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Effect of Non-Thermal Treatments of Clear Apple Juice on Exogenous Pectinases

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Abstract

Pulsed electric field (PEF) and high-pressure processing (HPP) are non-thermal treatments, developed to ensure preservation of food products whilst maintaining taste and valuable nutrients. In this study, we investigated their potential for the inactivation of 3 commercial exogenous pectinases (polygalacturonase, pectin transeliminase, pectin esterase) commonly used in juice processing for clarification of juices. The inactivation of these enzymes after processing is mandatory by European law. Clear apple juice was treated with both non-thermal processing methods, as well as with thermal pasteurization as the standard method. For HPP, 3 pressures (250, 450, and 600 MPa) and different holding times (from 2 to 12 min) were tested. For PEF, 3 electric field intensities (10, 13, and 15 kV/cm) and different specific energy values (from 121 to 417 kJ/kg). Standard thermal pasteurization resulted in a complete inactivation of all tested pectinases. HPP treatment only showed marginal effects on polygalacturonase and pectin transeliminase at the highest pressure and holding times, which are beyond levels used in industrial settings. For PEF, dependence upon high electric field strength and specific energy values was evident; however, here too, the effect was only moderate at the levels attainable within the scope of this study. Assuming a continued linear relationship, usable results could be achieved in an industrial setting, albeit under more extreme conditions.

Keywords: enzyme inactivation; pectinase; fruit juice; HPP; PEF; thermal pasteurization; non-thermal treatment; exogenous enzyme

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1. Introduction

Fruit juices are beloved products, rich in vitamins, antioxidants, and fiber. When consumed in moderate amounts, they can help reduce reactive oxygen species (ROS), promote good health, and reduce cholesterol levels [1,2]. A certain loss of these beneficial nutrients cannot be avoided during fruit processing, due to the multiple steps involved in production. Milling and pressing destroy cell integrity and increase the amount of oxygen present in the pulp, accelerating the oxidation of polyphenols. The pasteurization process, necessary to extend shelf life, has a negative impact on thermolabile compounds, like vitamin C and phytochemicals [3–5]. The degree of phytochemical loss varies based on fruit and juice matrix composition. For instance, Kilara et al. [6] reported a 15% reduction in vitamin C in cloudy apple juice after a short pasteurization cycle of 30 s at 85 °C; similar results were published for sweet lime juice [7], while a loss of 44% vitamin C and 15% in phenols was observed in pear juice [8]. Likewise, blends of strawberry-blackcurrant and strawberry-chokeberry nectars underwent a 25% reduction in vitamin C, although phenol levels remained stable after 15 s at 95 °C [9]. In addition to thermal treatments, clarification with bentonite or gelatin, which is frequently added to remove suspended solids, further removes fiber and nutrients bound to it [6]. While most of these steps are essential and cannot easily be replaced, novel methods for pasteurization have been investigated.

During the past two decades, a lot of effort has gone into researching alternative pasteurization methods in order to increase the availability of bioactive compounds while still ensuring microbial reduction and enzyme inactivation. High pressure processing (HPP) and pulsed electric field (PEF) are two non-thermal processes that have gained a lot of traction due to the mild conditions of the treatment [10–14]. PEF technology inactivates enzymes through the application of short, high-intensity electric pulses that induce irreversible structural changes in the enzyme molecules, while HPP inactivates enzymes by subjecting juices to high pressures, up to 600 MPa commercially, which disrupt the protein structure without the drawbacks of heat exposure [15,16]. However, while the efficacy of both processes has been proven for microbial inactivation, PEF and HPP are less effective than thermal treatments for inactivating resistant enzymes and efficacy on enzymes, which may vary depending on the enzyme source and the juice matrix [17]. Previous reports on these technologies have mostly focused on inactivation of endogenous enzymes and have shown, for example, a reduction of polyphenol oxidase (PPO) activity of over 50% in strawberry nectar after treatment with HPP (600 MPa for 3 min) [18–23]. While these findings are deemed positive, the results cannot be blindly applied transversally to every production chain where, for example, addition of exogenous pectinase enzymes has been implemented since the early 1930s, when the first commercial product was released [24]. These enzymes help with clarification, reducing viscosity, improving juice and phenolic and polysaccharidic extraction from the pulp, and producing juices and nectars with more vibrant colors and rheological properties [25,26]. The inactivation of exogenous pectinases in fruit juice processing is a major concern, as these enzymes can degrade juice quality by altering clarity, viscosity, and overall quality during storage. Their usage is regulated in accordance with European legislation [27,28] and the upcoming Food Improvement Agents Package (FIAP) regulations for food enzymes, in which EFSA developed updated scientific guidance to assist applicants in the preparation of applications for food enzymes. A union list is planned for enzymes, which are used in food production, authorized by EFSA after being positively evaluated [29]. Moreover, only those enzymes that are used in the food processing line that still present activity in the final product must be declared. Thus, the use of exogenous enzymes as processing aids, not as additives, is allowed, but inactivation must be ensured before product packaging [30]. In this study, we measured the inactivation percentage on 3 commercial pectinases in clarified apple

