., 2010) and specifically in  
107 countries such as Spain (Dávila et al., 2016). Vine shoots are considered novel biomass agricultural  
108 residues and their management under a biorefinery perspective is attracting notice (Nabais et al.,  
109 2010; Dávila et al., 2016). Numerous studies are available in the literature focused on the valorization  
110 of vine shoots to obtain high added value products, such as activated carbon (Nabais et al., 2010),  
111 polyphenols (Gullón et al., 2017), ethanol (Jiménez et al., 2007), lactic acid and/or xylitol (Rivas et al.,  
112 2007). However, to the best of our knowledge, the environmental sustainability of these production  
113 schemes has not been assessed yet. Only one environmental study has been published with special  
114 focus on the environmental footprints of different production schemes of soluble saccharides of  
115 polymeric and oligomeric nature from woody residual streams (González-García et al., 2016b) using

116 the Life Cycle Assessment (LCA) methodology. This methodology is used to understand and address  
117 environmental impacts throughout a production system (ISO 14040, 2006).

118 In this study, the assessment of the environmental impacts derived from the valorization of vine shoots  
119 from the winery sector into high added value products has been performed considering five different  
120 and alternative valorizing schemes. The evaluation of the schemes would permit the identification  
121 of environmental *hotspots* responsible of the largest environmental impacts. These scenarios differ on  
122 the extraction, delignification and/or hydrolysis routes and are based on experiments and protocols  
123 carried out at semi-pilot scale. The LCA models for each scenario were developed in accordance with  
124 the requirements of ISO 14040 (2006).

125

## 126 **2. Materials and methods**

127 Life cycle assessment (LCA) is a standardised framework for the systematic evaluation of  
128 environmental aspects of a product or service system through all stages of its life cycle (ISO 14040,  
129 2006) by means of the assessment of environmental impacts of a product from its extraction of raw  
130 materials to its disposal.

### 131 **2.1 Goal and scope definition**

132 This study aims to assess the environmental performance of different valorizing routes of vine shoots  
133 to obtain high added value products with potential uses from biopolymers to energy production from  
134 their three major constituents; cellulose, lignin and hemicelluloses. Production batches for each  
135 valorization scenario have been specifically designed and performed to analyse in detail the  
136 conversion routes. Different side-streams are derived from the valorizing systems which could be used  
137 for further applications such as cellulose, lignin, antioxidants as well as biomass rich on nitrogen. The  
138 description of the different valorization scenarios is included below.

139 In this study, a functional unit based on the amount of biomass valorized per batch, i.e. 100 kg of  
140 oven-dried vine shoots at the semi-pilot plant gate provided by a local farmer, has been defined and  
141 considered for comparison. The semi-pilot facility is located in the Chemical and Environmental  
142 Engineering Department from the University of the Basque Country (San Sebastián, Spain). All the  
143 scenarios under assessment are multi-outputs systems where more than one product is obtained.  
144 According to the functional unit chosen, no allocation procedure has been required and a system  
145 expansion approach has been considered (if necessary). The consideration of a reference unit based  
146 on the amount of waste-mass valorized is commonly used in LCA studies involving waste  
147 management systems (González-García et al., 2017). In addition, this functional unit will allow the  
148 comparison of the different schemes in spite of yielding on different high added value products.

## 149 **2.2 Description of the valorizing scenarios under study**

150 The base scenario (Scenario I) consisted of seven main subsystems: autohydrolysis (SS1),  
151 centrifugation (SS2), oligosaccharides extraction (SS3), ethyl acetate recovery (SS4), concentration  
152 and freeze drying (SS5), delignification (SS7) and wastewater treatment (SS6). The subsystems and  
153 stages of the process included within the system boundaries are represented in **Figure 1a** and  
154 described below.

155

156 <**Figure 1a** around here>

157

158 The vine shoots at an optimum size ( $\approx 0.4$ mm) are received at the semi-pilot plant directly supplied by  
159 a local farmer and are treated with water in a stainless steel Parr reactor (SS1) at a liquid-to solid ratio  
160 of 8 kg/kg (oven-dried basis) and under non-isothermal reaction at 200 °C (15.54 bar), conditions  
161 reported as optimal for hemicellulose solubilisation by Dávila et al. (2016). Once the treatment is  
162 completed, the reaction media was cooled by internal refrigeration for approximately 10 min, and the  
163 solid and liquid fractions are separated and recovered by centrifugation (SS2). The liquid stream  
164 (autohydrolysis liquour) is a mixture of hemicellulosic oligosaccharides and its composition is reported  
165 in **Table 1**. It is important to note that this stage is common to all the valorization sequences proposed  
166 for analysis.

