# Study on high pressure homogenization and high power ultrasound effectiveness in 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.

# 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 ($US_{ct}$) 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. $US_{ct}$ 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-$US_{ct}$ and HPH-US combined treatments led to enzyme residual activities similar to those caused by the application of HPH and $US_{ct}$, and US individual treatments, respectively. US provided to the apple juice less energy density to obtain PPO inactivation than $US_{ct}$ and HPH, due to the contribution of the *in situ* generated heat. Also, US showed the lowest energy consumption, thus confirming its appropriateness.

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

## 1. Introduction

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

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

## 2. Materials and methods

### *2.1. Apple juice preparation*

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

### *2.2. HPH and ultrasound treatments*

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

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

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

### *2.3. Thermal treatment*

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

### *2.4. Temperature measurement*

The sample temperature was measured just before and immediately after (i.e. before the cooling 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$^3$) 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 $$E_v = \Delta P \qquad (1)$$
125
126 where $\Delta P$ is the pressure difference operating at the nozzles.
127 As the power density ($P_v$, W/m$^3$) 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 $$P_v(T) = \frac{mc_p(\partial T / \partial t)}{V} \qquad (2)$$
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$^3$), 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 $$E_v = \int P_v(T)dt \qquad (3)$$

The energy density of multiple passes HPH and combined treatments was calculated as the sum of the energy density values of the corresponding single pass HPH and HPH plus $US_{ct}$ or US (Calligaris et al., 2016). The energy density of the thermal treatment was estimated according to eq. 4:

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

*2.6. Electrical energy consumption measurement*

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

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

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

*2.8. PPO activity assay*

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

*2.9. Data analysis*

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

## 3. Results and discussion

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

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

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

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

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

process to inactivate the enzyme (Fig. 1) and lower energy density values were computed (eq. 3). Moreover, as can be seen in Fig. 3, to achieve a same inactivation value, both $US_{ct}$ and US treatments provided higher energy density than $TT_{ct}$ and TT treatments, respectively. This discrepancy can be attributed to the different modality of delivering the energy. During heating alone, the energy provided to the closed system merely contributed to temperature increase. By contrast, ultrasound process was likely responsible for inducing other (mechanical) changes besides temperature rise. Some of these changes could positively contribute to apple juice stabilization. In fact, ultrasonication would favour the enzyme release from the cell walls making it more susceptible to thermal inactivation (Başlar and Ertugay, 2013). This is especially true for the $US_{ct}$ treatment when compared to the $TT_{ct}$ one. In fact, temperature control in $US_{ct}$ was performed by cooling the sample during continuous ultrasound treatment, while in $TT_{ct}$ heating once the desired temperature was achieved it was kept constant, thus leading to a notably lower energy density.

Overall, data confirmed that both HPH and $US_{ct}$ are scarcely effective in inactivating PPO, unless high energy density values were provided by applying a high number of passes of sample in the homogenization valve or long ultrasonication times. However, these conditions are far from to be applicable at the industrial level. On the contrary, the heat generated *in situ* during US greatly contributed to inactivate PPO at energy density and process time likely compatible with the industrial process. In the light of these results, further experiments were carried out to investigate the effect of combinations of single-pass HPH at 150 MPa and ultrasounds with (HPH-$US_{ct}$) and without (HPH-US) temperature control on PPO activity (Table 2). By comparing these results with those relevant to the individual treatments (Table 1 and Figs. 1 and 3), it can be noted that HPH-$US_{ct}$ led enzyme residual activities not dissimilar from those caused by the application of HPH and $US_{ct}$ providing comparable energy density values. Similarly, for a same energy density value, only slight differences in enzyme inactivation were observed between HPH-US and US. Therefore, it can be concluded that combined HPH and ultrasound treatment did not allow to reduce PPO activity compared to the single treatments. Finally, the effect of HPH, $US_{ct}$, US and their combinations as

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

## 4. Conclusions

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

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## Caption for figures

**Fig. 1.** Changes in PPO activity in apple juice subjected to ultrasound process with (a) ($US_{ct}$) or (b) without (US) temperature control as a function of time. $TT_{ct}$ and TT: heat treatments obtained by providing the sample the same time-temperature combinations received during $US_{ct}$ and US, respectively.

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

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

**Fig. 4.** PPO residual activity *vs* electrical energy consumption of high pressure homogenization (HPH), ultrasound with ($US_{ct}$) or without (US) temperature control and combinations of HPH and $US_{ct}$ and US. Data relevant to heat treatment ($TT_{ct}$ and TT) providing the sample the same time-temperature combinations received during $US_{ct}$ and US respectively are also shown.

**Table 1**

Temperature and PPO residual activity of apple juice subjected to HPH. Starting temperature: 8.0 ± 1.0.

