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Study on high pressure homogenization and high power ultrasound effectiveness in inhibiting polyphenoloxidase activity in apple juice

This is a pre print version of the following article:

*Original*

Study on high pressure homogenization and high power ultrasound effectiveness in inhibiting polyphenoloxidase activity in apple juice / Bot, Francesca; Calligaris, Sonia; Cortella, Giovanni; Plazzotta, Stella; Nocera, Francesco; Anese, Monica. - In: JOURNAL OF FOOD ENGINEERING. - ISSN 0260-8774. - 221:(2018), pp. 70-76. [10.1016/j.jfoodeng.2017.10.009]

*Availability:*

This version is available at: 11381/2919421 since: 2022-03-19T11:17:13Z

*Publisher:*

*Published*

DOI:10.1016/j.jfoodeng.2017.10.009

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note finali coverpage

(Article begins on next page)

02 May 2026

Manuscript Number:

Title: Can high pressure homogenization and high power ultrasound effectively replace heating for inhibiting polyphenoloxidase activity in apple juice?

Article Type: Research Article

Keywords: PPO inactivation, High pressure homogenization, Ultrasound, Heat, Energy density, Energy consumption

Corresponding Author: Professor Monica Anese,

Corresponding Author's Institution: University of Udine

First Author: Francesca Bot

Order of Authors: Francesca Bot; Sonia Calligaris; Giovanni Cortella; Stella Plazzotta; Francesco Nocera; Monica Anese

Abstract: High pressure homogenization (HPH) and ultrasound with (USct) or without (US) temperature control were applied to apple juice individually or in combination for inactivating polyphenoloxidase (PPO). Ten passes HPH at 150 MPa were needed to achieve 50% PPO inactivation. USct led to 90% PPO decrease at the longest time (45 min), whereas total enzyme inactivation was achieved by subjecting samples to 6 min US. Results showed that temperature affected enzyme inactivation rather than the process applied. Moreover, the HPH-USct and HPH-US combined treatments led to enzyme residual activities similar to those caused by the application of HPH and USct, and US individual treatments, respectively. US provided to the apple juice less energy density to obtain PPO inactivation than USct and HPH, due to the contribution of the in situ generated heat. Also, US showed the lowest energy consumption, thus confirming its appropriateness.

Dear Editor,

I would like to submit the manuscript entitled “Can high pressure homogenization and high power ultrasound effectively replace heating for inhibiting polyphenoloxidase activity in apple juice?” by Francesca Bot, Sonia Calligaris, Giovanni Cortella, Stella Plazzotta, Francesco Nocera, Monica Anese, for consideration for publication in Journal of Food Engineering.

Best regards

Monica Anese

## Highlights

- High pressure homogenization scarcely affected polyphenoloxidase activity in apple juice.
- Ultrasound without temperature control effectively inactivated polyphenoloxidase.
- Ultrasound *in situ* generated heat mainly contributed to inactivate polyphenoloxidase.
- Ultrasound without temperature control was the least energy consuming treatment.

1 **Can high pressure homogenization and high power ultrasound effectively replace heating for**  
2 **inhibiting polyphenoloxidase activity in apple juice?**

3

4 Francesca Bot<sup>13</sup>, Sonia Calligaris<sup>1</sup>, Giovanni Cortella<sup>2</sup>, Stella Plazzotta<sup>1</sup>, Francesco Nocera<sup>1</sup>,  
5 Monica Anese<sup>1\*</sup>

6

7 <sup>1</sup>Dipartimento di Scienze AgroAlimentari, Ambientali e Animali, University of Udine, via Sondrio  
8 2/A, 33100 Udine, Italy

9 <sup>2</sup>Dipartimento Politecnico di Ingegneria e Architettura, University of Udine, via delle Scienze 206,  
10 3100 Udine, Italy

11 <sup>3</sup>Present address: School of Food and Nutritional Sciences, University College Cork, Cork, Ireland

12

13 \* Direct correspondence to: Monica Anese

14 Tel +390432558571

15 Fax +390432558130

16 e-mail: monica.anese@uniud.it

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18

19 **Abstract**

20 High pressure homogenization (HPH) and ultrasound with (US<sub>ct</sub>) or without (US) temperature  
21 control were applied to apple juice individually or in combination for inactivating  
22 polyphenoloxidase (PPO). Ten passes HPH at 150 MPa were needed to achieve 50% PPO  
23 inactivation. US<sub>ct</sub> led to 90% PPO decrease at the longest time (45 min), whereas total enzyme  
24 inactivation was achieved by subjecting samples to 6 min US. Results showed that temperature  
25 affected enzyme inactivation rather than the process applied. Moreover, the HPH-US<sub>ct</sub> and HPH-US  
26 combined treatments led to enzyme residual activities similar to those caused by the application of  
27 HPH and US<sub>ct</sub>, and US individual treatments, respectively. US provided to the apple juice less  
28 energy density to obtain PPO inactivation than US<sub>ct</sub> and HPH, due to the contribution of the *in situ*  
29 generated heat. Also, US showed the lowest energy consumption, thus confirming its  
30 appropriateness.