juice processed with PEF and HPP to test the viability of these treatments in the food industry. The parameters chosen for PEF in this study are consistent with those reported in the literature for the inactivation of endogenous and exogenous enzymes while for HPP extended treatment times (up to 9–12 min at 600 MPa), exceeding typical commercial durations were adopted to assess the limits of enzyme reduction despite reduced energy efficiency. This cocktail of pectinases is routinely employed in the juice industry and further knowledge of their behavior with new non-thermal treatments would be of great interest for producers to evaluate adoption of these newer technologies in the production chain given the current scarcity of data on the field and higher costs compared to conventional thermal processing [31].

2. Materials and Methods

2.1. Material Sourcing and Sample Preparation

Commercial clear apple juice from Solevita (Lidl) was purchased at a Lidl grocery store in Parma, Italy, in January 2024. The pH was measured to be 3.51 ± 0.01, conductivity as 2.28 ± 0.01 mS/cm, and Brix as 10.83 ± 0.29°. The methodology involved direct measurements using calibrated instruments and calculation of mean and standard deviation from three independent measurements. A blend of 3 commercial pectinases, polygalacturonase (PGX, EC 3.2.1.15), pectin transeliminase (PTF, EC 4.2.2.10), and pectin esterase (PE EC 3.1.1.11), was obtained from AB Enzymes (Darmstadt, Germany) and mixed on the day of treatment with the juice to a final concentration of 5% (*v*/*v*) (50,000 ppm). The dosage chosen was 200–5000 fold higher than the industrially relevant dosages (0.001–0.025%, 10–250 ppm), and while this might lead to unexpected side effects, such as aggregation due to low ionic strength in the juice along with binding of polyphenols in the active site derived by lack of native substrate in solution, it was a necessary step to ensure that potential residual activities would be still detectable. Samples were then directly processed with the respective technique, as described in Section 2.2, and stored at −20 °C until analysis. All chemicals were of the highest purity and purchased from Merck/Sigma-Aldrich (Darmstadt, Germany).

2.2. Processing Parameters

2.2.1. Thermal Pasteurization

Thermal treatment was carried out on a lab scale using a water bath (Salvis Lab WB20, Rotkreuz, Switzerland). Two different conditions were applied: heating for 1 min at 70 °C (TT1) and for 15 s at 90 °C (TT2). These parameters were selected based on scientific literature, which provided guidance on commonly adopted practices and demonstrated effective outcomes in similar studies [32]. Juice was poured into 100 mL PET containers and thermally treated. To monitor the internal temperature, a T-type thermocouple (TERSID) was inserted into a control sample. Immediately after the heating process, the bottles were placed in ice water to quickly bring the juice down to 4 °C, thereby reducing the risk of thermal degradation.

2.2.2. Pulsed Electric Field (PEF)

The PEF experiments took place at the Food Technology laboratory of the University of Parma. Apple juice was processed using a PEF pilot system (EPULSUS®-LBM1B-15, Energy Pulse System, Lisbon, Portugal) fitted with a co-linear treatment chamber and operating in continuous mode. The generator within the device can deliver up to 15 kV/400 A, providing pulses with widths ranging from 2 µs to 200 µs, with a maximum frequency of 50 Hz. A volumetric pump was used to circulate the apple juice between the inlet tank and system. The juice in the inlet tank was maintained at an initial temperature of

8.1 ± 0.5 °C using a cooling jacket to ensure process consistency. Following PEF processing, a control valve enabled precise regulation of system pressures as indicated on the gauges. The treated juice was then rapidly cooled using a spiral cooling coil submerged in an ice bath to minimize thermal degradation. Temperature was continuously monitored using thermocouples positioned at the entrance to the PEF system, at the exit of the PEF system, and at the exit of the cooling unit. Samples were collected after the juice had reached room temperature. The tests were conducted at three electric field strength values (10, 13, and 15 kV/cm), while the specific energy input to the samples ranged from 121 kJ/kg to 417 kJ/kg. These values were selected from those reported in the literature for enzyme inactivation [33]. Table 1 provides a comprehensive overview of all relevant parameters. For each test, 2 L juice were used.