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

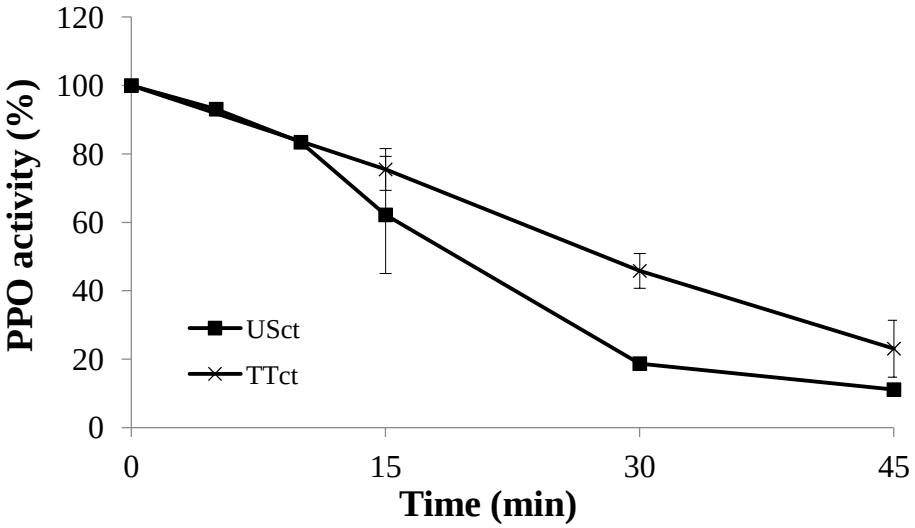
Values are the mean of three repetitions on two replicates ± standard error.

**Table 2**

Temperature, PPO residual activity and energy density of apple juice subjected to combinations of 1 pass HPH at 150 MPa and ultrasound process under controlled ($US_{ct}$) and uncontrolled (US) 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 |

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


120
100
80
60
40
20
0
PPO activity (%)
USct
TTct
0
15
30
45
Time (min)

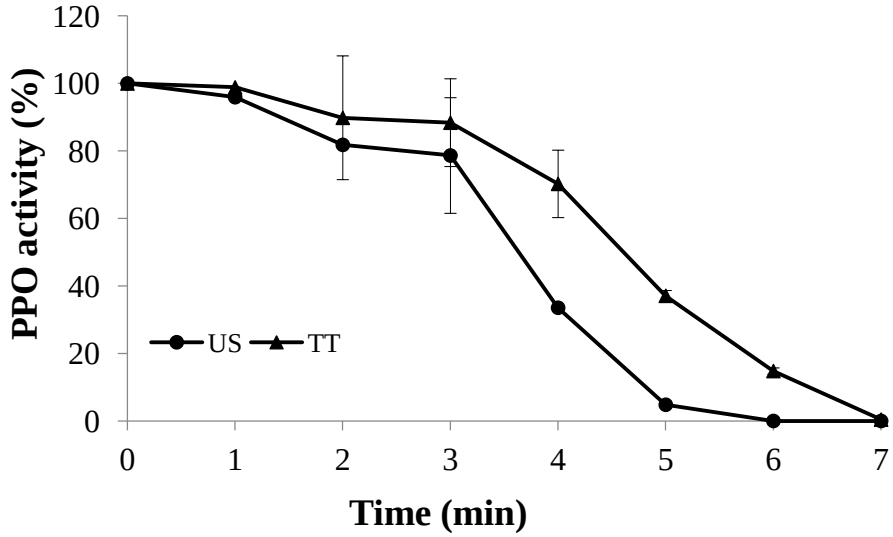

120
100
80
60
40
20
0
PPO activity (%)
US
TT
0
1
2
3
4
5
6
7
Time (min)

**Fig. 1.**

**Figure**

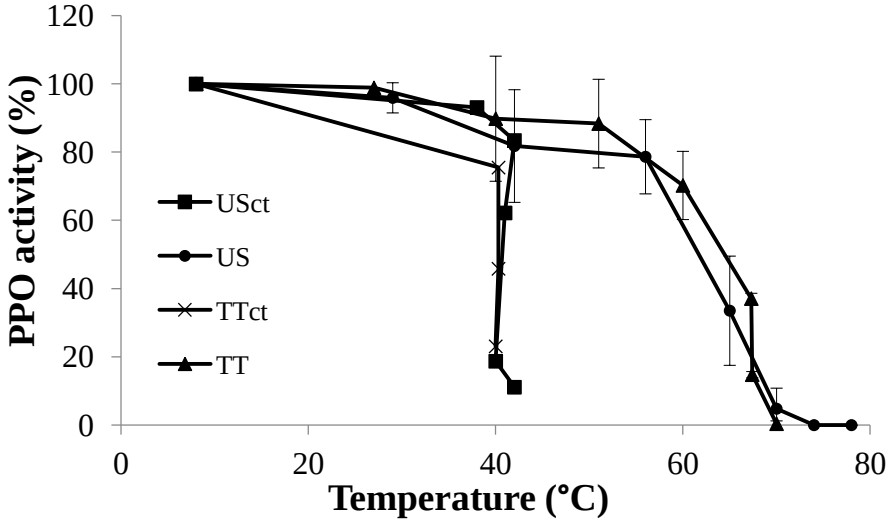

120
100
80
60
40
20
0
PPO activity (%)
USct
US
TTct
TT
0
20
40
60
80
Temperature (°C)