167

168 <Table 1 around here>

169

170 In order to obtain two different streams of high-added value compounds, one rich on antioxidant and  
171 the other one rich on oligosaccharides with potential nutraceutical and pharmaceutical applications,  
172 these liquors are processed in two stages. First of all, the liquors are extracted with ethyl acetate (1:3  
173 (v/v) hydrolysate: solvent ratio) in a single extraction stage under stirring for 15 min at room  
174 temperature (SS3). The organic and aqueous phases are separated by decantation and processed as  
175 follows: a) the organic phase is vacuum-evaporated at 40 °C to recover the solvent and to obtain an  
176 extract rich in antioxidants (SS4) and, b) the aqueous phase is concentrated to obtain a stream rich in  
177 oligomeric compounds using a membrane processing unit (SS5) as described previously by González-  
178 García et al. (2016b). Briefly, the experiments were performed using a regenerated cellulose  
179 membrane of 1 kDa cutoff (Millipore) operating at a pressure of 4 bar. The concentration was  
180 performed up to reach a ratio of overall retentate to feed of 1:4. The processing was carried out at  
181 room temperature. At the end of process, the retentate is freeze-dried and the residual stream  
182 (permeate) is sent to wastewater treatment (SS6). The solid fraction obtained after the hydrothermal  
183 processing (SS1), composed mainly by cellulose (27.7%) and lignin (52%), is subjected to alkaline  
184 delignification to obtain both fractions separately enabling their individual use for applications of added  
185 value (SS7). Briefly, the alkaline delignification stage is carried out using a ratio liquid:solid of 10  
186 kg/kg, NaOH 12% (w/w) at 124 °C (pressure= 2.32 bar) during 105 min in a autoclave (Dávila et al.,  
187 2017a). After the pretreatment, the slurry was vacuum filtered to separate the black liquors (containing  
188 the dissolved lignin) from the delignified solid. The lignin is precipitated from the black liquors using  
189 H<sub>2</sub>SO<sub>4</sub> at 96% until pH 2 and then it is recovered by filtration and washed several times first with  
190 acidified water and then with water. The delignified solid is washed several times with water to remove  
191 alkali until its pH is about 7 and then it is filtered. Thus, lignin and cellulose are also obtained as co-  
192 products from this valorization scheme.

193

194 *Alternative valorization scenarios*

- 195 • Scenario II: this scenario follows the same scheme as the one depicted in Scenario I with only  
196 one remarkable difference, it is that in this scenario the autohydrolysis liquors obtained in the



197 centrifugation step (SS2) are directly sent to the concentration unit (SS5), so that the antioxidants are  
198 not finally extracted (**Figure 1b**).

199

200 <**Figure 1b** around here>

201

202 • Scenario III: the scenario I is extended including an enzymatic hydrolysis step (SS8) of the  
203 cellulose from the delignification treatment (SS7) to obtain glucose (susceptible of being used in the  
204 production of ethanol by a fermentative via). For this purpose, the delignified solid (enriched in  
205 cellulose) is mixed with water at a liquid to solid ratio of 30 w/w, and the enzymatic hydrolysis is  
206 carried out by the addition of cellulase (Celluclast 1.5 L) at a ratio of 25 FPU<sup>1</sup> per g of dry solid, and  $\beta$ -  
207 glucosidase (Novozymes 188) at a ratio of 5 IU<sup>2</sup> per FPU of cellulase during 48 h at 48.5 °C. Citric  
208 acid-sodium citrate buffer was used at 0.05 N to maintain the medium at pH~ 4.85. The fraction of  
209 cellulose that has not been converted (so called solid waste fraction **in Figure 1c**) is separated by  
210 centrifugation (SS9) as additional product stream for energy production. The production of this energy  
211 has been included within the system boundaries of this scenario (SS14). Moreover, an avoided  
212 product approach has been considered with regard to the production of the same amount of electricity  
213 (taking into account the Spanish electricity profile) as well as heat (considering its production from  
214 natural gas) in a CHP unit.

