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# **Control of the sugar/ethanol conversion rate during moderate pulsed electric field-assisted fermentation of a *Hanseniaspora* sp. strain to produce low-alcohol cider**

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## 1 **Abstract**

2 The effect of moderate pulsed electric fields (PEF) on *Hanseniaspora* sp. fermentations in apple  
3 juice was examined. The treatments were applied prior to or during the fermentation using a field  
4 strength of 285 V/cm. A significant increase ( $p<0.05$ ) in the biomass growth was observed  
5 during all the treatments along with a significant decrease ( $p<0.05$ ) in the ethanol yield. The  
6 optimal impact of PEF on ethanol reduction by 1.6% (v, v) was obtained during the treatment of  
7 the pre-culture for 6 h. The greatest rate was observed for the treatment during the first 12 h of  
8 the fermentation. During this fermentation performed at 28 °C under agitation at 250 rpm, the  
9 time to reach the stationary phase was reduced by 10 h, and the maximum biomass growth rate  
10 was ten-fold higher than that of the control. In addition, the sensitivity of *Hanseniaspora* sp.  
11 yeast to PEF treatment was more pronounced during the lag phase rather than the log phase in  
12 term of shortening the fermentation time, and reducing the ethanol content. The results obtained  
13 here demonstrated the promising efficiency of stimulated yeast by PEF in reducing the ethanol  
14 content in fermented alcoholic beverages.

15

16 **Keywords:** *Hanseniaspora* sp.; pulsed electric fields; fermentation; cider; low alcohol content

## 17 **1. Introduction**

18 Over the last decade, an increase of the alcohol content in some alcoholic beverages such as  
19 wines has been observed worldwide (Godden, Wilkes, & Johnson, 2015; Tilloy, Ortiz-Julien, &  
20 Dequin, 2014). This behavior is most probably largely related to the intensification of global  
21 warming, which leads to the accumulation of higher sugar contents in the fruits (Alston, Fuller,  
22 Lapsley, & Soleas, 2011; Samson et al., 2016). Increasing the alcohol content in the fermented  
23 fruit juices is undesired due to the associated drawbacks such as the deceleration of the  
24 fermentation kinetics (Malacrinò, Tosi, Caramia, Prisco, & Zapparoli, 2005), the reduced yeast  
25 activity, and the alteration of the beverage sensory quality (King, Dunn, & Heymann, 2013).  
26 Producing low alcoholic and non-alcoholic beverages such as wine, beer, and cider is attracting  
27 more and more attention due to the societal and health issues related to alcohol consumption.

28 Several approaches have been described in the literature leading obtaining low-alcohol  
29 beverages (Longo et al., 2017; Schmidtke et al., 2012). One of the proposed solutions to decrease  
30 the alcohol level consists of reducing the sugar content in the fruits by for example modifying the  
31 cultivation practices, the fermentation of early-harvested fruits, or by diluting or membrane  
32 filtration of the juice (Novello & de Palma, 2013; Palliotti et al., 2014). However, it was  
33 observed that these methods seem to have a low impact on the ethanol concentration of the  
34 fermented juice (de Toda et al., 2013; Heymann et al., 2013; Novello & de Palma, 2013; Palliotti  
35 et al., 2014). However, some of these techniques may alter the aroma and the mouthfeel of the  
36 product (Cassano, Mecchia, & Drioli, 2008; Echavarría, Torras, Pagán, & Ibarz, 2011; Heymann  
37 et al., 2013; Kontoudakis, Esteruelas, Fort, Canals, & Zamora, 2011; Salgado, Fernández-  
38 Fernández, Palacio, Hernández, & Prádanos, 2015). Another solution is to generate low-alcohol  
39 engineered yeasts, which are able to redirect the ethanol biosynthesis towards the production of

40 by-products such as glycerol, acetaldehyde, and acetoin during the fermentation (Lopes et al.,  
41 2000; Tilloy, Cadière, Ehsani, & Dequin, 2015; Varela et al., 2015, 2012). Yet, another approach  
42 reducing the ethanol concentration during the fermentation is to stop the process before  
43 completion. Post-fermentation membrane separation technique including reverse osmosis (Bui,  
44 Dick, Moulin, & Galzy, 1986; Catarino, Mendes, Madeira, & Ferreira, 2007; Pilipovik &  
45 Riverol, 2005), evaporative perstraction (de Francesco, Freeman, Lee, Marconi, & Perretti, 2014;  
46 Varavuth, Jiraratananon, & Atchariyawut, 2009), and pervaporation technology (del Olmo,  
47 Blanco, Palacio, Prádanos, & Hernández, 2014; Takács, Vatai, & Korány, 2007) are generally  
48 the most applied methods that are agreed to reduce the ethanol concentrations in the fermented  
49 juices.