Table 1. Values of electric field intensity, pulse width, frequency, current, and total specific energy applied during PEF treatments.

Test	Electric Field Intensity (kV/cm)	Pulse Witdth (μs)	Frequency (Hz)	Current (A)	Specific Energy (kJ/kg)
PEF 1	15	10	10	210	121
PEF 2	15	10	20	336	389
PEF 3	15	10	15	228	189
PEF 4	13	10	30	277	417
PEF 5	10	10	40	161	251

For all the tests, the outlet temperature was lower than 40 °C, reducing in this way the thermal effects that usually accompany PEF treatment. Immediately following treatment, samples were transferred to sterile plastic (PET) containers and stored at −20 °C to maintain quality before enzyme testing. Each treatment was conducted in triplicate.

2.2.3. High-Pressure Processing (HPP)

High-pressure processing (HPP) utilized a 300-L high-pressure unit (Avure Technologies Inc., Erlanger, KY, USA) housed within the facilities of HPP ITALIA SRL in Traversetolo (Parma, Italy). Cold water maintained at 4 °C served as the medium for pressure transfer. The HPP treatments were conducted at three pressure levels: 250, 450, and 600 MPa, with varying durations of 2, 4, and 6 min for 250 MPa, 4 min for 450 MPa, and 4, 8, and 12 min for 600 MPa. Except for the treatments at 600 MPa for 8 and 12 min, all other treatment combinations investigated in this study fall within the range of conditions commonly and practically used in real-world applications [34–36]. These parameters were selected based on industry standards and previously successful applications. The inclusion of more severe treatments at 600 MPa for extended durations was based on preliminary tests, where standard HPP conditions failed to significantly inactivate the target enzymes. These intensified parameters were explored to assess whether harsher conditions could achieve partial inactivation and better define the limits of HPP technology. Decompression was executed immediately following the specified treatment period. Post-treatment, the samples were swiftly placed in refrigerated storage at 4 °C. To ensure the validity and reproducibility of the results, each HPP treatment was carried out in triplicate. Table 2 provides a comprehensive overview of all relevant parameters. For each test, 75 mL of juice were used.

Table 2. Values of pressure and holding time applied during HPP treatments.

Test	Pressure (MPa)	Time (min)
HPP 1	250	2
HPP 2	250	4
HPP 3	250	6
HPP 4	400	4
HPP 5	600	4
HPP 6	600	8
HPP 7	600	12

2.3. Analysis of Residual Enzyme Activity

Treated and control samples were thawed overnight in a refrigerator at 4 °C. PGX and PE enzymes were assayed in technical duplicates and biological triplicates while PTF was assayed in technical and biological triplicates.

2.3.1. Polygalacturonase (PGX) Assay

Activity for PGX was measured following the method of Neogen Megazyme [37] with a minor modification to the substrate used. Briefly, 1.8 mL of 0.7% (*w*/*w*) pectin in sodium acetate buffer pH 3.9 solution was placed in a conical tube and equilibrated at 40 °C in a water bath, and 200 µL of sample diluted in 0.1 M pH 3.9 acetate buffer was added after equilibration, vortexed, and incubated at the same temperature for 10 min. The reaction was stopped by addition of 2 mL of a 1% (*w*/*v*) 3,5-dinitrosalicylic acid (DNS) solution. Samples were boiled at 100 °C for 5 min and then plunged into ice cold water for 5 min. For blank samples, the DNS solution was added prior to the addition of the enzyme dilutions. A portion of the cold sample was transferred to a 1.5 mL tube and centrifuged for 3 min to pellet the residual pectin. Supernatant was used to measure the absorption at 540 nm.

A standard curve with D-(+)-galactose was used to calculate the concentration of galactose in the sample.

The final activity was calculated with the formula:

$$\mathrm{PGX} \times \mathrm{g}^{-1} = \frac{(\mathrm{a} + \mathrm{b} \times \Delta \mathrm{E}_{540}) \times 1000}{\mathrm{c_s} \times \mathrm{t}}$$

where:

a = intercept of galactose calibration curve;
b = slope of galactose calibration curve;
ΔE_{540} = difference in extinction between sample and blank sample;
c_s = concentration sample [mg/mL];
t = reaction time [min].