215

216 <**Figure 1c** around here>

217

218 • Scenario IV: in this case, the scenario III is extended with the incorporation of an alcoholic  
219 fermentation (SS11) of the glucose that is converted into ethanol and CO<sub>2</sub> as displayed in  
220 **Figure 1d**. For this goal, the strain *Saccharomyces cerevisiae* DSM 70449, obtained from the  
221 DSMZ collection (Braunschweig, Germany), is used in the fermentation. For the inoculum  
222 preparation, cells of *S. cerevisiae* are grown (SS10) at 30 °C and 120 rpm for 24 h in a  
223 medium containing 10 g glucose/L, 5 g peptone/L, 3 g malt extract/L, and 3 g yeast extract/L.  
224 After growth, cells are recovered by centrifugation, resuspended in a phosphate buffer solution

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<sup>1</sup> FPU - Filter Paper Activity

<sup>2</sup> U: unit of enzymatic activity. It is defined as the amount of enzyme catalyzing the formation of 1  $\mu$ mol of D-galacturonic acid per minute at 37 °C and pH5.

225 and inoculated to achieve a concentration of 2.5 g yeast/L (dry weight basis) in the medium  
226 together with the nutrients (without glucose) to start the fermentation. The fermentation is  
227 carried out at 35 °C and 120 rpm for 48 h. Once the fermentation is completed, the yeast cells  
228 are separated from the medium containing the ethanol by centrifugation (SS12). The spent  
229 yeast cells of *S. cerevisiae* could be used as a cheap nitrogen source in other biotechnological  
230 processes (Rivas et al., 2004). In this study, the further management of this biomass based  
231 stream has not been considered within the system boundaries since it is out of the factory  
232 scope. Finally, to obtain ethanol for chemical uses it is necessary to carry out a purification  
233 step of the fermentation broth. Among all the alternatives available for this purpose, in this  
234 research we have selected a process of distillation (SS13). The data of energy consumptions  
235 of this stage were obtained from the database of Aspen Plus® of our research group. Taking  
236 into account the perspective considered concerning the solid waste fraction management  
237 obtained in SS9 (avoided energy production), the same approach has been established for the  
238 ethanol obtained in SS13. Thus, avoided ethanol production has been computed in the  
239 environmental evaluation.

240

241 &lt;Figure 1d around here&gt;

242

243 • Scenario V: the scheme proposed in this scenario is similar as the one reported in Scenario I  
244 with only one difference in the delignification stage (SS6). In this case, an organosolv pre-treatment is  
245 performed to obtain cellulose and lignin as isolated fractions (see **Figure 1e**). In summary, the  
246 organosolv process is performed at a liquid/solid of 8 kg/kg (oven dried basis) with ethanol at 50%  
247 (w/w) at 200 °C in a 1.5 L stainless steel PARR reactor during 90 min (Dávila et al., 2017b). At the end  
248 of the treatment, the reactor is cooled down and the suspension is filtered obtaining two streams: the  
249 black liquors (liquid fraction) and the delignified solid (solid fraction). The lignin is obtained by  
250 precipitation from the black liquors with three volumes of acidified water and then is filtered and  
251 washed several times with water until neutralization. The delignified solid is washed several times with  
252 water and filtered.

253

254 &lt;Figure 1e around here&gt;

255

256 **2.3 Inventory data acquisition**

257 A consistent environmental assessment requires the collection of high quality Life Cycle Inventory  
258 (LCI) data. In this study and regardless of the valorizing scenarios under evaluation, inventory data for  
259 the foreground systems (i.e. direct inputs to and outputs from each subsystem) such as electricity  
260 requirements in all equipment (reactors, fermenter, distiller, centrifuges, membrane units and freeze-  
261 dryers), chemical doses – when necessary (H<sub>2</sub>SO<sub>4</sub>, NaOH, ethyl acetate, ethanol), tap water, nutrients  
262 and enzymes consumption correspond to primary data which have been directly taken from the semi-  
263 pilot plant taking into account the average data per batch (100 kg vine shoots – dry matter). As  
264 previously indicated, specific batches have been performed for each valorization route to compile all  
265 mass and energy data.

266 All electricity requirements have been directly estimated considering the power of equipment as well  
267 as the duration of its use in order to obtain the specific requirement for each subsystem. A summary of  
268 primary data managed for each valorizing scenario is displayed in **Table 2**.