50 Due to all of the above-mentioned issues, developing new strategies that preserve the  
51 sensory and organoleptic properties of the fermented fruit juices along with reducing the ethanol  
52 content is of paramount importance. Pulsed electric field (PEF) assisted fermentation process  
53 seems to be a promising technique to solve the above-mentioned problems. In fact, the  
54 interaction of microorganisms with electric fields has intrigued microbiologists for many years  
55 since electrical current can increase or inhibit the growth of microorganisms, depending on the  
56 experimental conditions (Cho, Yousef, & Sastry, 1996). The influential parameters include the  
57 intensity of the electric field, its temporal function (e.g. constant and pulsed), the treatment time,  
58 the cell type, and the characteristics of the medium (Castro, Oliveira, Domingues, Teixeira, &  
59 Vicente, 2005). Thus, the PEF technology may represent a challenging technique to control the  
60 sugar/ethanol conversion rate in the fermented fruit juices. The review of the literature shows  
61 that the application of moderate PEF during the fermentation processes was the subject of a  
62 minor number of studies. Most of them were focused on the effect of PEF on the microbial

63 cultures when treated prior to fermentation (Ki, Parameswaran, Popat, Rittmann, & Torres, 2015;  
64 Mattar et al., 2015). In this regard, the aim of this work was to investigate the effect of moderate  
65 pulsed electric field treatment on apple juice fermentation using the yeast *Hanseniaspora* sp.  
66 towards cider production. In fact, the genus *Hanseniaspora* was found as a major yeast  
67 developed during spontaneous apple juice fermentation (Pando Bedriñana, Querol Simón, &  
68 Suárez Valles, 2010; Valles, Bedriñana, Tascón, Simón, & Madrera, 2007). In addition, the  
69 positive impact of *Hanseniaspora* sp. in alcoholic fermentation has been demonstrated (Capozzi  
70 et al., 2019), which may positively contribute to the aroma profile of the fermented apple juice  
71 (de Arruda Moura Pietrowski, dos Santos, Sauer, Wosiacki, & Nogueira, 2012).

## 72 **2. Materials and methods**

### 73 *2.1. Chemicals, yeast, and apples*

74 Acetonitrile (CH<sub>3</sub>CN), ethanol (C<sub>2</sub>H<sub>6</sub>O), and peptone were purchased from Fisher Scientific  
75 (Illkirch, France). Yeast extract was bought from Sigma-Aldrich (Saint-Quentin-Fallavier,  
76 France). Fructose and glucose were supplied by Merck Millipore (Guyancourt, France).  
77 *Hanseniaspora* sp. strain used in this work belongs to the authors' laboratory collection. The  
78 strain was isolated previously from spontaneous fermentation of a Lebanese apple juice obtained  
79 from the "Ace Spur" variety (Al Daccache, Salameh, Maroun, & Louka, 2017). The Yeast-  
80 Extract-Peptone-Dextrose (YPD) medium (1 % yeast extract, 2 % peptone, 2 % dextrose) was  
81 used for the pre-culture yeast growth. The apples used in this work ("Ace spur" variety) were  
82 collected from Bqaatouta village (Keserwan region, Lebanon).

### 83 *2.2. Fermentation processes*

#### 84 *2.2.1. Hanseniaspora* sp. pre-culture

85 The pre-culture of the *Hanseniaspora* sp. strain was performed by taking one colony from  
86 YPD agar (1 % yeast extract, 2 % peptone, 2 % dextrose, and 2 % agar) plate into 250 mL of  
87 YPD (1 % yeast extract, 2 % peptone, 2 % dextrose) medium under sterile conditions (20 min at  
88 121 °C in an autoclave HMC HV-110L (HMC Europe GmbH, Germany)). The pre-culture was  
89 incubated during 45 h at 30 °C temperature and 250-rpm agitation on a shaker (Thermo  
90 Scientific, France). A volume of 1.5 L of sterile YPD (1 % yeast extract, 2 % peptone, 2 %  
91 dextrose) medium was introduced into a 2-L fermenter (LSL Biolafitte S.A.). After sterilization  
92 and cooling to 30 °C, the medium was inoculated by the pre-culture to get an initial  
93 concentration of  $3.0 \times 10^6$  cells/mL. After 24-h incubation at 30 °C and 250 rpm, the pre-culture  
94 was used to inoculate the apple juice.

#### 95 2.2.2. *Apple juice control fermentations*

96 The apples were first washed and then used to extract the juice using a fruit juice centrifuge  
97 separator (Moulinex, France). A volume of 1.5 L of apple juice was introduced into a 2-L sterile  
98 fermenter and inoculated by the pre-culture to get an initial concentration of  $3.0 \times 10^6$  cells/mL.  
99 Cider production was conducted for 100 h in batch mode, without medium circulation, at 30 °C  
100 and 250 rpm (Figure 1.A). The kinetics of growth, substrate consumption, and ethanol  
101 production were recorded during the fermentation. The content of soluble compounds was  
102 followed by measuring the °Brix using a digital refractometer (Anggur DR401, France). After  
103 recording the kinetics of growth and substrate consumption in batch mode without medium  
104 circulation, a treatment chamber was used to allow the circulation of the medium and the yeast  
105 treatment by PEF during the fermentation (Figure 1.B). A peristaltic pump allowed the medium  
106 circulation from the reactor to the treatment chamber and back into the reactor. A circulation  
107 flow rate of 75 mL/min, allowing obtaining the same growth and glucose consumption kinetics

108 than in batch mode without circulation (Figure 1.A) was used. The total length of the tubes used  
109 was equal to 90 cm. The treatment chamber and the tubes were sterilized as above-mentioned.