31

32

33 *Keywords:* PPO inactivation, High pressure homogenization, Ultrasound, Heat, Energy density,  
34 Energy consumption

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## 37 **1. Introduction**

38 Polyphenoloxidase (PPO) is a widely distributed enzyme in nature and plays an important role in  
39 catalyzing the hydroxylation of monophenols to *o*-diphenols and dehydrogenation of *o*-diphenols to  
40 *o*-quinones in the presence of oxygen (Espin et al., 1998). As known, the aforementioned final  
41 products are responsible for the formation of browning compounds and thus cause quality loss of  
42 vegetable products. Traditionally, PPO inactivation is achieved by the application of thermal  
43 treatments, which, however, may cause loss of sensory and nutritional quality of vegetable  
44 products. To tackle these issues, non-thermal technologies have gained significant interest over the  
45 last decades for their ability of reducing enzyme activity while minimizing detrimental effects on  
46 food quality. A number of studies has been reported on the effects of high pressure homogenization  
47 (HPH) and high power ultrasound on this food quality-related enzyme, due to their ability to change  
48 the enzymatic activity by the application of mechanical stresses and cavitation phenomena to a fluid  
49 (Liu et al., 2009a; Liu et al., 2009b; Suarez-Jacobo et al., 2012; Lacroix et al., 2005; Tribst and  
50 Cristianini, 2012; Terefe et al., 2015). Both activation and inactivation effects on PPO in fruit juices  
51 and model systems subjected to HPH or ultrasound treatments are described in the literature, due to  
52 differences in equipment, process conditions, enzyme source, among others (Liu et al., 2009a; Liu  
53 et al., 2009b; Costa et al., 2013; Yu et al., 2013; Silva et al., 2015; Suarez-Jacobo et al., 2012). As a  
54 rule, PPO inactivation can be obtained by applying intense HPH and ultrasound processes, that can  
55 be achieved by providing the matrix with very high pressures/number of passes and long times  
56 (Suarez-Jacobo et al., 2012; Abid et al., 2014). It is noteworthy that these process conditions might  
57 not fit the industrial needs as they can contribute to increase the ownership total cost. In the attempt  
58 to overcome these drawbacks, combined technologies have been taken into consideration. As an  
59 example, the simultaneous application of ultrasound with mild heat (thermosonication) and pressure  
60 (200-500 kPa; manothermosonication) or UV light (photosonication) has been demonstrated to  
61 improve ultrasound efficacy in inactivating PPO (López et al., 1994; Sulaiman et al., 2015; Başlar  
62 and Ertugay, 2013; Abid et al., 2014; Terefe et al., 2015). However, from these data a clear

63 indication on the most suitable treatment for PPO inactivation can be hardly obtained in terms of  
64 energy efficiency and applicability at the industrial level. Therefore, the objective of this research  
65 work was to compare the effectiveness of HPH and ultrasound processes in inactivating PPO in  
66 apple juice. As heat may be generated during ultrasonication, its contribution to enzyme  
67 inactivation was also considered. To this purpose, apple juice was subjected to HPH or ultrasound  
68 treatments with and without temperature control. Moreover, the effect of combinations of HPH and  
69 ultrasound processes on the enzyme activity was studied for the first time. Processes efficiency was  
70 evaluated in terms of energy density transferred to the juice during treatments and electrical energy  
71 consumption of the HPH and ultrasound devices.

72

## 73 **2. Materials and methods**

### 74 *2.1. Apple juice preparation*

75 A 20 kg batch of fresh apples (*Malus domestica* Borkh., cv. Golden Delicious) were purchased at  
76 the local market and maintained at 7 °C until use. Apples were peeled and the juice was extracted  
77 using a household table top juice extractor (Ariston Hotpoint Slow Juicer, Fabriano, Italy). The  
78 extract was filtered through a filter cloth to remove impurities and coarse particles, centrifuged at  
79 4000 g for 5 min at 4 °C (Beckman Avanti tm J-25, Beckman Instruments Inc., Palo Alto, CA,  
80 USA) and filtered again by using a filter cloth. Apple juice was prepared fresh for every trial from  
81 the same batch of fruits to minimize sample variability. The resulting clear apple juice having a  
82 soluble solid content of  $14.5 \pm 0.2$  °Brix and pH of  $3.6 \pm 0.2$  was immediately subjected to HPH  
83 and/or ultrasonication with or without temperature control.