269 <**Table 2** around here>

270 With regard to the wastewater treatment plant where the wastewater produced in the different  
271 activities is treated, secondary data have been managed since this stage is not performed within the  
272 semi-pilot plant although it is located on the surroundings. The corresponding inventory data have  
273 been taken from the ecoinvent database © (Doka, 2007).

274 Concerning the background processes, the inventory data corresponding to the production of the  
275 different inputs to the systems (electricity from Spanish grid, chemicals, nutrients and tap water), have  
276 been taken from Ecoinvent © database version 3 (werner et al., 2016). Regarding the production of  
277 electricity, the process included in the database was updated using data for the average electricity  
278 generation and import/export data from Spain in 2014 (Red Eléctrica de España, 2014). Moreover,

279 inventory data related with the process in which the solid fraction is valorized into energy (SS14) in  
280 Scenario III and Scenario IV have been taken from Dones et al. (2007). Data for enzyme production  
281 have been found in Nielsen et al. (2007) and Feijoo et al. (2017).

282 Since bioethanol is also produced as co-product in scenario IV and bioethanol can be used for energy  
283 and chemical purposes, a system expansion perspective has been assumed and the production of this  
284 amount of ethanol by means of a conventional way has been included within the system boundaries  
285 and considered as “avoided product”. The data managed have been taken from Jungbluth et al.  
286 (2007). The same perspective has been assumed regarding to the solid waste fraction produced in  
287 Scenarios III and IV in the centrifugation stage (SS9). Inventory data managed regarding the avoided  
288 heat production corresponds to the production of heat from natural gas as fossil source (Dones et al.,  
289 2007). Regarding electricity, the production of electricity in the national grid has been computed as  
290 previously described. Detailed information with regard to the background processes managed is  
291 summarized in **Table 3**.

292 <**Table 3** around here>

## 293 **2.4 Impact assessment methodology**

294 The characterization factors reported by the Centre of Environmental Science of Leiden University -  
295 CML 2001 method v2.05 (Guinée et al. 2001) have been considered in this study for the analysis. The  
296 following impact categories have been evaluated: abiotic depletion potential (ADP), acidification  
297 potential (AP), eutrophication potential (EP), global warming potential (GWP), ozone layer depletion  
298 potential (ODP), human toxicity potential (HTP), freshwater aquatic ecotoxicity potential (FEP), marine  
299 aquatic ecotoxicity potential (MEP), terrestrial ecotoxicity potential (TEP) and photochemical oxidation  
300 potential (POP). The choice of these environmental impacts is due that all together give a full and  
301 comprehensive overview of the environmental effects related to activities involved in the valorization  
302 schemes under evaluation. In addition, this selection should allow the comparison with other studies  
303 where environmental profiles of other valorization systems were also environmentally evaluated  
304 (González-García et al., 2016b, 2017). SimaPro 8.02 has been the software used for the

305 computational implementation of the inventory data and estimation of the characterization stage (PRé  
306 Consultants, 2017).

### 307 **3. Interpretation of results and discussion**

#### 308 **3.1. Global environmental results**

309 First of all, the environmental burdens of the different scenarios proposed for analysis have been  
310 quantified to estimate the comparative profiles and identify the most environmentally friendly one, that  
311 is, this scenario that reports the lowest environmental burdens. Next, the main environmental *hotspots*  
312 have also been identified since the environmental improvements should be proposed under their  
313 consideration.

314 From the analysis of data summarized in **Table 2** for the different valorization routes as well as taking  
315 into account the description of the considered strategies, it is evident that they share a number of  
316 steps in common such as autohydrolysis (SS1), centrifugation (SS2), concentration and freeze drying  
317 (SS5) and wastewater treatment (SS6). Although delignification step (SS7) is also present in all  
318 routes, the delignification strategy considerably differs between Scenario I-IV (alkaline delignification)  
319 and Scenario V (organosolv), being different not only in terms of operating conditions but also taking  
320 into account the goals. Whereas alkaline delignification allows a higher delignification ratio deriving on  
321 a solid stream rich on cellulose and susceptible to be used for glucose obention by enzymatic  
322 hydrolysis, the organosolv based delignification is almost negligible being the lignin extraction one of  
323 the main roles.

324 Additional stages (enzymatic hydrolysis, inoculum preparation, fermentation, distillation, ...) have been  
325 computed taking into account the desired final products. Therefore, the specificity of the final products  
326 is different between scenarios.