### 110 2.2.3. Apple juice PEF-assisted fermentations

111 The impact of moderate pulsed electric fields on the growth, substrate consumption, and  
112 ethanol production by *Hanseniaspora* sp. yeast during apple juice fermentation was studied. For  
113 this purpose, the two electrodes of the PEF-treatment chamber were connected to a PEF  
114 generator made by the “Service électronique” of the University of Technology of Compiègne  
115 (France). This generator provides bipolar pulses of near rectangular shape, with the respective  
116 maximal delivered voltage and current of 400 V and 40 A. As the distance between the  
117 electrodes was set to 1.4 cm, the maximum field strength applied and used was 285 V/cm. A  
118 succession of  $N$  trains, each of them composed of  $n = 10$  pulses was applied. Each pulse duration  
119 was set to  $t_i = 100 \mu\text{s}$ , with a pulse period time of  $\Delta t = 1 \text{ ms}$ . The time between the trains was set  
120 to  $\Delta t_i = 1 \text{ s}$ . First, the PEF treatments were carried out during the lag and the log fermentation  
121 phases, and then during each phase separately. The impact of PEF-treatment of the pre-culture,  
122 followed by apple juice fermentation without PEF-treatment was also studied. The effective PEF  
123 treatment  $t_{PEF}$  was calculated as follows:  $t_{PEF} \text{ (s)} = N \cdot n \cdot t_i \text{ (s)}$ . Thus, four PEF-assisted  
124 fermentations were investigated (Figure 2): 1) PEF-treatment for 12 h from the beginning of  
125 fermentation including the lag phase ( $t_{PEF} = 21.43 \text{ s}$ ), 2) PEF-treatment for 12 h during the log  
126 phase (after 12 h of fermentation) ( $t_{PEF} = 21.43 \text{ s}$ ), 3) fermentation of the medium inoculated  
127 with treated pre-culture during 6 h ( $t_{PEF} = 10.72 \text{ s}$ ) (during the lag and the log phases), and 4)  
128 fermentation of the medium treated for 12 h during the log phase (after 12 h of fermentation),  
129 which was inoculated by a pre-culture previously PEF-treated for 6 h ( $t_{PEF} = 10.72 \text{ s} + 21.43 \text{ s}$   
130  $= 32.15 \text{ s}$ ).



131 The current  $I$  (A) and the voltage  $U$  (V) were recorded using a data logger and software  
132 developed by the “Service électronique” of the University of Technology of Compiègne  
133 (France). The specific power consumption of PEF treatment  $W$  (J/mL) was estimated by the  
134 summation of the power consumptions during each pulse as given in equation (1).

$$135 \quad W \text{ (J/mL)} = \frac{U \cdot I \cdot t_{PEF}}{V} \quad \text{Equation (1)}$$

136 where  $U$  is the voltage (V),  $I$  is the current intensity (A), and  $V$  is the volume of the culture  
137 medium in the bioreactor (mL).

#### 138 *2.2.4. Determination of the growth, substrate consumption, and ethanol production kinetics*

139 The kinetics of growth, substrate consumption, and ethanol production were recorded for all  
140 fermentations (control and PEF-assisted).

##### 141 *2.2.4.1. Determination of the growth kinetics*

142 The growth kinetics were followed by the determination of the cell concentration (cells/mL)  
143 during the fermentation phases. Samples were taken periodically from the bioreactor, diluted  
144 several times in ultrapure water, and the cells were counted using a Thoma cell counting  
145 chamber (Preciss, France). To detect the viable cells, the samples were mixed volume to volume  
146 with a solution of 0.01 % methylene blue. The biomass concentration  $X$  (cells/mL), the ethanol  
147 production rate  $\gamma$  (g<sub>ethanol</sub>/g<sub>biomass</sub>.h), the biomass yield  $Y_{X/S}$  (g<sub>biomass</sub>/g<sub>substrate</sub>), and the product yield  
148 ( $Y_{P/S}$  (g<sub>ethanol</sub>/g<sub>substrate</sub>)) were calculated respectively according to equations (2), (3), (4) and (5).

$$149 \quad X \text{ (cells/mL)} = \frac{a \cdot 250000 \cdot d}{b} \quad \text{Equation (2)}$$

150 where  $a$  represents the number of cells counted,  $d$  represents the dilution factor, and  $b$  represents  
151 the number of squares counted in the chamber.