**Fig. 2.**

**Figure**

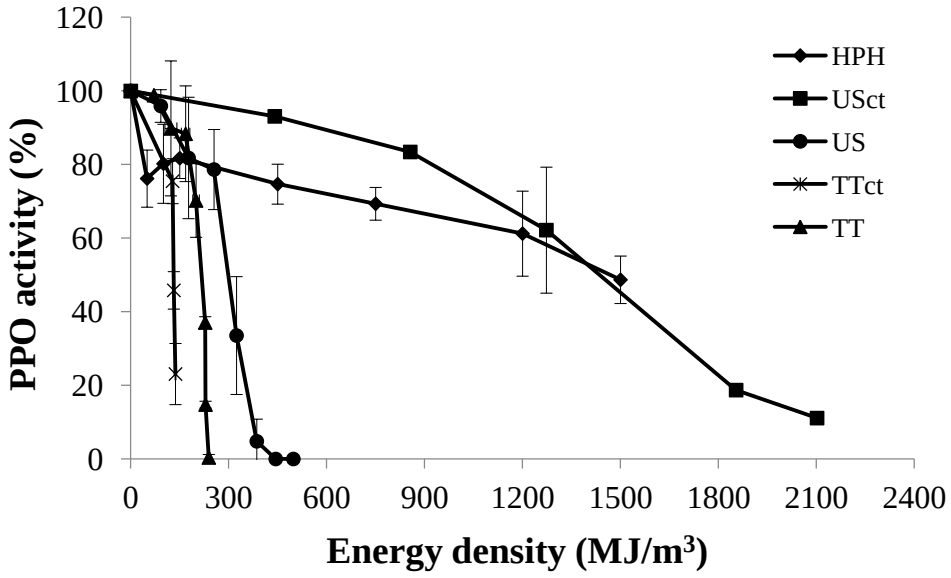

120
100
80
60
40
20
0
PPO activity (%)
0
300
600
900
1200
1500
1800
2100
2400
Energy density (MJ/m3)
HPH
USct
US
TTct
TT

**Fig. 3.**

**Figure**

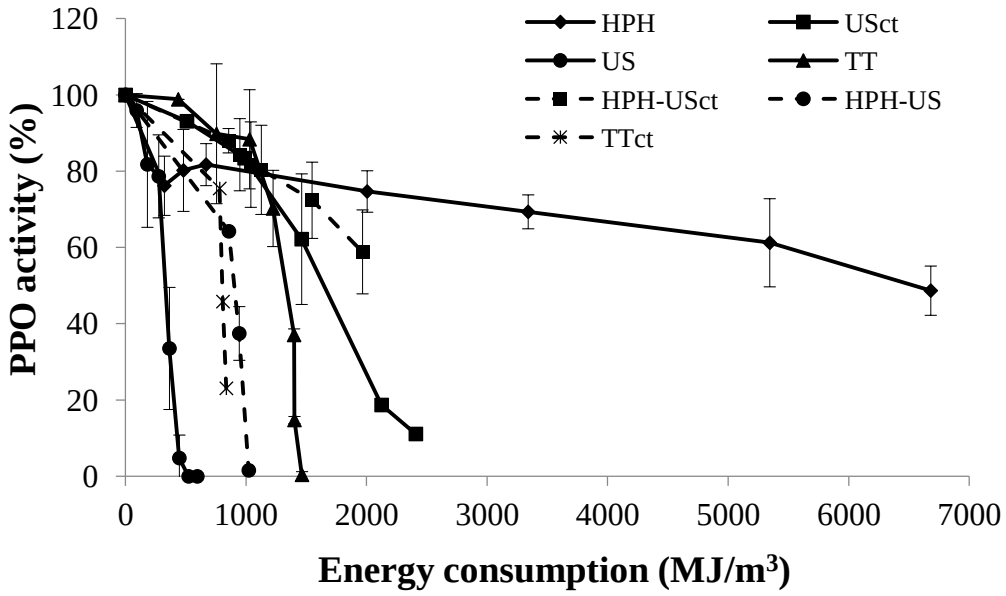

120
100
80
60
40
20
0
PPO activity (%)
0
1000
2000
3000
4000
5000
6000
7000
Energy consumption (MJ/m3)
HPH
USct
US
TT
HPH-USct
HPH-US
TTct

**Fig. 4.**