327 **Figure 2a** displays the comparative environmental profiles for the different valorization scenarios  
328 proposed for study in terms of each impact category selected for assessment. According to the results

329 reported, two scenarios derived on the worse results in different impact categories: Scenario IV in  
330 ADP, AP, EP, GWP, ODP, HTP, FEP and MEP and, Scenario V in TEP and POP. Thus, two  
331 scenarios (I and II) show similar environmental profiles with no outstanding differences (reductions  
332 ranging from 3% to 8% depending on the impact category for Scenario II with regard to Scenario I).  
333 However, the valorization strategies considerably differ between them. As displayed in **Figure 1a**, the  
334 liquid fraction from the centrifugation step (SS2) rich on antioxidant compounds and hemicellulosic  
335 oligosaccharides is further processed by means of an extraction and decantation unit to obtain both  
336 components separately (hemicellulosic oligosaccharides and antioxidant extract). On the contrary (see  
337 **Figure 1b**), this stream is not further treated and both components with market applications cannot be  
338 separated, obtaining a mixed stream.

339 <**Figure 2a** around here>

340 Among the outstanding differences on the environmental profiles, it is important to highlight that an  
341 increment on the number of valorization steps included in the valorization route involves higher energy  
342 and chemical requirements which derives on worse environmental profiles. Thus, it must be taken into  
343 account that if enhancing “closing the loop” strategies under a biorefinery concept through better  
344 recycling and re-using the waste streams, balances out the worse environmental burdens obtained as  
345 result from their higher intensive stages. However, this trend on the results is also linked to the  
346 functional unit selected to report the environmental results that is, the amount of waste biomass  
347 valorized per batch. Therefore, it could be expected according to the available literature that this trend  
348 would entirely change if a unit based on the economic revenues was considered (González-García et  
349 al., 2016b; 2017) since the increment of valorization routes derives on more pure streams with  
350 economic and industrial value.

### 351 **3.2. Contributions per subsystems involved**

352 **Figure 2b** displays the distribution of environmental burdens per subsystems involved in the different  
353 valorization strategies of vine shoots into high added value products. Firstly, two scenario must be

354 analyzed in detail, these are Scenario I and Scenario V since stages such as SS1-SS6 are common in  
355 both scenarios and differences are only identify in terms of SS7 contributions.

356 <Figure 2b around here>

357 According to **Figure 2b.(1)** and **Figure 2b.(5)**, activities involved in SS7 intended for delignification of  
358 the solid fraction from SS2 are the environmental *hotspots* with contributions ranging from 29% to 77%  
359 and from 46% to 96% respectively for Scenario I and Scenario V, depending on the category.

360 Moreover, the effect from SS7 in the profile associated with Scenario V is more outstanding in  
361 categories such as EP, HTP, TEP and POP (see **Figure 3**), which is directly linked to the  
362 delignification procedure, in this case organosolv (it is expected due to the chemicals requirement in  
363 this case, ethanol).

364 <Figure 3 around here>

365 The alkaline delignification involves a ratio liquid:solid of 10 kg/kg considering NaOH 12% (w/w) at 124  
366 °C during 105 min. The organosolv stage is performed at a liquid/solid of 8 kg/kg with ethanol at 50%  
367 at 200 °C during 90 min. These different operating conditions derive on a different electricity  
368 consumption (7.4 times higher in Scenario I than in Scenario V) and chemical doses as summarized in  
369 **Table 2**. In the case of Scenario I, the main responsible of the impacts derived from SS7 is the large  
370 electricity requirements in the autoclave as displayed in **Figure 4a**, which contribute from 75% to 96%  
371 of the total. On the contrary in Scenario V, the production of the ethanol requirements is the key issue  
372 with contributing ratios ranging from 72% to 100% of the total burdens derived from SS7 (see **Figure**  
373 **4b**). Thus, further improvements in these scenarios should be focused on optimizing the electricity  
374 consumption and the ethanol requirements in Scenario I and V, respectively. In addition, alternative  
375 chemicals to ethanol in the organosolv process could also be evaluated.

376 <Figure 4 around here>

377 Contributions from SS1 (autohydrolysis) and SS5 (concentration and freeze drying) in scenarios I and  
378 V are also remarkable as depicted in **Figures 2b.1** and **2b.5**. In both cases, electricity requirements in  
379 the reactor and the freeze dryer are the key issues responsible of these results.