152  $\gamma (g_{ethanol}/g_{biomass} \cdot h) = \frac{P_f - P_0}{X \cdot (t_f - t_0)}$  Equation (3)

153  $Y_{X/S} (g_{biomass}/g_{substrate}) = \frac{X_f - X_0}{S_0 - S_f}$  Equation (4)

154  $Y_{P/S} (g_{ethanol}/g_{substrate}) = \frac{P_f - P_0}{S_0 - S_f}$  Equation (5)

155 where  $P_0$  and  $P_f$  represent respectively the initial and the final ethanol concentrations,  $X_0$  and  $X_f$   
156 represent respectively the initial and the final biomass concentrations, and  $S_0$  and  $S_f$  represent  
157 respectively the initial and the final substrate concentrations.

158 The maximal specific growth rate  $\mu_{max}$  ( $h^{-1}$ ) was determined by plotting  $\ln X$  (cells/mL)  
159 *versus* the time (h).  $\mu_{max}$  ( $h^{-1}$ ) values corresponded to the slopes of the linear curves obtained  
160 during the exponential phases.

#### 161 2.2.4.2. Carbon substrates quantification

162 The concentrations of carbon substrates were determined using high-performance liquid  
163 chromatography instrument LC-20AD (Shimadzu, Japan). The separation was performed in  
164 isocratic mode (a mixture of 1:4 (20:80) (v/v) water/acetonitrile with a flow rate of 3 mL/min),  
165 and using a Luna 5  $\mu m$   $NH_2$  250\*4.6 mm column. The separation temperature was set to 40 °C  
166 and the molecules were detected using a low-temperature evaporative light scattering detector  
167 ELSD-LT II (Shimadzu, Japan). The samples taken during the fermentation processes were  
168 diluted 10 times in ultrapure water and a volume of 1  $\mu L$  was injected. The compounds'  
169 assignation and quantification were performed using pre-established standard curves of glucose  
170 (5-30 g/L) and fructose (2.5-30 g/L).

#### 171 2.2.4.3. Ethanol quantification

172 Samples taken at different times of the fermentation processes were centrifuged at 6000 rpm  
173 for 10 min in order to eliminate the yeast suspensions and other apple particles. The ethanol  
174 content was determined by density measurements using a 5-mL glass pycnometer (Thermo  
175 Fisher, France). Calibration curve for ethanol quantification was performed using reconstituted  
176 solutions of apple juice/ethanol (1-10%, v/v).

#### 177 2.2.5. Statistical analyses

178 Each experiment was repeated, at least, three times. One-way ANOVA was used for the  
179 statistical analysis of the data with the help of Statgraphics Plus software (version 5.1, Statpoint  
180 Technologies Inc., Warrenton, VA, USA). A significance level of 5% was taken for each  
181 analysis. The error bars presented in the figures correspond to the standard deviations.

182

### 183 3. Results and discussion

184 The impact of moderate PEF on ethanol accumulation was evaluated by treating  
185 *Hanseniaspora* sp. yeast during cider production. First, the different growth phases were  
186 identified during the conventional fermentation of the apple juice (without and with medium  
187 circulation, and without PEF application), which served as a control.

188 Results in figure 3.A show an increase in the biomass concentration by around 25% when the  
189 apple juice yeast suspension was PEF-treated during or after the first twelve hours of  
190 fermentation (Figure 2.A-B) and by around 45% when the inoculum was previously treated  
191 (Figure 2.C). These results concur with that of Mattar et al., (2014) who reported the stimulation  
192 of *Saccharomyces cerevisiae* cells under moderate PEF. In addition, many other works have  
193 been described in the literature reporting the increase in the biomass concentration by the

194 application of moderate PEF, which were recently summarized and reviewed (Barba et al., 2015;  
195 Koubaa, Barba, Roohinejad, J., & Lorenzo, 2019; Mota et al., 2018). This increase in the  
196 biomass concentration could be explained by the increase of the yeast cells' division during the  
197 PEF treatment. The values of the maximal growth rate,  $\mu_{max}$  (Table 1) indicate a ten-fold  
198 acceleration of the growth kinetics for the PEF-assisted fermentation treated during the first  
199 twelve hours (Figure 2.A). Consequently, the time to reach the stationary phase was reduced by  
200 10 h. In addition, the treatment of the inoculum (Figure 3.C) showed a significant impact on the  
201 fermentation kinetics. In fact, higher  $\mu_{max}$  values were obtained compared to the control, which  
202 indicates that the stress applied to the yeasts during the pre-culture may influence the  
203 fermentation kinetics. The review of the literature shows that few studies have described the  
204 effect of electric field on the fermentation kinetics, which demonstrate mainly a significant  
205 acceleration of the PEF-assisted fermentations (Berovic, Potocnik, & Strus, 2008; Loghavi,  
206 Sastry, & Yousef, 2008; Mattar et al., 2015; Nakanishi, Tokuda, Soga, Yoshinaga, & Takeda,  
207 1998).