2.3.2. Pectin Transeliminase (PTF) Assay

PTF activity was measured according to Albersheim [38] with a modified buffer. In a 1 cm quartz cuvette, 1 mL of citrate-phosphate buffer pH 5.8 containing 0.5% (*w*/*w*) pectin was pre-tempered at 30 °C directly in a multicuvette spectrophotometer, which was connected to a water bath. Samples diluted in the same buffer were transferred directly into the cuvette and the change in absorbance at 235 nm, caused by the formation of unsaturated bonds between galacturonic acid units, was measured for 8 min. The linear part of the curve was used for the calculation of the activity with the formula:

$$\mathrm{PTF} \times \mathrm{mg}^{-1} = \frac{\Delta \mathrm{E}_{235}/\Delta \mathrm{t}}{0.01 \times \mathrm{c_s} \times \mathrm{V_s}}$$

where:

ΔE_{235} = difference in absorbance recorded at 235 nm;
Δt = difference of time in linear part of the curve [min];
c_s = concentration of the sample [mg/mL];
V_s = sample volume [mL].

2.3.3. Pectin Esterase Assay (PE)

PE activity was measured by titration in an automated system equipped with a Metrohm dosing robot (Herisau, Switzerland) and the "tiamo" software v3.0 according to Kertesz [39] with minor modifications. Pectin solution was buffered to pH 3.9, and the starting point for titration was pH 4.5 and kept constant by addition of 0.025 M NaOH after formation of free carboxyl groups. Samples were diluted in distilled water to a final concentration between 4.5 and 5 mg/mL. After sample injection, the linear NaOH consumption was recorded continuously for 6 min and used for the calculation of activity.

Activity was calculated with the formula:

$$\text{PE} \times \text{g}^{-1} = \frac{\text{consumption NaOH[mL]} \times 25 \times 1000}{t \times c_s \times V_s}$$

where:

c_s = concentration sample [mg/mL];
V_s = volume sample [mL];
t = time [min].

2.4. Data Analysis, Plotting, and ANOVA Statistics

Inactivation of the enzymes was calculated with the equation:

$$\text{Inactivation \%} = 100 - \left(\frac{\text{Mean activity treated sample}}{\text{Mean activity control sample}}\right) \cdot 100$$

Data were plotted in OriginPro 2023 with the average of 3 biological replicates and their standard deviation (SD).

One-way ANOVA with Tukey mean comparison was performed on the 3 technical replicates of each biological sample for PGX, PE, and PTF.

3. Results and Discussion

3.1. Inactivation of Pectinases

3.1.1. PEF Inactivation

The data presented in Figure 1 illustrates the inactivation percentage for PGX, PE, and PTF across three biological replicates for five PEF treatments, compared to traditional thermal pasteurization at 70 °C and control samples. The findings indicate that the highest inactivation rate occurred in the second test with a 60% decrease in mean activity. Compared to the other conditions, PEF2 had the strongest electric field and the highest specific energy value. PE inactivation appeared to be slightly lower with PEF1 and PEF2 conditions than that observed for PGX with a reduction in activity of 16% and 53% compared with 21% and 60%. PEF3, PEF4, and PEF5, on the other hand, had an impact comparable to what was previously seen. For PTF, a similar trend was noticeable with treatments 1 and 5, with values around 15% and 20% compared with 21% and 25%.