84

### 85 *2.2. HPH and ultrasound treatments*

86 The methodology of Bot et al. (2017) was followed. Briefly, HPH processing was performed by  
87 means of a continuous lab-scale high-pressure homogenizer (Panda Plus 2000, GEA Niro Soavi  
88 Spa, Parma, Italy) supplied with two Re+ type tungsten carbide homogenization valves, with a flow

89 rate of 2.5 cm<sup>3</sup>/s. Aliquots of 150 mL of apple juice were subjected to increasing pressures from 0  
90 (control) to 150 MPa, or for up to 10 successive passes at 150 MPa. Ultrasound treatments were  
91 carried out with (US<sub>ct</sub>) and without (US) temperature control by using an ultrasonic processor  
92 (Hieschler Ultrasonics GmbH, mod. UP400S, Teltow, Germany) operating at 24 kHz frequency and  
93 100 μm amplitude, and equipped with a titanium horn tip diameter of 22 mm. During the  
94 ultrasonication experiment, the temperature was either controlled using a cryostatic bath, to  
95 dissipate the heat generated during treatment, or uncontrolled, leaving the temperature to rise due to  
96 heat dissipation. The US<sub>ct</sub> and US treatments were performed on 150 mL apple juice for increasing  
97 time periods up to 45 and 7 min, respectively. Following the treatments, the samples were cooled in  
98 an ice bath.

99 Further experiments were carried out by subjecting 150 mL apple juice to HPH at 150 MPa  
100 followed by ultrasound with (HPH-US<sub>ct</sub>) and without (HPH-US) temperature control for up to 15  
101 and 4 min, respectively. The time between the two treatments did not exceed 30 s. Samples were  
102 cooled in an ice bath at the end of the second treatment.

103

### 104 *2.3. Thermal treatment*

105 The total temperature-time combination received by the sample during ultrasonication was applied  
106 to the sample in the absence of the ultrasound treatment. To this purpose, aliquots of 150 mL of  
107 apple juice were introduced into 250 mL capacity glass vessels and heated in a thermostatic water  
108 bath (Ika Werke, MST BC, Staufen, Germany) under continuous stirring, by mimicking the same  
109 temperature profile produced during ultrasound treatment with (TT<sub>ct</sub>) and without (TT) temperature  
110 control. Following the treatments, the samples were cooled in an ice bath.

111

### 112 *2.4. Temperature measurement*

113 The sample temperature was measured just before and immediately after (i.e. before the cooling  
114 step) each treatment by a copper-constantan thermocouple probe (Ellab, Hillerød, Denmark)

115 immersed in the fluid, connected to a portable data logger (mod. 502A1, Tersid, Milan, Italy). In  
116 addition, during ultrasound and thermal treatments, the temperature was recorded as a function of  
117 time, by immersing (50 mm) the thermocouple tip in the fluid, half way between the solution centre  
118 and the inside wall of the vessel.

119

## 120 2.5. Energy density computation

121 The energy density ( $E_v$ , MJ/m<sup>3</sup>) transferred from the homogenization valve to the sample during  
122 HPH treatment was computed as described by Stang et al. (2001), according to eq. 1:

123

$$124 \quad E_v = \Delta P \quad (1)$$

125

126 where  $\Delta P$  is the pressure difference operating at the nozzles.

127 As the power density ( $P_v$ , W/m<sup>3</sup>) transferred from the probe to the sample during ultrasound  
128 treatment is markedly affected by temperature (Raso et al., 1999), this parameter was first  
129 determined calorimetrically by means of eq. 2,

130

$$131 \quad P_v(T) = \frac{mc_p(\partial T / \partial t)}{V} \quad (2)$$

132

133 where  $m$  is the sample mass (kg),  $c_p$  is the sample heat capacity (3870 J/kg K as given by Ashrae,  
134 2002),  $T$  is temperature (K),  $V$  is the sample volume (m<sup>3</sup>), and  $t$  (s) is the time frame of treatment  
135 considered. Temperature values were recorded in quasi-adiabatic conditions at various temperature  
136 levels as suggested by Raso et al. (1999). The energy density was then estimated by integration  
137 according to eq. 3 on the whole treatment time:

138

$$139 \quad E_v = \int P_v(T)dt \quad (3)$$

140

141 The energy density of multiple passes HPH and combined treatments was calculated as the sum of  
142 the energy density values of the corresponding single pass HPH and HPH plus US<sub>ct</sub> or US  
143 (Calligaris et al., 2016). The energy density of the thermal treatment was estimated according to eq.  
144 4:

145

$$146 \quad E_v = \frac{mc_p \Delta T}{V} \quad (4)$$

147

#### 148 *2.6. Electrical energy consumption measurement*

149 The measurement of electrical energy consumption was performed as in Bot et al. (2017). The  
150 energy requirement was estimated by measuring the electrical consumption at the mains supply.  
151 The high pressure homogenizer was supplied with three-phase 400 V electrical power, thus a three-  
152 phase energy logger was inserted (Kilo Box, Electrex, Reggio Emilia, Italy) to measure the  
153 electrical consumption (MJ/m<sup>3</sup>). The ultrasonic processor was instead supplied with single-phase  
154 230 V electrical power, and a power meter (PC-300, Lafayette, Taiwan) was connected to measure  
155 the electrical power and thus calculate the electrical energy (MJ/m<sup>3</sup>) for the whole treatment. The  
156 same power meter was employed for measuring the electrical power and energy consumption of the  
157 thermal treatment.