208 Furthermore, the results presented in figure 3.A demonstrate that the impact of moderate PEF  
209 treatment may differ from one growth phase to another. For instance, although the same energy  
210 (J/mL) was consumed for the treatments during either the first 12 h (lag phase) (Figure 2.A) or  
211 the second 12 h (log phase) of fermentation (Figure 2.B), the results obtained are significantly  
212 different (Table 1). In fact, the fermentation rate was significantly faster for the PEF-treated  
213 culture during the lag phase. These results concur with those reported by Mattar et al., (2013)  
214 who noticed an acceleration of the fermentation kinetics when stimulating *S. cerevisiae* cells by  
215 PEF during the lag phase. It has been also reported that the electroporation effectiveness was  
216 greater when PEF was applied during the first hours of fermentation, compared to that applied

217 after 24 h of fermentation (Mattar et al., 2013). These observations also concur with that of  
218 McDonald et al. (2000), who demonstrated that PEF has different effects on microorganisms  
219 depending on the growth phase.

220 The results in figure 3.A show as well that the impact of moderate PEF treatment of the  
221 inoculum for 6 h and the log phase of the apple juice culture for 12 h (Figure 2.D) was similar to  
222 that of the PEF treatment of the inoculum alone (Figure 2.C) for 6 h. In fact, no significant  
223 differences were observed between the cell growth kinetics.

224 The impact of PEF treatment was also studied in term of ethanol accumulation. The results  
225 presented in figure 3.B show that the ethanol content in the fermented apple juice depended on  
226 the PEF treatment applied, with significant differences observed. In all cases, the application of  
227 PEF led to decrease the ethanol content after 100 h of apple juice fermentation. To the best of  
228 our knowledge, this is the first time that moderate PEF is demonstrated to reduce the ethanol  
229 content in fermented beverages. In fact, some previous works such as that of Nakanishi and co-  
230 workers have reported an increase in the ethanol production when *S. cerevisiae* cells were treated  
231 by direct electric current (Nakanishi et al., 1998). These differences could be related to many  
232 factors including the PEF parameters and treatment duration, as well as the characteristics of the  
233 microorganism and the growth phase. The lowest ethanol decrease compared to the control was  
234 by 0.5% (v, v), observed for the fermentation assisted by PEF during the log phase. The highest  
235 ethanol decrease was by 1.6% (v: v), compared to the control, and was observed for the  
236 fermentations inoculated with treated yeasts during the pre-culture, without any PEF treatment of  
237 the apple juice yeast suspension (Figure 2.C). Furthermore, lower ethanol production rates  $\gamma_{\max}$   
238 (Table 1) were obtained for all the pre-treated and treated samples. The lowest rate values were  
239 related to the pre-treated yeast and treated juice during the first 12 h of fermentation. The fastest

240 growth kinetics observed for the treatment during the first 12 hours and the pre-culture treatment  
241 (Figure 3.A) are associated to the fermentations having the lowest ethanol production rate and  
242 content (Figure 3.B). The reasons behind could be explained by the fact that the yeast cells are  
243 consuming sugars to produce more biomass rather than producing ethanol. In fact, when the  
244 biomass yield is significantly higher for the treated fermentations compared to that of the  
245 untreated ones, this was compensated by a decrease in the ethanol yield (Table 1). It was recently  
246 reported that applying an electric field could affect the reaction pathways by different  
247 mechanisms (Jiang, Feng, Zeng, & Luo, 2019). For instance, the electric field can have a direct  
248 effect on the charged particles that can pull them away from the reaction zone (Jiang et al.,  
249 2019), which may disturb the reactions of ethanol biosynthesis in the current study. In fact,  
250 during glycolysis, it is crucial to recycle the  $\text{NAD}^+$  and oxidize the NADH, otherwise, a decrease  
251 in the glycolytic flux may occur, knowing that glycolysis is an important metabolic pathway for  
252 the ethanol production. A significant part of the NADH produced during the glycolysis is  
253 afterward oxidized during the ethanol formation (Kutyna, Varela, Henschke, Chambers, &  
254 Stanley, 2010). The alteration of the balance  $\text{NAD}^+/\text{NADH}$  can redirect the carbon flux towards  
255 different end-points. This alteration could take place during the PEF treatment since it has an  
256 impact on the charged particles as mentioned above.

257 The yeast growth and ethanol production were obviously associated with the consumption of  
258 substrates. Here, mainly glucose and fructose were consumed during the apple juice  
259 fermentation. Similar to the above-discussed kinetics, the decrease in the concentrations of  
260 substrates depended on the PEF treatment (Figure 4). This behavior was previously observed  
261 when using mutants for ethanol production. For instance, Tilloy et al. (2015) cultivated strains of  
262 *S. cerevisiae* wine yeasts under hyperosmotic stress in order to redirect the ethanol synthesis

263 towards the glycerol formation. All the strains showed a reduced ethanol yield compared to the  
264 reference strain. In addition, the mutants showed reduced sugar consumption, and the authors  
265 established a correlation between the reduced ethanol yield, the high glycerol yield, and the  
266 decrease in the fermentative properties.

