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

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

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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

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6

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18

19 **Abstract**

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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_{ct} led to 90% PPO decrease at the longest time (45 min), whereas total enzyme
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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 ± 0.2 °Brix and pH of 3.6 ± 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 cm³/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_{ct}) 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_{ct} 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_{ct}) 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_{ct}) 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/m³) 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 ΔP is the pressure difference operating at the nozzles.

127 As the power density (P_v , W/m³) 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 (m³), 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_{ct} 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/m³). 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/m³) 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/m³, while 90% inactivation was obtained through US_{ct} at
241 the highest energy density (i.e. 2102 MJ/m³). 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_{ct} and US
245 treatments provided higher energy density than TT_{ct} 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_{ct}
252 treatment when compared to the TT_{ct} one. In fact, temperature control in US_{ct} was performed by
253 cooling the sample during continuous ultrasound treatment, while in TT_{ct} 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_{ct} 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_{ct}) 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_{ct} led enzyme residual activities not dissimilar from those caused by the application of HPH and
265 US_{ct} 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_{ct}, US and their combinations as

269 well as TT_{ct} 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_{ct} treatments were the most energy wasting due to the
271 long application times, followed by US_{ct} 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_{ct}). 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_{ct} contribution to PPO inactivation in apple
280 juice, even when used in combination. Thus, HPH and US_{ct} 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/m³) 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_{ct}) or (b)
379 without (US) temperature control as a function of time. TT_{ct} and TT: heat treatments obtained by
380 providing the sample the same time-temperature combinations received during US_{ct} and US,
381 respectively.

382

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

387

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

392

393 **Fig. 4.** PPO residual activity vs electrical energy consumption of high pressure homogenization
394 (HPH), ultrasound with (US_{ct}) or without (US) temperature control and combinations of HPH and
395 US_{ct} and US. Data relevant to heat treatment (TT_{ct} and TT) providing the sample the same time-
396 temperature combinations received during US_{ct} 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 ($^{\circ}\text{C}$)	PPO residual activity (%)
50	1	27.5 ± 2.3	$76 \pm 8^{\text{a}}$
100	1	35.6 ± 1.7	$80 \pm 11^{\text{ab}}$
150	1	42.6 ± 1.2	$82 \pm 6^{\text{ab}}$
150	3	44.7 ± 1.2	$75 \pm 5^{\text{b}}$
150	5	51.6 ± 3.0	$69 \pm 5^{\text{b}}$
150	8	52.4 ± 0.9	$61 \pm 12^{\text{b}}$
150	10	56.4 ± 0.6	$49 \pm 7^{\text{c}}$

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_{ct}) and uncontrolled (US)
 412 temperature regime. Starting temperature 8.0 ± 1.0 .

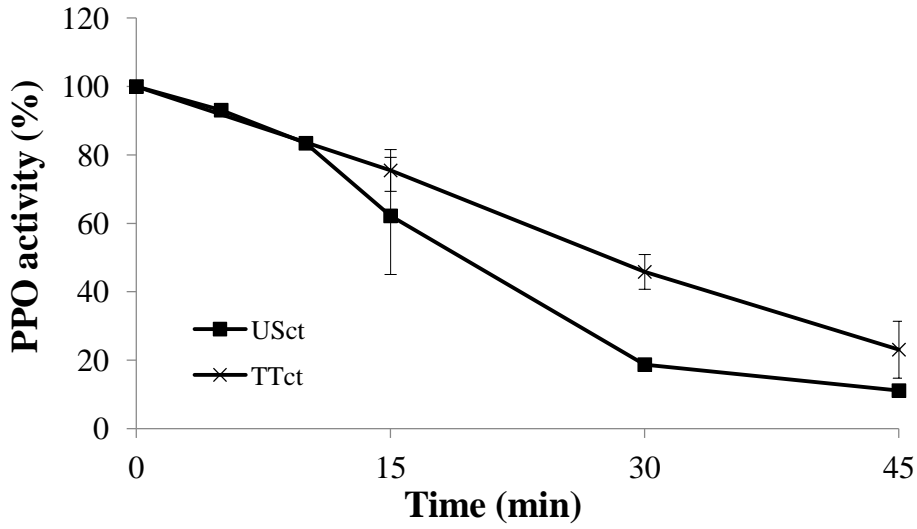
Treatment	Temperature control	Sonication time (min)	Temperature (°C)	PPO residual activity (%)	Energy density (MJ/m ³)
HPH-US _{ct}	yes	2	41.3±1.1	90±3 ^a	315
		3	41.3±3.3	84±10 ^a	397
		4	44.9±1.4	82±11 ^a	479
		5	46.2±1.3	80±11 ^{ab}	558
		10	47.4±1.8	72±10 ^b	953
		15	46.7±1.2	59±11 ^c	1348
HPH-US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