158

#### 159 *2.7. Apple juice soluble solids content and pH determinations*

160 Soluble solid content (° Brix) was measured using a table refractometer (Unirefrax, Bertuzzi, Milan,  
161 Italy) calibrated with distilled water. The pH was measured at 25 °C using a using a Basic 20 pH  
162 meter (Crison Instruments, S.A., Barcelona, Spain) equipped with a combination of glass electrodes  
163 and a temperature probe.

164

## 165 2.8. PPO activity assay

166 The PPO activity was determined spectrophotometrically immediately after each treatment  
167 (Shimadzu UV-2501PC, UV-Vis recording spectrophotometer, Shimadzu Corporation, Kyoto,  
168 Japan) at 25 °C (Kahn, 1985). Aliquots of 0.5 mL of apple juice were added to 2.5 mL of  $1.5 \cdot 10^{-3}$  M  
169 L-DOPA (Sigma-Aldrich, Milano, Italy). The absorbance at 420 nm was monitored each minute for  
170 10 min. The changes in absorbance per min were calculated by linear regression in the linearity  
171 interval by applying the pseudo zero order kinetic model. PPO activity (%) was calculated as the  
172 percentage ratio between the rate constants (Abs/min) of the enzymatic activity of the treated and  
173 untreated samples.

174

## 175 2.9. Data analysis

176 The results are the average of at least three measurements carried out on two replicated experiments  
177 ( $n \geq 6$ ). Data are reported as mean value  $\pm$  standard error. Statistical analysis was performed using R  
178 v.2.15.0 (The R foundation for Statistical Computing). Bartlett's test was used to check the  
179 homogeneity of variance, one-way ANOVA was carried out and Tukey test was used to determine  
180 statistically significant differences among means ( $p < 0.05$ ). Linear regression analysis was  
181 performed by using Microsoft Excel 2013. The goodness of fitting was evaluated based on visual  
182 inspection of residual plots and by calculation of  $R^2$  and  $p$ .

183

## 184 3. Results and discussion

185 Table 1 shows the temperature and PPO residual activity of apple juice subjected to single-pass HPH  
186 at 50 to 150 MPa and up to 10 passes HPH at 150 MPa. During HPH, temperature increased linearly  
187 with the increasing of pressure ( $R^2 > 0.99$ ,  $p < 0.05$ ) or number of passes ( $R^2 > 0.89$ ,  $p < 0.05$ ) up to  
188 56 °C. No significant reduction ( $p > 0.05$ ) of PPO activity was achieved by applying a single pass  
189 treatment at pressures increasing from 50 to 150 MPa. It is likely that the fluid-mechanical stresses  
190 (i.e. elongational, shear stresses, turbulence and cavitation) generated during the homogenization

191 (Donsi et al., 2009; Flourey et al., 2004) were not able to induce modifications of enzyme structure  
192 and activity. By submitting the apple juice to multiple passes through the homogenization valve,  
193 PPO activity decreased to a residual value of 50%. Either activation or inactivation effects have been  
194 reported in the literature for HPH pressures ranging from 80 to 300 MPa (Liu et al., 2009a; Liu et  
195 al., 2009b; Suarez-Jacobo et al., 2012). In particular, the PPO inactivation has been attributed to loss  
196 of the native structure, due to temperature increase and mechanical forces generated by the passage  
197 of the fluid through the homogenization valve. In our experimental conditions, the modest  
198 temperature increase (up to 43 °C) together with the short residence time (approximately  $10^{-4}$  s) in  
199 the homogenization valve (Jafari et al., 2007) may have been responsible for the inefficacy of single-  
200 pass HPH treatments in inactivating PPO. On the contrary, the efficacy of multiple HPH passes in  
201 reducing PPO activity by up to 50% can be attributable to increases in shear stress, cavitation and  
202 turbulence, as well as to the multiplication of treatment time by the number of passes and to the  
203 higher temperature reached (up to 56 °C after 10 passes at 150 MPa). Results are in agreement with  
204 literature data showing that mushroom PPO remained fully active up to 40 °C, whereas inactivation  
205 occurred at temperatures between 50 °C and 70 °C (Baltacıoğlu et al., 2015).

206 With regard to ultrasound treatments, upon 45 min  $US_{ct}$ , the temperature never exceeded 42 °C.  
207 When performed without temperature control, US treatment was responsible for a linear  
208 ( $R^2 > 0.93$ ,  $p < 0.05$ ) temperature increase up to 78 °C (data not shown). Fig. 1 shows the changes in  
209 PPO activity in apple juice subjected to  $US_{ct}$  or US as a function of time. The effects of heat alone,  
210 i.e. simulating the temperature increase obtained during  $US_{ct}$  ( $TT_{ct}$ ) and US (TT) without sonication,  
211 are also shown. In all cases, a decrease in enzyme activity with increasing process time was  
212 observed. As expected, US was more effective in reducing PPO activity than  $US_{ct}$ . These results are  
213 in agreement with those reported in the literature for PPO inactivation by ultrasonication in apple and  
214 pineapple juices (Costa et al., 2013; Abid et al., 2014; Silva et al., 2015). Enzyme inactivation caused  
215 by ultrasound processing has been attributed to different mechanisms, including acoustic cavitation,  
216 which is responsible for localized increase of pressure and temperature, and strong shear stress,