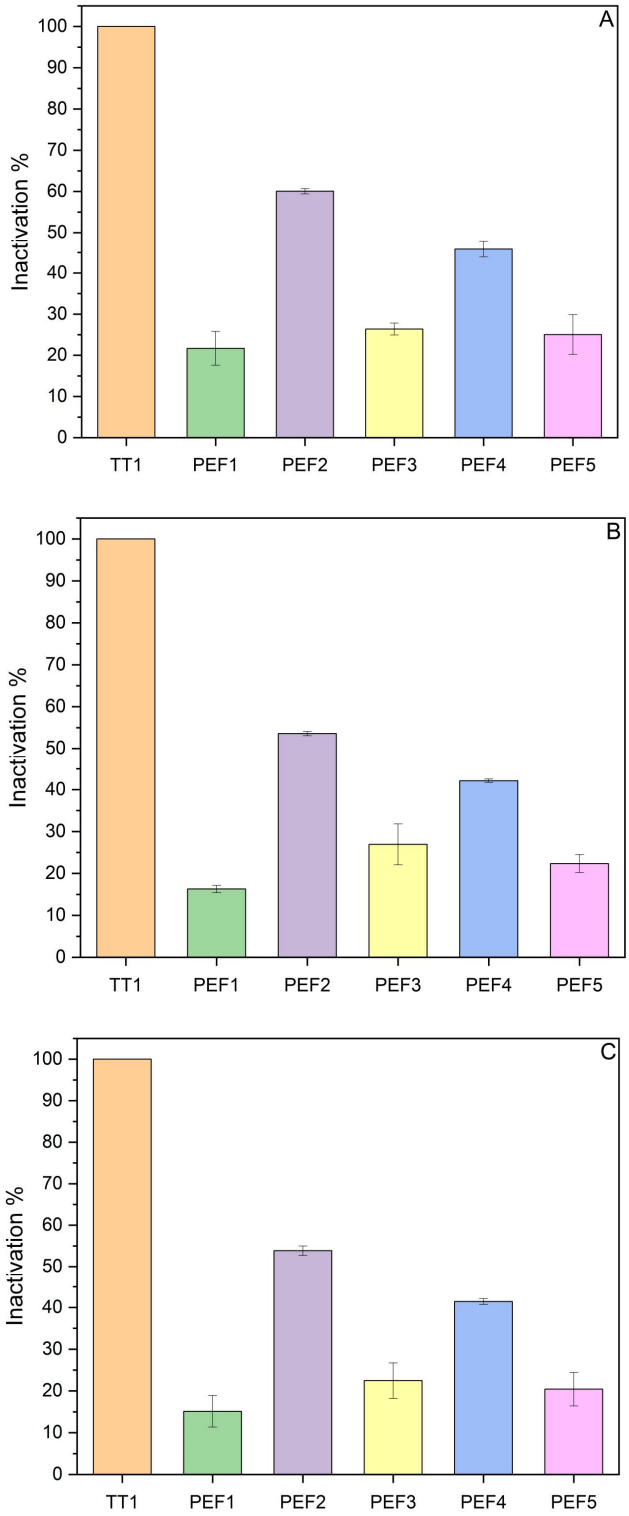


Figure 1. Inactivation (%) of the enzymes (**A**) PGX, (**B**) PE, and (**C**) PTF by PEF compared to standard thermal pasteurization at 70 °C (TT1) for 1 min. Data plotted as the average of biological triplicates ± SD. Specific values for electric field and energy input for each PEF conditions were: (PEF1) 15 kV/cm–121 kJ/kg; (PEF2) 15 kV/cm–389 kJ/kg; (PEF3) 15 kV/cm–189 kJ/kg; (PEF4) 13 kV/cm–417 kJ/kg; (PEF5) 10 kV/cm–251 kJ/kg.

While research on PEF has primarily focused on food microbiology and the impact of PEF processing on enzyme activity has been less studied, it has been established that enzymes are generally less sensitive to PEF than microbes, and both seem to share similar critical factors [40]. In fact, its effectiveness is influenced by several key factors, with the

two most significant being the intensity of the electric field and the specific energy [41]. Specifically for food microbiology, the specific energy applied during PEF treatment correlates directly with the degree of microbial inactivation. As specific energy increases, the reduction in microbes becomes more pronounced. However, greater effects can be achieved at the same energy levels by increasing the electric field strength [42,43].

In this study, a clear trend emerged when comparing different specific energies for the same electric field strength (Figure 2): an increase in specific energy corresponds to a higher inactivation rate.