267

268       Regarding the energy consumption, the PEF-treatment of the pre-culture during 6 h  
269 consumed less energy (Table 1) than the other PEF-assisted fermentations and provided the  
270 highest ethanol reduction in the fermented apple juice. Applying 82 J/mL reduced the final  
271 ethanol concentration by 1.6 % (v: v), compared to the control. The conventional methods used  
272 to remove partial ethanol from beverages such as vacuum distillation requires higher energy  
273 consumption and higher cost than that demonstrated here (Margallo et al., 2015; Schmidtke et  
274 al., 2012). In addition, the use of genetically modified yeasts producing less ethanol is not only  
275 restricted by the consumers' resistance to genetically modified food products (Chambers &  
276 Pretorius, 2010), but also by the complexity of the biological systems and their understanding  
277 which can limit the engineering processes (Williams, Pretorius, & Paulsen, 2016). From another  
278 perspective, the application of the conventional methods, involving the use of heat, may induce  
279 the loss of flavors and the organoleptic properties (Scott & Jarvis, 1995). In addition, retention of  
280 the compounds affecting the product aroma may occur during the membrane filtration (Catarino  
281 et al., 2007). Compared to the results obtained in the current study, it could be underlined that  
282 moderate PEF treatment could be a challenging solution to reduce the ethanol content in  
283 alcoholic beverages and to control the sugar/ethanol conversion rate without affecting the  
284 sensory properties of cider. In fact, it has been previously demonstrated that PEF-treated cider  
285 had a better color and aroma retention than the thermally processed samples (Azhuvalappil, Fan,

286 Geveke, & Zhang, 2010). Nonetheless, organoleptic investigations are required in order to  
287 evaluate the volatile organic compounds and the aromatic profile of the untreated and PEF-  
288 treated ciders.

289

## 290 **4. Conclusions**

291 In this work, the impact of moderate PEF treatment on *Hanseniaspora* sp. cells was studied  
292 during the apple juice fermentation. For an effective PEF-treatment time of 10.716 s applied to  
293 the pre-culture during 6 h, the highest alcohol reduction was observed. The yeast concentration  
294 and the biomass yield increased significantly, while the ethanol production rate was reduced by  
295 the half. In addition, the yeast cells were more sensitive to moderate PEF treatment during the  
296 first hours of fermentation rather than during the log phase, even though the same energy was  
297 used. The yeast concentration and the biomass yield increased significantly for all the treatments.  
298 However, the fastest kinetic was observed for the treatment during the first 12 h of fermentation.  
299 The increased growth rates were accompanied by decreased ethanol rates and contents.

300 The results indicate the great potential for practical implementation of PEF-assisted  
301 fermentation technology to reduce the ethanol content during the fermentation processes and  
302 especially using *Hanseniaspora* sp. yeast cells. However, further investigations are required in  
303 order to understand the mechanisms behind the microbial stimulation and ethanol reduction.

304

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310

## 311 **References**

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461 **Figure captions:**

462 **Figure 1.** **A.** Control batch mode fermentation without medium circulation, **B.** Control batch  
463 mode fermentation with medium circulation (without and with PEF treatment). Insert represents  
464 the dimensions of the PEF treatment chamber.

465

466 **Figure 2.** PEF treatment during the fermentation processes (pre-culture and apple juice PEF-  
467 assisted fermentations). The symbols in the dashed inserts represent those used in figures 3 and 4  
468 of the results and discussion section.

469

470 **Figure 3.** Growth kinetics (**A.**) and ethanol kinetics (**B.**) for the control and the PEF-assisted  
471 fermentations, “a” denotes significant differences at the end of the fermentation, in comparison  
472 with the untreated samples.

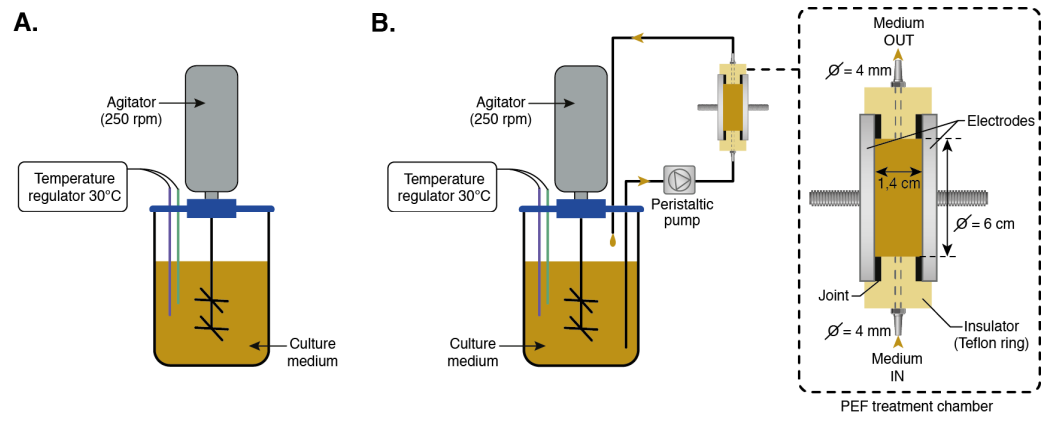
473

474 **Figure 4.** Fructose (**A.**) and glucose (**B.**) consumption kinetics for the control and the PEF-  
475 assisted fermentations.