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

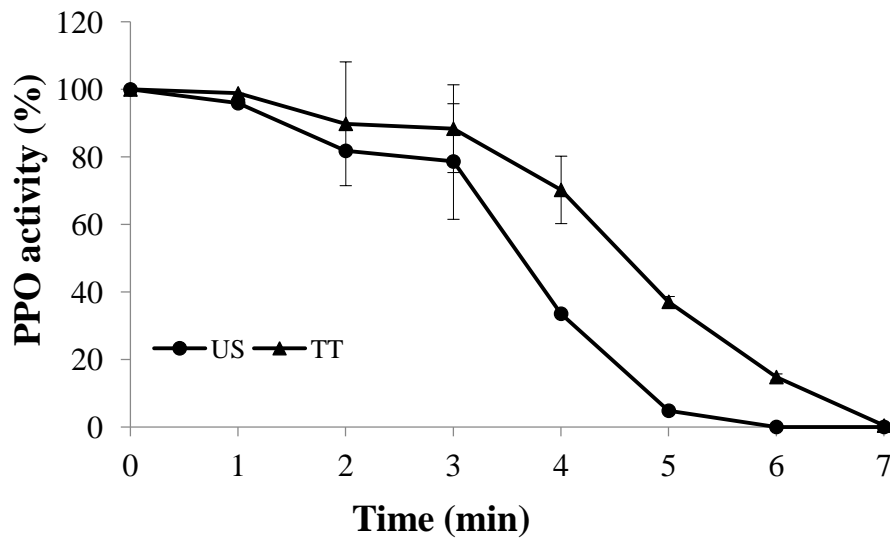
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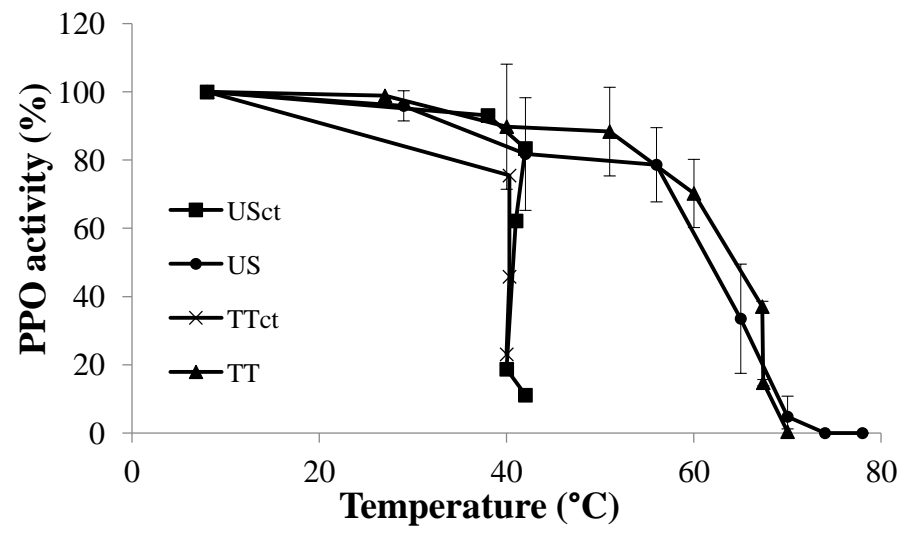


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Fig. 1.