217 leading to modification of secondary and tertiary changes of protein (Feng et al., 2008; Mawson et  
218 al., 2011). Enhanced enzyme inactivation by heat provided during sonication has also been reported  
219 by several authors (Abid et al., 2014; Sulaiman et al., 2015). From Fig. 1, it can be also noted that to  
220 obtain a same inactivation level,  $US_{ct}$  and US required less time than the corresponding heat  
221 treatments. In order to investigate whether an acoustic effect can be distinguishable from a thermal  
222 one, PPO activity was reported as a function of the temperature reached by the apple juice during the  
223 different processes (Fig. 2). It can be observed that the curves describing the changes in PPO activity  
224 as a function of temperature reached by the apple juice during  $US_{ct}$  and US were almost overlapping  
225 with those of the corresponding heat treatments ( $TT_{ct}$  and TT, respectively). These results clearly  
226 indicate that temperature affected enzyme inactivation rather than the process applied, in agreement  
227 with previous findings (Başlar and Ertugay, 2013). Moreover, these data show that as long as the  
228 treatments did not allow the enzyme denaturation temperature to be overcome (40-50 °C), no  
229 significant activity reduction was detected. It is worthy to note that when  $US_{ct}$  and  $TT_{ct}$  treatments  
230 were applied, PPO inactivation was achieved at 40 °C, provided the time was sufficiently long. In the  
231 light of these findings, it is likely that an acoustic effect during ultrasound treatment was negligible  
232 and heat directly contributed to enzyme inactivation.

233 To compare the results among the different technologies, the energy density was taken a reference  
234 indicator of the treatment intensity because it incorporates the transferred power, the duration of the  
235 treatment and the treated sample volume (Stang et al., 2001; Hulsmans et al., 2010). Fig. 3 shows  
236 the effects of HPH,  $US_{ct}$  and US, as well as those of the corresponding  $TT_{ct}$  and TT treatments, on  
237 PPO activity of apple juice as a function of energy density. US process provided much less energy  
238 density to the fluid to obtain PPO inactivation than  $US_{ct}$ , the latter delivering energy density within  
239 the same order of magnitude of HPH. For instance, 100% PPO inactivation was achieved by US  
240 delivering an energy density of 444 MJ/m<sup>3</sup>, while 90% inactivation was obtained through  $US_{ct}$  at  
241 the highest energy density (i.e. 2102 MJ/m<sup>3</sup>). In fact, due to the contribution of the *in situ* generated  
242 heat, which raised the temperature up to 70 °C, less sonication time was necessary in the US

243 process to inactivate the enzyme (Fig. 1) and lower energy density values were computed (eq. 3).  
244 Moreover, as can be seen in Fig. 3, to achieve a same inactivation value, both US<sub>ct</sub> and US  
245 treatments provided higher energy density than TT<sub>ct</sub> and TT treatments, respectively. This  
246 discrepancy can be attributed to the different modality of delivering the energy. During heating  
247 alone, the energy provided to the closed system merely contributed to temperature increase. By  
248 contrast, ultrasound process was likely responsible for inducing other (mechanical) changes besides  
249 temperature rise. Some of these changes could positively contribute to apple juice stabilization. In  
250 fact, ultrasonication would favour the enzyme release from the cell walls making it more  
251 susceptible to thermal inactivation (Başlar and Ertugay, 2013). This is especially true for the US<sub>ct</sub>  
252 treatment when compared to the TT<sub>ct</sub> one. In fact, temperature control in US<sub>ct</sub> was performed by  
253 cooling the sample during continuous ultrasound treatment, while in TT<sub>ct</sub> heating once the desired  
254 temperature was achieved it was kept constant, thus leading to a notably lower energy density.  
255 Overall, data confirmed that both HPH and US<sub>ct</sub> are scarcely effective in inactivating PPO, unless  
256 high energy density values were provided by applying a high number of passes of sample in the  
257 homogenization valve or long ultrasonication times. However, these conditions are far from to be  
258 applicable at the industrial level. On the contrary, the heat generated *in situ* during US greatly  
259 contributed to inactivate PPO at energy density and process time likely compatible with the  
260 industrial process. In the light of these results, further experiments were carried out to investigate  
261 the effect of combinations of single-pass HPH at 150 MPa and ultrasounds with (HPH-US<sub>ct</sub>) and  
262 without (HPH-US) temperature control on PPO activity (Table 2). By comparing these results with  
263 those relevant to the individual treatments (Table 1 and Figs. 1 and 3), it can be noted that HPH-  
264 US<sub>ct</sub> led enzyme residual activities not dissimilar from those caused by the application of HPH and  
265 US<sub>ct</sub> providing comparable energy density values. Similarly, for a same energy density value, only  
266 slight differences in enzyme inactivation were observed between HPH-US and US. Therefore, it can  
267 be concluded that combined HPH and ultrasound treatment did not allow to reduce PPO activity  
268 compared to the single treatments. Finally, the effect of HPH, US<sub>ct</sub>, US and their combinations as