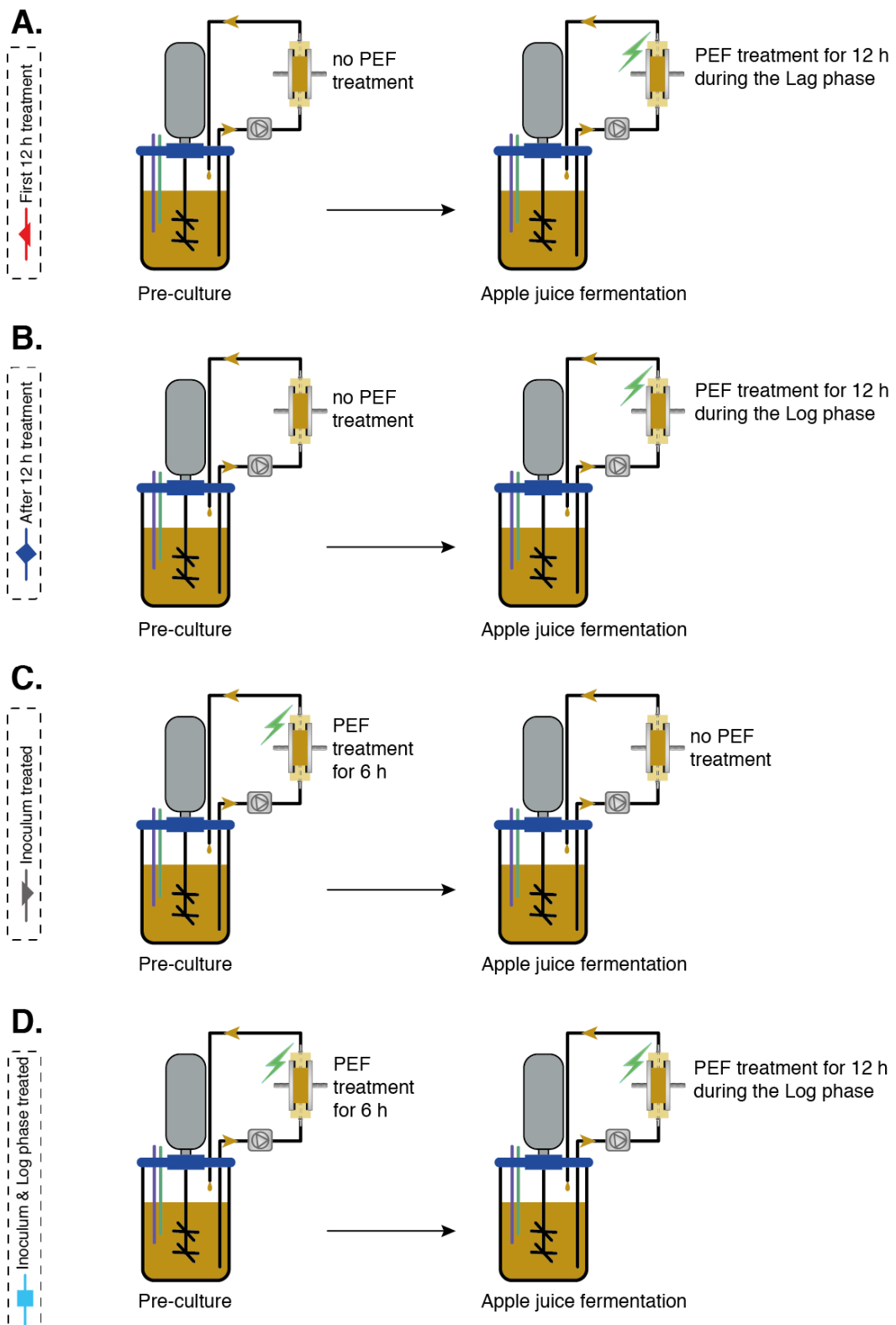


476 **Figures**

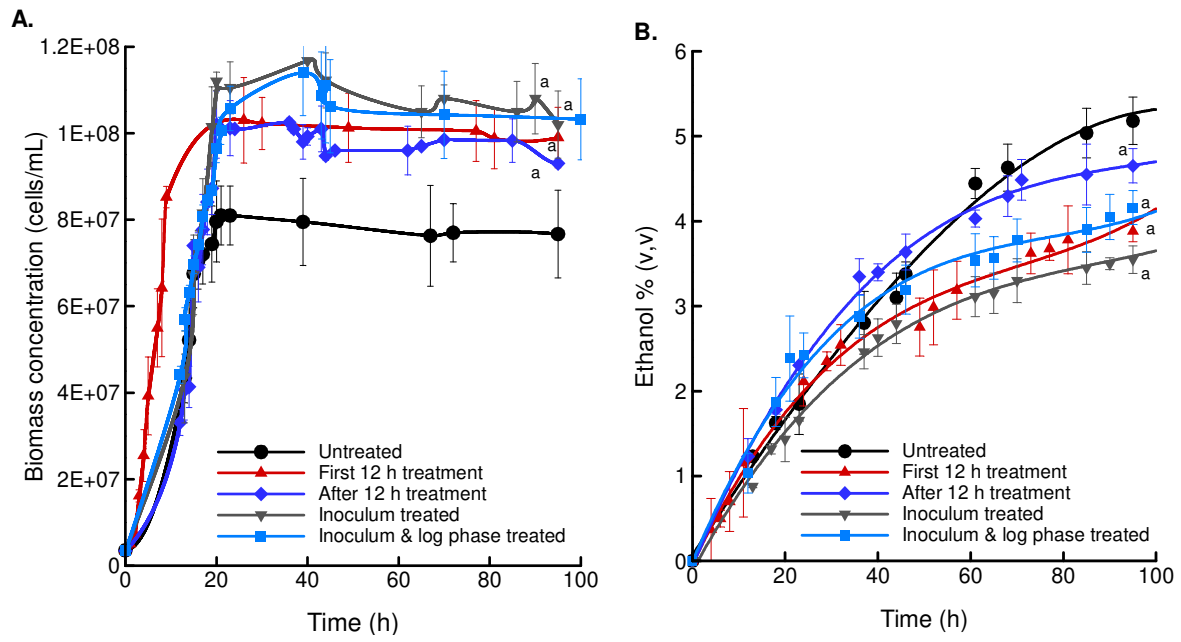
477 **Figure 1.**



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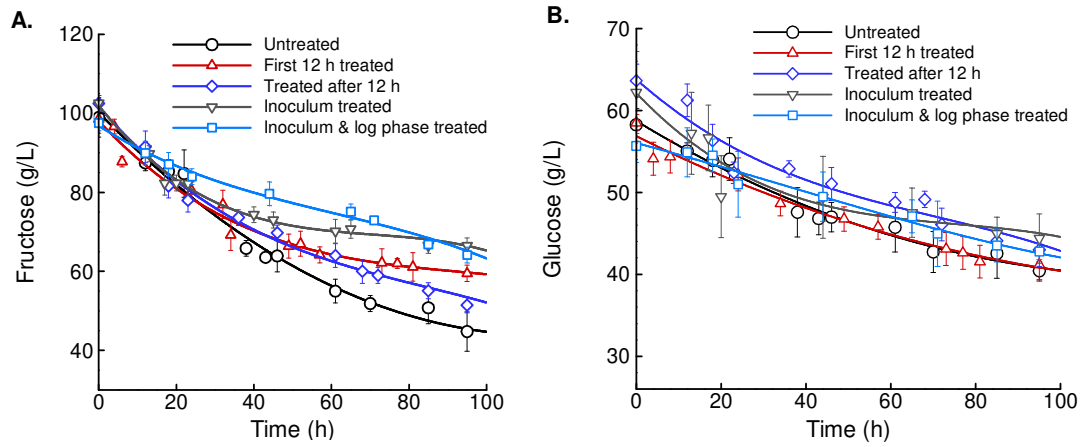


481 **Figure 3.**



482

483 **Figure 4.**



484

485 **Tables**486 **Table 1.** Fermentation process performances, and energy consumptions during the PEF treatments. SD denotes standard deviation.

<b>Treatment</b>	$\mu_{max}$ (h <sup>-1</sup> )		$\gamma_{max}$ (g ethanol/g biomass.h)		Y (g biomass/ g fructose)		Y (g biomass/ g glucose)		Y (g ethanol/ g fructose)		Y (g ethanol/ g glucose)		W (J/mL)	
	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD
<b>Untreated</b>	0.03	0.002	0.30	0.01	0.01	0.002	0.04	0.001	0.07	0.007	0.27	0.01	0	0
<b>Treatment for 12 first hours</b>	0.30 <sup>a</sup>	0.003	0.15 <sup>a</sup>	0.01	0.06 <sup>a</sup>	0.004	0.05	0.008	0.19 <sup>a</sup>	0.001	0.14 <sup>a</sup>	0.01	165	1.8
<b>After 12 hours (log phase treatment)</b>	0.07 <sup>a</sup>	0.001	0.28	0.01	0.03	0.005	0.06	0.006	0.07	0.005	0.17 <sup>a</sup>	0.01	164	1.5
<b>Treatment of the inoculum</b>	0.10 <sup>a</sup>	0.002	0.16 <sup>a</sup>	0.01	0.03	0.004	0.06	0.004	0.08	0.004	0.17 <sup>a</sup>	0.01	82	1.2
<b>Treatment of the inoculum and log phase</b>	0.06 <sup>a</sup>	0.002	0.21 <sup>a</sup>	0.01	0.03	0.004	0.06	0.006	0.10 <sup>a</sup>	0.001	0.26	0.02	247	2.6

487 “a” denotes significant differences with the untreated sample

488