380 Regarding Scenario II, improvements on the global environmental profile of up to 8% are identified  
381 with regard to Scenario I (**Figure 2a**), although pure oligosaccharides and antioxidant extract based  
382 streams are not obtained. The main responsible of these results is the avoidance of SS3  
383 (oligosaccharides extraction) and SS4 (ethyl acetate recovery), both of them with electricity  
384 requirements.

385 Scenario III reports a more complex valorization scheme with enzymatic hydrolysis and energy  
386 production as main new incorporated activities to obtain glucose liquors as well as heat and electricity  
387 from solid waste fractions. As expected, the incorporation of these processes within the system  
388 boundaries of the valorization route derives on higher environmental burdens that is, on a worse  
389 environmental profile than Scenario I as depicted in **Figure 2a**. Having a look into the distribution of  
390 the environmental burdens derived from Scenario III, three stages can be managed as environmental  
391 *hotspots* as displayed in **Figure 2b.3**. These are SS5 (concentration and freeze drying) and SS7  
392 (delignification) – in the same line as the previous scenario presented and, SS8 (enzymatic hydrolysis)  
393 –specific to this one. The key issues responsible of contributions from SS5 and SS7 are one again,  
394 electricity requirement in the freeze dryer and, in the autoclave, respectively. Regarding SS8, the  
395 distribution of contributing burdens is depicted in **Figure 5**. Electricity requirement for the agitation as  
396 well as the consumption of both enzymes and buffer are the key factors considered as environmental  
397 *hotspots*. The effect from these factors depends on the category evaluated. Thus, production of  
398 enzymes required in the hydrolysis is the responsible of near 100% in categories such as MEP and  
399 TEP and around 60% in ODP. Enzymes production process requires large amount of energy as well  
400 as chemicals (Feijoo et al., 2017) which are responsible of these high impacts. In the remaining  
401 categories, the main contributor is the production of electricity requirement, directly taken from the  
402 national grid and which presents a fossil origin. Production of buffer reports outstanding contributions  
403 (ranging from 15% to 20%) in AP, EP, GEP, HTP, FEP and POP mainly because of background  
404 activities involved in its production.



405 Scenario IV reports a full valorization scheme with enzymatic hydrolysis, fermentation (including  
406 inoculum preparation), energy production and distillation as the main incorporated processes to obtain  
407 bioethanol for chemical applications as well as heat and electricity from solid waste fractions. Once  
408 again, the incorporation of these activities within the valorization route derives on higher environmental  
409 burdens that is, on a worse environmental profile than Scenario I as depicted in **Figure 2a**. However,  
410 comparing all the scenarios, Scenario IV reports the worst environmental results in only five  
411 categories, ADP, EP, GWP, FEP and MEP. Looking into the distribution of the environmental burdens  
412 derived from Scenario IV (**Figure 2b.4**), SS8 is the environmental key factor followed by SS7, SS11  
413 (fermentation) and SS13 (distillation). It is important to highlight in this scenario the environmental  
414 credits associated with the avoided processes specifically in terms of TEP and POP. In this case, the  
415 avoided production of ethanol is the main responsible of this positive effect. As in Scenario III, the  
416 production of the enzymes requirement for the hydrolysis is the responsible of the high contributions  
417 from SS8. Electricity requirement in the autoclave, fermenter and distiller are the key issues  
418 responsible for SS7, SS11 and SS13 contributing burdens, respectively.

419 Finally, if profiles from Scenario III and IV are compared –since the second one involves the  
420 integration of additional biorefining activities, the profiles are increased 1.3-1.7 times in all the  
421 categories except in MEP and TEP, where negligible differences are identified. The increment of the  
422 environmental burdens is related with the additional activities included in Scenario IV as indicated in  
423 **Figure 2b.4**. In MEP and TEP the effect of these activities is negligible since the main responsible of  
424 the environmental burdens is SS8, which is a shared activity in both scenarios.