269 well as TT<sub>ct</sub> and TT only on PPO activity were compared in terms of electrical energy consumption  
270 (Fig. 4). It appears that the HPH and HPH-US<sub>ct</sub> treatments were the most energy wasting due to the  
271 long application times, followed by US<sub>ct</sub> and HPH-US processes. On the contrary, the US process  
272 was more advantageous and much less energy consuming than both the corresponding thermal  
273 treatment (TT) and the temperature controlled thermal treatment (TT<sub>ct</sub>). Reasonably, this gap will  
274 be maintained also after scaling up to an industrial continuous plant, because the US treatment  
275 supplies energy directly to food very efficiently, while heating is indirectly provided in TT from  
276 outside by means of another working fluid.

277

#### 278 **4. Conclusions**

279 Acquired results confirmed the negligible HPH and US<sub>ct</sub> contribution to PPO inactivation in apple  
280 juice, even when used in combination. Thus, HPH and US<sub>ct</sub> do not represent suitable technologies  
281 for PPO inactivation in apple juice. On the contrary, US, which was provided without temperature  
282 control, allowed PPO total inactivation to be achieved at energy density (444 MJ/m<sup>3</sup>) and process  
283 time (6 min) likely compatible with the industrial needs. Moreover, results clearly indicated that US  
284 *in situ* generated heat mainly contributed for more efficient enzyme inactivation, whereas an  
285 acoustic effect was negligible. Thus, US would be a feasible alternative technology for enzymatic  
286 inactivation in fruit derivatives. Instead of increasing ultrasound power input and dissipate the heat  
287 produced during the treatment, enzymatic inactivation can be achieved by US process providing  
288 low energy density to the fluid and exploiting the *in situ* generated thermal effect. The same  
289 conclusion can be drawn from the point of view of energy consumption, since the US was the least  
290 energy wasting treatment among all those considered. The results of this study highlighted that not  
291 only the effectiveness in terms of PPO inactivation but also energy related issues and application  
292 time should be considered to estimate process efficiency and thus steer the technology choice.

293

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377 **Caption for figures**

378 **Fig. 1.** Changes in PPO activity in apple juice subjected to ultrasound process with (a) (US<sub>ct</sub>) or (b)  
379 without (US) temperature control as a function of time. TT<sub>ct</sub> and TT: heat treatments obtained by  
380 providing the sample the same time-temperature combinations received during US<sub>ct</sub> and US,  
381 respectively.

382

383 **Fig. 2.** Changes in PPO activity in apple juice subjected to ultrasound process with (US<sub>ct</sub>) or  
384 without (US) temperature control as a function of temperature. TT<sub>ct</sub> and TT: heat treatments  
385 obtained by providing the sample the same time-temperature combinations received during US<sub>ct</sub> and  
386 US, respectively.

387

388 **Fig. 3.** Changes in PPO activity in apple juice subjected to ultrasound process with (US<sub>ct</sub>) or  
389 without (US) temperature control as a function of energy density. TT<sub>ct</sub> and TT: heat treatments  
390 obtained by providing the sample the same time-temperature combinations received during US<sub>ct</sub> and  
391 US, respectively.

392

393 **Fig. 4.** PPO residual activity vs electrical energy consumption of high pressure homogenization  
394 (HPH), ultrasound with (US<sub>ct</sub>) or without (US) temperature control and combinations of HPH and  
395 US<sub>ct</sub> and US. Data relevant to heat treatment (TT<sub>ct</sub> and TT) providing the sample the same time-  
396 temperature combinations received during US<sub>ct</sub> and US respectively are also shown.

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401 **Table 1**

402 Temperature and PPO residual activity of apple juice subjected to HPH. Starting temperature:  $8.0 \pm$   
 403 1.0.

Pressure (MPa)	Number of passes	Temperature (°C)	PPO residual activity (%)
50	1	27.5±2.3	76±8 <sup>a</sup>
100	1	35.6±1.7	80±11 <sup>ab</sup>
150	1	42.6±1.2	82±6 <sup>ab</sup>
150	3	44.7±1.2	75±5 <sup>b</sup>
150	5	51.6±3.0	69±5 <sup>b</sup>
150	8	52.4±0.9	61±12 <sup>b</sup>
150	10	56.4±0.6	49±7 <sup>c</sup>

404 Values are the mean of three repetitions on two replicates  $\pm$  standard error.

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409 **Table 2**

410 Temperature, PPO residual activity and energy density of apple juice subjected to combinations of 1  
 411 pass HPH at 150 MPa and ultrasound process under controlled (US<sub>ct</sub>) and uncontrolled (US)  
 412 temperature regime. Starting temperature  $8.0 \pm 1.0$ .