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Fig. 2.

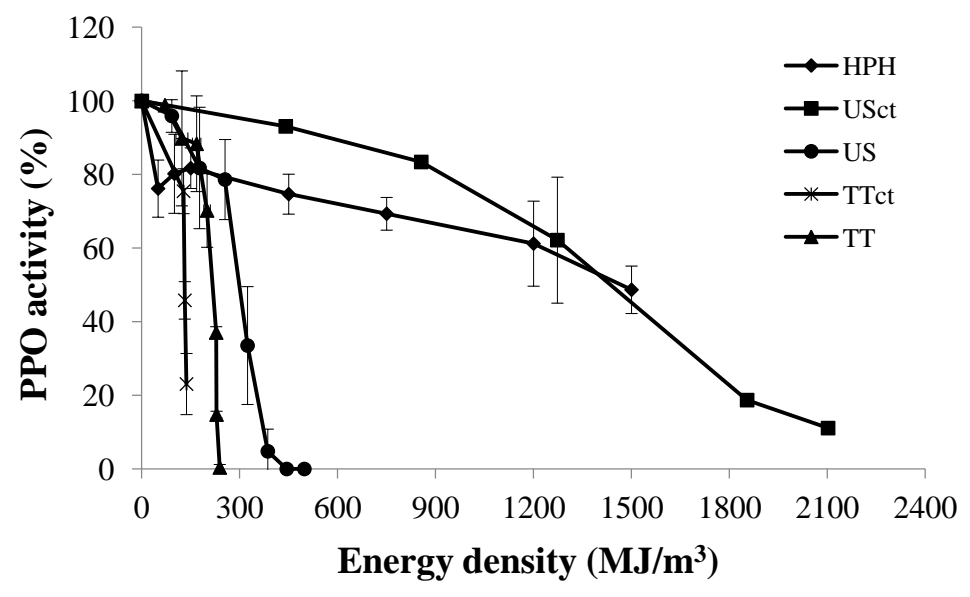


Fig. 3.

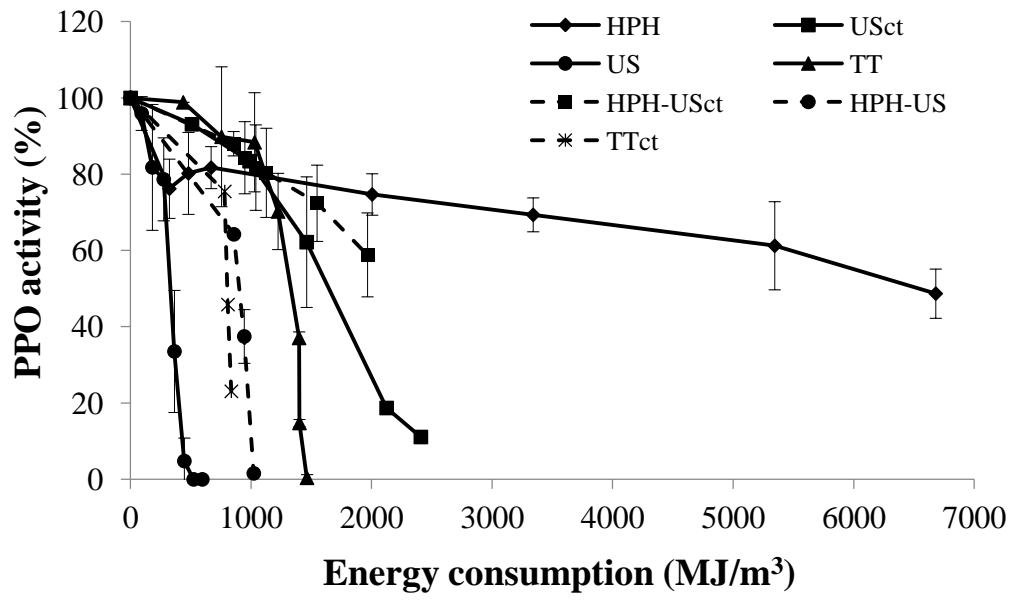


Fig. 4.