### 425 **3.3. Discussion of results**

426 According to the results displayed above it can forecast that “closing the loop” strategies to introduce  
427 biorefinery strategies and thus, better recycling and re-using the waste streams from production  
428 systems should derive on the worse environmental profiles (see **Figure 2a**) in numerous impact  
429 categories since the number of valorizing activities is increased and therefore, the chemicals and  
430 energy requirements. However, it must be taken into account that this expected behavior is also  
431 related with the functional unit selected to report the environmental results. If a functional unit based

432 on economic approach was considered, where not only the production amount of the different added-  
433 value products was considered but also the potential revenues associated to their market, a different  
434 environmental performance could be expected (González-García et al., 2016b, 2017) balancing out  
435 the worse environmental burdens obtained as result from their higher intensive stages. Therefore, the  
436 analysis will be completed with an economic functional unit and 1€ of economic revenue will be  
437 selected to report the environmental profiles. However in this analysis only three scenarios will be  
438 managed and these are Scenario I, Scenario IV and Scenario V. Scenario II is excluded since there is  
439 not an increment on the valorization strategies regarding Scenario I. Scenario III is not considered  
440 since the glucose liquors should be further valorized into bioethanol, which is carried out in Scenario  
441 IV. A description of market prices of the different coproducts obtained in the different scenarios is  
442 displayed in **Table 4**.

443 <**Table 4** around here>

444 However and in contrast to expecting, no differences have been found on the environmental behavior  
445 regardless the functional unit considered. Therefore, Scenario IV reports the worst profile in all the  
446 impact categories except in terms of TEP and POP, where Scenario V is the worst option as displayed  
447 in **Figure 6a**. The low market prices for ethanol, electricity and heat together with their low yields,  
448 unlike the other co-products, derive on negligible alterations on the economic revenue per batch.  
449 Therefore, the effect of their production is not outstanding on the economic functional unit.

450 <**Figure 6** around here>

451 *Organosolv chemicals: sensitivity analysis*

452 Taking in mind the results obtained for the organosolv based scenario (Scenario V), the ethanol  
453 requirement in the pre-treatment is considered an environmental hotspot being the responsible of the  
454 highest environmental burdens regardless the impact category (contributing ratios ranging from 36%  
455 to 95% of the global results depending on the category). However and according to the literature  
456 (Martín et al., 2011), alternative chemicals could be managed in the pre-treatment stage under

457 organosolv conditions. Therefore, different alternative scenarios have been proposed with the aim of  
458 studying their effect on the environmental impacts associated to this stage.

459 The base scenario, which corresponds to the original case study (Scenario V), is named as scenario  
460 A. Scenarios B and C correspond to the use of glycerol-water at 60% (v/v) and ethylene glycol-water  
461 at 60% respectively, as they are both chemicals typically used in organosolv (Martín et al., 2011,  
462 González et al., 2008).

463 The outcomes of the sensitivity analysis (**Figure 6b**) showed that scenario B yields to the highest  
464 environmental burdens, except for ADP, HTP, MEP and POP categories. Scenario C is the worst  
465 option in terms of ADP and MEP, being Scenario A (the case base) the responsible of the highest  
466 impacts in HTP and POP. Thus, the use of glycerol as organosolv solvent is not recommended from  
467 an environmental point of view in line with the results obtained.

468 Scenario C could be classified as the best option when compared to the other two scenarios since  
469 environmental improvements are reported in terms of seven of the ten categories analyzed,  
470 Consequently, it would be convenient the use of ethylene glycol as solvent instead of ethanol. This  
471 selection is also supported by the consideration of the normalization phase (Guinée et al. 2001), which  
472 allows us to compare all environmental impacts using the same scale as well as to add the  
473 normalization values of impact categories to obtain a single number per scenario. According to it, the  
474 normalization values are:  $1.40 \cdot 10^{-7}$ ,  $1.77 \cdot 10^{-7}$  and  $9.38 \cdot 10^{-7}$  for Scenario C, Scenario A and Scenario B  
475 respectively.

#### 476 *Comparison with literature*

477 Nowadays the interest on the biorefinery concept is receiving special attention for multiple reasons  
478 mainly due to a great fraction of energy carriers and materials come from fossil fuel refineries  
479 (Cherubini, 2010), as well as because the European Commission is adopting strategies to “closing the  
480 loop” of product life cycles in industrial production systems from a circular economy approach. By  
481 applying the “closing the loop” strategy the production of waste could be reduced, the environmental

482 profiles could be improved and the the economic profits could increase (Liguori et al., 2016). Different  
483 types of products can be obtained such as fuels, fibres (Nascimento et al.,2016), fine chemicals  
484 (Ekman and Börjesson, 2011), antioxidants (Ekman et al., 2013) and even proteins (Halloran et al.,  
485 2016). However, special attention is currently being paid on oligosaccharides production due to their  
486 interest as prebiotic functional food ingredients (González-García et al., 2016b) and biomaterials  
487 (Gullón et al., 2016).