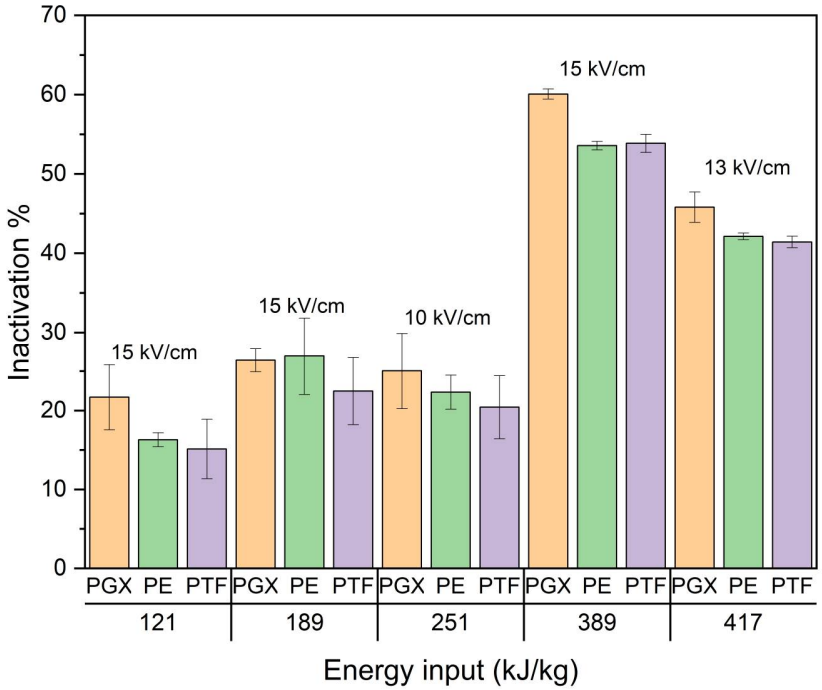


Figure 2. Enzyme inactivation (%) in relation to energy input and strength of electric field for PGX, PE, and PTF clustered with each energy input. Specific values for each energy input are reported on the x axis. Electric field intensities are specified on top of each cluster and were 15 kV/cm for 121 kJ/kg, 15 kV/cm for 189 kJ/kg, 10 kV/cm for 251 kJ/kg, 15 kV/cm for 389 kJ/kg, and 13 kV/cm for 417 kJ/kg.

For the samples treated with the same electric field strength—PEF 1, PEF 2, and PEF 3—the percentage of inactivation increased with the rising specific energy. Conversely, despite PEF samples being processed with higher specific energy values, they exhibited lower inactivation rates than PEF 2. This suggests the presence of a threshold specific energy value, as PEF 1 and PEF 2, with the highest value of electric field strength but with the lowest specific energy, showed lower or comparable inactivation percentages to PEF 4 and PEF 5.

As mentioned previously, the mechanism for enzyme inactivation is still not fully understood, although a synergy of localized thermal and electrochemical effects is believed to be the leading cause of inactivation. PEF might induce localized oxidation of amino acids, such as cysteine, and disrupt disulfide bridges [44], and it has also been reported to create pH shifts along with knockout of hydrogen bonds [45]. Disulfide bridges are integral for enzyme folding and resistance to denaturation and pH changes. Pectinases from microbial sources commonly present such covalent interactions, and this could partially explain the effectiveness of the treatment [46,47]. For example, the crystal structure of PGX from *Aspergillus aculeatus* presents a total of four disulfide bridges, with 3 positioned directly at the extremities, plausibly enhancing susceptibility to oxidation, hence possibly explaining the lower residual activity we observed [46]. In industry, stronger electric fields

from 25 kV/cm to 35 kV/cm tend to be employed, increasing the total energy output. With such setups, previous research on endogenous PE reported a decrease of 88% and 93% in orange and tomato juice, respectively [48,49]. For commercial pectinases, a study by Giner et al. focused on the impact of the electric field (19 to 38 kv/cm) and number of pulses on the activity of PE in aqueous solutions and reported an exponential decay with up to 90% enzyme inactivation at 38 kV/cm and 100 pulses and of circa 50% with similar energy input of PEF2 [50]. Unfortunately, previous data on the inactivation of PTF are not available, but due to the structural similarity with PE [51], it is likely to react similarly during the treatment. Overall, all the pectinases showed a comparable degree of inactivation, indicating that the PEF technique has the potential to partially inactivate the tested enzymes and might lead to full denaturation with higher energy input and electric fields, particularly considering that the enzyme concentrations in the samples were significantly higher than typical values used in the food industry.

3.1.2. HPP Inactivation

Treatment with HPP did not result in enzyme inactivation. All tested enzymes showed good stability at 250 MPa and 450 MPa of pressure up to 6 min of holding time with activity ranges comparable to the control (Figure 3).

At 600 MPa, however, PGX and PTF showed significant differences in long holding periods. After 12 min, PTF's activity was reduced by 15%, while PGX's increased by roughly the same amount. While a 15% reduction can be deemed positive, it is still far off from the 100% inactivation required by law. On top of that, 12 min at 600 MPa are not parameters commonly used in industry due to the cost/effectiveness ratio.

Most studies published on the inactivation of endogenous enzymes with HPP technology are focused on oxidase enzymes and showed either minor reduction or an increase in specific activity, and this seems to hold true also for the exogenous pectinases used in this study. This might be influenced by factors such as pH, temperature, and juice composition, which can stabilize the enzyme structure or promote refolding after pressure release [18–22,52].

From our findings, PE was the least impacted by the treatment at every pressure tested, with activities remaining within ±5% of the control. This barotolerance has been previously reported in literature on apple juice, pitaya-pineapple juice, and in the work of Wibowo et al., which describes an increase of activity in apple juice at 600 MPa after 3 min of treatment [22,53,54].