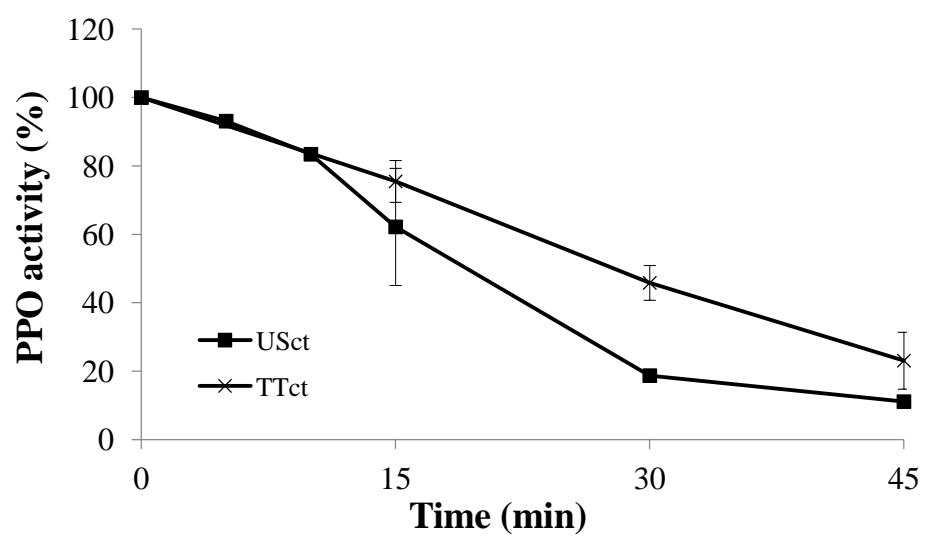
Treatment	Temperature control	Sonication time (min)	Temperature (°C)	PPO residual activity (%)	Energy density (MJ/m <sup>3</sup> )
HPH-US <sub>ct</sub>	yes	2	41.3±1.1	90±3 <sup>a</sup>	315
		3	41.3±3.3	84±10 <sup>a</sup>	397
		4	44.9±1.4	82±11 <sup>a</sup>	479
		5	46.2±1.3	80±11 <sup>ab</sup>	558
		10	47.4±1.8	72±10 <sup>b</sup>	953
		15	46.7±1.2	59±11 <sup>c</sup>	1348
HPH-US	no	2	58.4±2.3	64±1 <sup>a</sup>	304
		3	67.0±4.8	37±7 <sup>b</sup>	371
		4	73.9±5.8	2±0 <sup>c</sup>	430

413 Values are the mean of three repetitions on two replicates ± standard error.

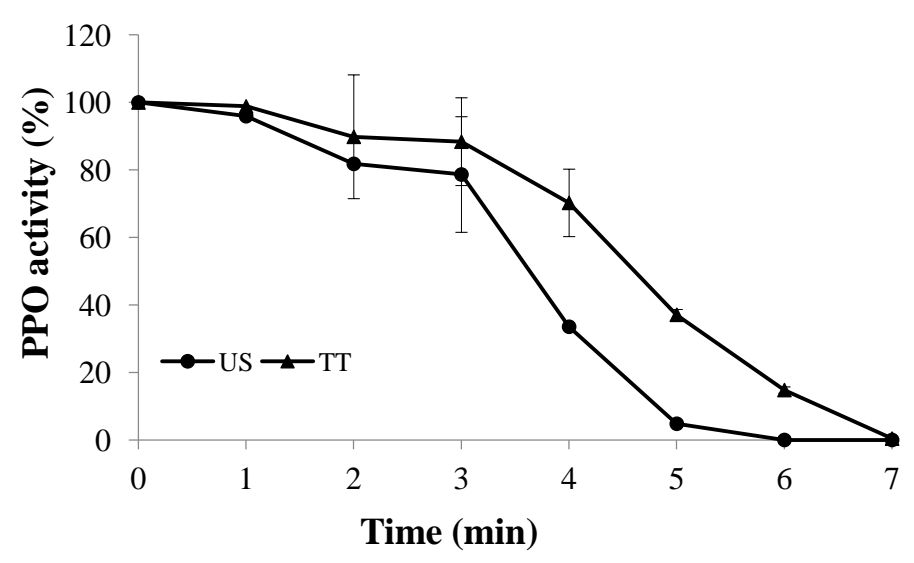
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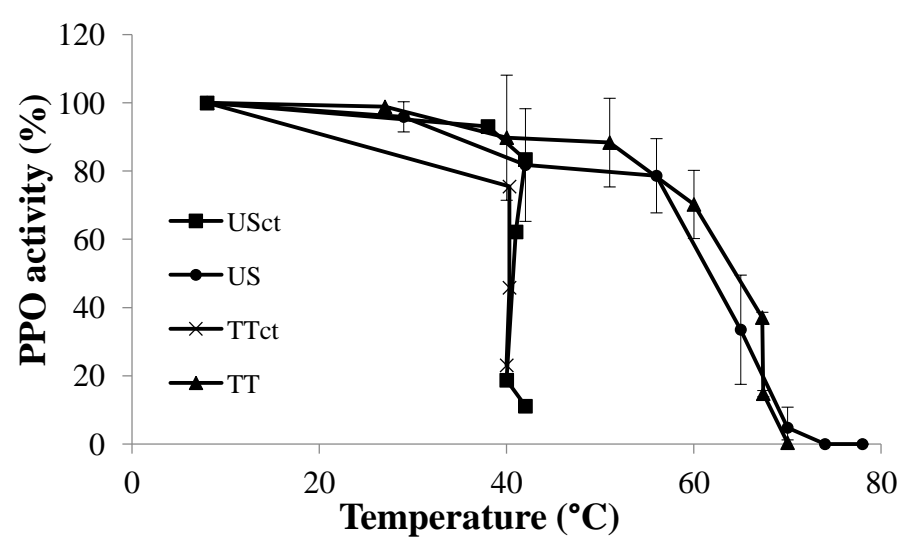