488 In this sense, several studies can be found in the literature where LCA methodology has been applied  
489 to determine the environmental improvements linked to biorefinery strategies (Ekman and Börjesson,  
490 2011; Ekman et al., 2013; González-García et al., 2016b; 2017). However, only some studies can be  
491 found focused on the environmental analysis of the oligosaccharides production at semi-pilot scale, as  
492 driving force of the valorization schemes. González-García et al. (2016b) reported the environmental  
493 analysis of different schemes under thermal treatment conditions of wood chips valorization into  
494 soluble saccharides of polymeric and oligomeric nature (also known as galactoglucomannas) or  
495 levulinic acid as the main products. González-García et al. (2017) considered the production of pectin-  
496 derived oligosaccharides from sugar beet pulp valorization under thermal and enzymatic treatments.  
497 To the best of our knowledge, no other environmental studies have been published in this topic. Both  
498 studies were performed from a cradle-to-gate approach, excluding from the system boundaries further  
499 uses as well as distribution. Therefore, no studies can be found in the literature in which the  
500 production of xylooligosaccharides –as in this study-, had been previously environmentally evaluated.  
501 Other essential limitations in performing a LCA comparison of valorization systems in a biorefinery  
502 approach could be the use of different characterization methods (CML and ReCiPe) (Guinée, 2002;  
503 Goedkoop et al., 2012), different impact categories, system boundaries or even system expansion  
504 approaches. Although different types of oligosaccharides streams have been assessed in the literature  
505 which involve different uses and properties (galactoglucomannas, pectin based and  
506 xylooligosaccharides in our study), a comparative environmental profile in terms of greenhouse gases  
507 emission throughout the whole life cycle that is, carbon footprint is proposed for consideration in this  
508 section. The carbon footprint is compared per kilogram of oligosaccharide produced and the results  
509 are displayed in **Figure 6c**.

510 According to Figure 6c, the carbon footprint ranges from 49 to 218 kgCO<sub>2</sub>eq·kg oligosaccharide<sup>-1</sup>. The  
511 best profiles are obtained in scenarios A and B, which correspond to the production of pectin-based  
512 oligosaccharides under thermal and enzymatic treatments considering a waste from sugar industry as  
513 raw material (González-García et al., 2017). The worst profiles correspond to the extraction of  
514 galactoglucomannans from residual wood waste under thermal treatment conditions (scenario C and D)  
515 (González-García et al., 2016b). The remaining scenarios (E-I) correspond to the xylooligosaccharides  
516 extraction from vine shoots considering different thermal pre-treatments and different valorization  
517 routes. Thus, the carbon footprints allocated to the systems under evaluation in this study are between  
518 the previous studies available in the literature.

519

#### 520 **4. Conclusions**

521 Biorefining systems cannot be per se favourable from an environmental perspective since according to  
522 the literature, they can be high intensive chemicals and energy demanding systems, deriving on high  
523 impacts on the environment. The use of renewable waste as raw material in biorefining activities under  
524 efficient and optimized conditions could be crucial to make future high added compounds and  
525 processes more sustainable not only from environmental but also from an economic perspective. The  
526 degree of waste valorization of the foreground systems is linked to the desired purity of the final  
527 product. The range of carbon footprint results (kgCO<sub>2</sub>eq·kg oligosaccharide<sup>-1</sup>) reported in this study  
528 must be carefully compared to others available in the literature since depending on the type of  
529 oligosaccharide, the production system requires complex and intensive foreground processes coming  
530 into larger impacts. The valorizing routes analyzed in this study appear to be attractive options to  
531 produce high-added value products with multiple applications from an agro-industry residue available  
532 in large amounts in Mediterranean countries. According to the outcomes, further research should be  
533 focused on improving the current valorizing techniques at pilot scale specifically in terms of chemicals  
534 and electricity requirements. LCA methodology can be considered as a valuable and useful tool to  
535 support decision making strategies, specifically for systems under development such as the one  
536 reported in this study. Other issues that can affect the implementation of biorefineries are policy issues

537 and incentives as well as legislation. It is important to take into account that implementing a biorefinery  
538 approach could be considered as a way to manage renewable wastes, avoiding their disposal which  
539 involves a cost for companies.

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