PGX and PTF presented an increase in activity, in agreement with literature for monomeric proteins [55,56]. The relatively moderate pressure is believed to induce a stabilizing effect, even when heat is applied to the system, as shown by Tomlin et al. on a cocktail of the same enzymes in ideal solutions [57,58]. The pressure also might ease unwanted interactions between polyphenols and active sites that might occur, considering the scarce presence of pectin in the juice matrix [59]. Typically, at higher pressures, such as 450 and 600 MPa, destabilizing forces overthrow this balance resulting in irreversible denaturation of the protein. While this holds partially true for PTF, with PGX the opposite was observed. Most papers focus on endogenous polygalacturonase stability and have reported a satisfactory degree of inactivation in tomato, avocado, and carrot [60–62], while multi-pulsed HPP on apple juice showed no difference in activity after 5 min at 600 MPa. A possible explanation for our observations could be disaggregation of PGX agglomerates caused by the high concentration of enzyme in the matrix along with an increased barotolerance of the microbial isoenzymes.

While quite effective for microbial reduction, high pressure processing has currently not proved to be a suitable technology for exogenous pectinase inactivation.

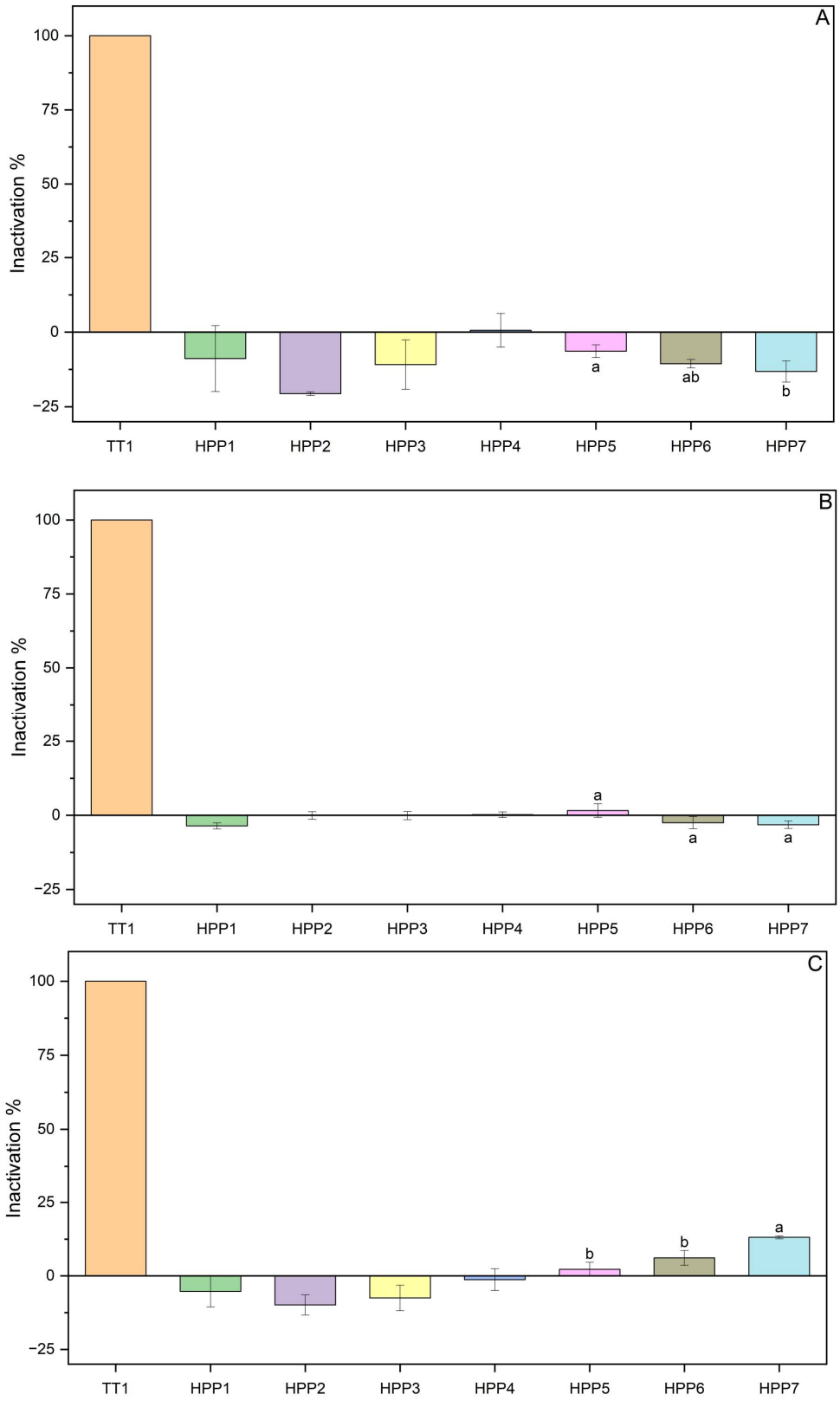


Figure 3. Inactivation (%) of the enzymes (**A**) PGX, (**B**) PE, and (**C**) PTF with HPP treatments at 3 pressures and 6 different holding times compared to standard thermal pasteurization at 70 °C (TT1) for 1 min. Letters a, ab, and b derived from ANOVA analysis ($p < 0.05$). Specific values for pressure and holding time were: (HPP1) 250 MPa—2 min; (HPP2) 250 MPa—4 min; (HPP3) 250 MPa—6 min; (HPP4) 400 MPa—4 min; (HPP5) 600 MPa—4 min; (HPP6) 600 MPa—8 min; (HPP7) 600 MPa—12 min.

3.1.3. Thermal Pasteurization

Two setups were used for thermal pasteurization: 1 min at 70 °C and 15 s at 90 °C. The mean activity of 3 biological replicates is shown in Figure 4 alongside the control samples. The mean value of activities in the controls were 343 ± 19 U/g for PGX, 348 ± 3 U/g for PE, and 4.2 ± 0.1 U/g for PTF.

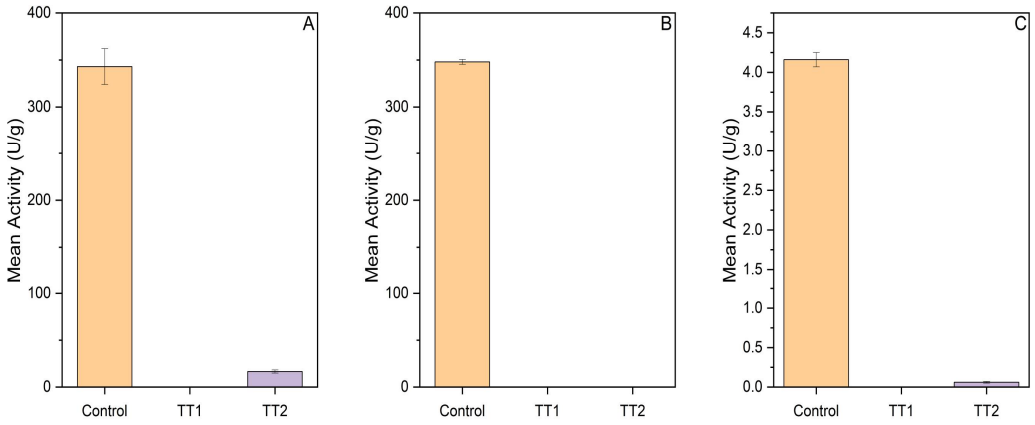