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Fig. 1.



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Fig. 2.

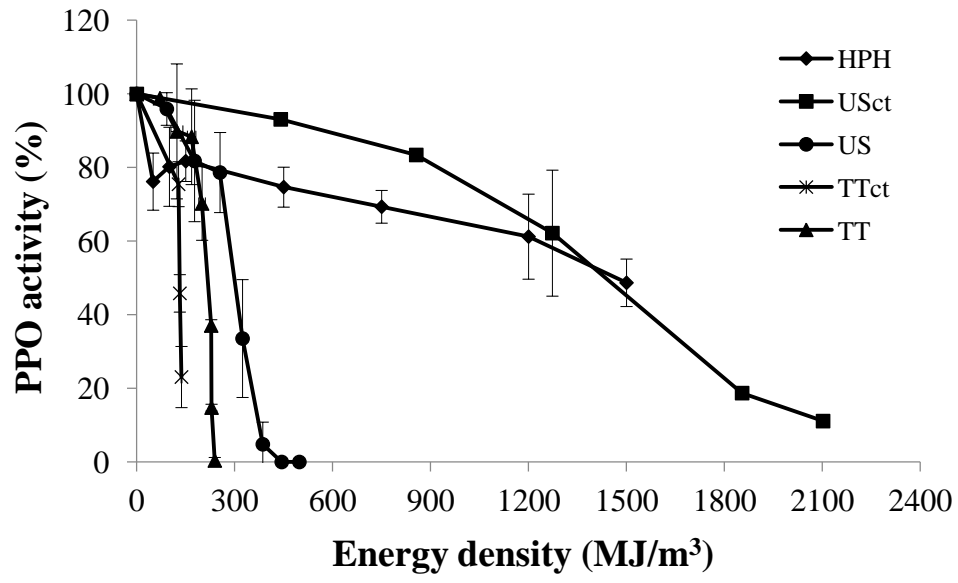


Fig. 3.

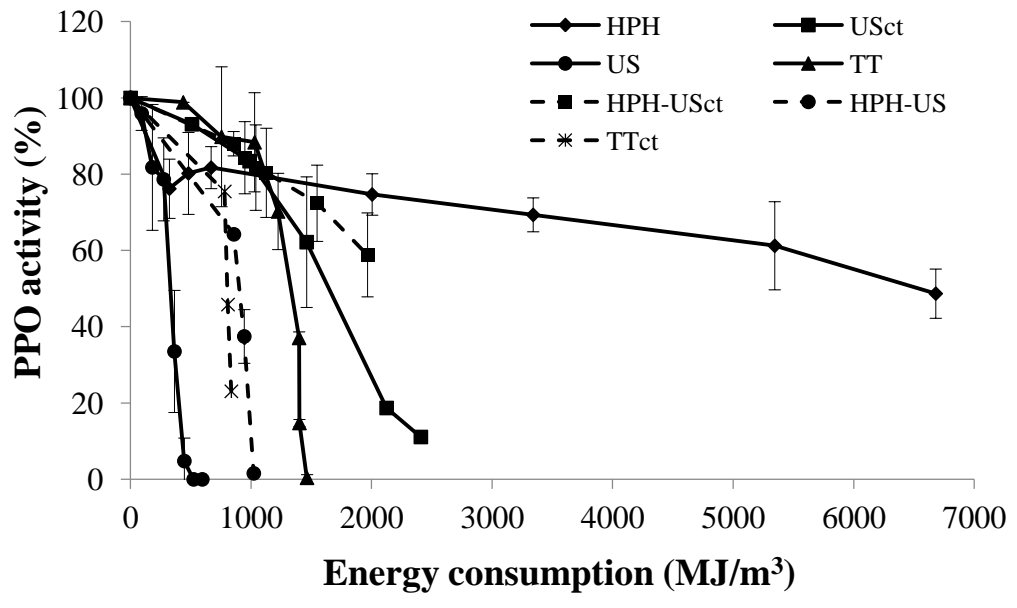


Fig. 4.