Figure 4. Effect of thermal pasteurization at 70 °C for 1 min (TT1) and at 90 °C for 15 (TT2) seconds on the investigated enzymes: (**A**) PGX, (**B**) PE, (**C**) PTF. Results as the average of mean activity of biological triplicates ± SD.

The lower temperature setting with longer holding time achieved the best results, with all enzymes completely inactivated. Higher temperature but shorter treatment inactivated PTF, while PGX and PTF still showed a residual activity of 16.5 ± 1.7 U/g for the former and 0.06 ± 0.1 U/g for the latter. Considering the high concentrations of enzymes in the samples and the juice matrix, it is possible for molecular crowding to be a stabilizing factor, producing unexpected inactivation kinetics that would not be present at normal 0.001–0.025% (v/v) working ranges. On the other hand, it is also worth noting that both PGX and PTF purified from microbial origins have shown to be more resilient to thermal treatments, possibly justifying the residual activity for such short contact time at 90 °C [63,64]. A longer incubation time, even at a lower temperature, seems to overcome this hurdle as shown in our results.

4. Conclusions

In this study, we measured the inactivation percentages of three commercial pectinases, polygalacturonase (PGX), pectin transeliminase (PTF), and pectin esterase (PE), in clarified apple juice processed using pulsed electric field (PEF) and high-pressure processing (HPP). The aim was to assess the viability of these treatments in the food industry.

The strategic choice was made to use a significantly oversized blend of the three enzymes to ensure that the residual activity after treatment was within a range measurable by analytical methods. The enzyme concentrations used in this study were, therefore, roughly 100–1000 times higher than those typically used in juice production. An analogous reduction to industry standard concentrations would have demonstrably resulted in residual activities well below the detectable range. It can therefore be concluded that the use of these enzymes is safe for the food industry when paired with thermal treatment, and perhaps even with future technological improvements of PEF treatment.

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