

## Article

# Effects of Equivalent Processing Conditions for Microbial Inactivation by Innovative Nonthermal Technologies on the Safety, Quality, and Shelf-Life of Reineta Parda Apple Puree

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**Abstract:** This study aimed to identify equivalent processing conditions using ultrasound (US), pulsed electric fields (PEF), and high-pressure processing (HPP) for shelf-life extension and to ensure the safety and quality of Reineta Parda apple puree (AP) in comparison to conventional thermal pasteurization (72 °C/15 s, CTP). The processing conditions were selected to achieve at least 5 log CFU/g inactivation of *Escherichia coli*. HPP (400 MPa/1 min), US (60 °C, 20 kHz/12 min), and PEF (57.2 °C, 10 kV/cm, 70 bipolar pulses of 8 µs each) reduced *E. coli* counts by 6.6, 6.1, and 5.8 log CFU/g, respectively, thus achieving the pasteurization status. After processing, HPP samples showed higher levels of total antioxidant activity (DPPH and ABTS) compared to the other samples. HPP and PEF samples showed lower browning degrees than the CTP samples. All treatments ensured indigenous microbial stability (below 1 log CFU/g) for at least 30 days under refrigeration. Principal component analysis showed that the HPP samples increased their similarity to the untreated fresh AP during storage based on the pH, total soluble content and water activity, retaining its fresh-like qualities. HPP and PEF were found to be potential alternatives to the CTP of AP, resulting in a safe, minimally processed product with improved antioxidant activity.

**Keywords:** nonthermal pasteurization; ultrasound; pulsed electric fields; high-pressure processing; *Escherichia coli*; apple puree



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## 1. Introduction

Apples (*Malus domestica*) are one of the most widely consumed fruits globally, with 93.1 million tonnes produced in 2021. In Europe, they represent 20% of the global production, with Portugal reporting 368,230 tonnes of apples produced. China is the largest producer with 45 million tonnes, followed by Turkey and the United States of America, both with 4.5 million tonnes produced [1]. Apples can be consumed in their natural state or processed into juice, jam, cider, and vinegar to minimize waste [2]. Apple puree (AP) is a common ingredient in many food items such as juices, beverages, fruit sauces, pie fillings, and infant foods. With its abundance of polyphenols, which are recognized for their antioxidant characteristics, and high fiber content, this food choice is both nutritious and beneficial for customers' health. Following the pressing of apples in the juice manufacturing process, the leftover solid residue, known as pomace, can make up 20–35% of the apple's original weight. This pomace is a combination of the apple's peel, core, seed, calyx, stem, and pulp [3]. Nevertheless, the quality of AP can vary based on genetics, storage, heating parameters, processing intensity, and refining. Consequently, it is essential to identify suitable and sustainable processing technologies that can guarantee food safety and preserve product quality [4].

Thermal processing has traditionally been employed in the food industry for AP pasteurization, with the aim of eliminating particular pathogenic bacteria including *Escherichia coli*, *Salmonella* spp., and *Listeria monocytogenes*, which are responsible for foodborne illness outbreaks. Consequently, pasteurization diminishes the likelihood of contracting foodborne diseases and extends the shelf-life of AP and other apple-based products [5]. Before undergoing pasteurization, the AP is exposed to a technique called blanching, which involves a brief but significant thermal shock (about 100 °C for 30 s to 5 min) to deactivate enzymes associated with browning, such as polyphenol oxidase and peroxidase [6]. However, these thermal processes can frequently cause undesirable quality changes to the product such as vitamin loss, changes in texture or colour, and the development of off-flavours [7]. Recently, emergent processing technologies, such as ultrasound (US), pulsed electric field (PEF) and high-pressure processing (HPP), have attracted interest due to their ability to ensure food safety with minimal quality detrimental effects. Therefore, it is necessary to select the appropriate technology and processing conditions that can achieve, at least, the same microbial inactivation level comparable to that obtained for the conventional heat-based pasteurization methods to ensure food safety [8]. Moreover, in general, the shelf-life of both nonthermal (US, PEF and HPP-treated) and thermally pasteurized foods is comparable. Like thermally and HPP processed foods, PEF-treated products are usually stored refrigerated.

In the application of US, food items are subjected to sonic waves at a frequency of approximately 20 kHz. These waves are generated either by probes or in a liquid bath. Ultrasound causes the formation of bubbles through pressure changes, and the subsequent collapse of these tiny bubbles leads to a localized rise in temperature and pressure. This phenomenon has a pasteurization effect without causing a substantial increase in overall temperature. PEF involves the administration of electric pulses (typically with a field strength ranging from 1 to 30 kV/cm in the food industry) to foods positioned between two electrodes for a brief duration. This process renders biological membranes permeable to small molecules, leading to swelling and eventual rupture of the cell membrane, thereby deactivating microorganisms. HPP uses water as a medium to exert significant pressure (usually ranging from 300 to 600 MPa in the food sector) on meals contained in flexible packets within a vessel. The rise in water volume inside the vessel leads to an increase in pressure, resulting in the inactivation of microorganisms. This is mostly achieved by irreversibly damaging cell membranes and cell walls, which alters their permeability and destroys their functionality [8,9].

When it comes to the application of the aforementioned processing technologies, previous studies from Tian et al. (2023) have demonstrated the feasibility of HPP (400 and 500 MPa for 2 min) to extend the shelf-life of an apple–kiwi–carrot puree, as well as to retain some nutritional attributes such as ascorbic acid, total carotenoids, and antioxidant capacity [10]. A study analysing the effects of HPP (600 MPa, 15 min, 48 °C) and US (460 W/cm<sup>2</sup>, 24 kHz, 15 min, 33 ± 7 °C) on strawberry puree was performed by Sulaiman et al. (2017) who reported that HPP was better than US to retain antioxidant activity over a period of 30 days at 3 °C, while the later performed better to retain colour [11]. When it comes to PEF, Geveke et al. (2015) reported a reduction of 7.3 log units in *E. coli* inoculated in strawberry puree after a PEF treatment at 30 kV/cm, 57.5 °C, 400 pulses/s and a pulse width of 1.8 µs [12].

The objective of this study was to identify equivalent US, PEF and HPP processing conditions that can achieve inactivation of at least 5 log units of pathogenic-surrogated microorganisms (using *E. coli* ATCC 25922 as a target microorganism) by conventional thermal pasteurization, and to evaluate the effectiveness of such processing conditions to ensure the safety during the shelf-life of Reineta Parda AP. In response to the practical demands of the food industry, our study emphasizes a practical approach, prioritizing the possible applicability of these technologies. By bridging the gap between innovative non-thermal technologies and their practical implementation in the food industry, particularly for Reineta Parda AP, we sought to provide insights that are directly relevant and readily

applicable for food processors and manufacturers, as well as using the ready-to-implement quality assessment analyses, such as microbiological parameters, pH, colour, total soluble solids and water activity ( $a_w$ ), as well as a more fundamental parameter (antioxidant activity accessed by two different methods) that could be used as a possible marketing argument for this type of product for the cases where the processing technologies increase the antioxidant activity.

## 2. Materials and Methods

### 2.1. Raw Material

The apples used in this study were of the Reineta Parda variety and originated in a selection of apples deemed unacceptable for fresh sale. The general nutritional composition of the apples used for the puree consisted of 3.73% of crude protein, <0.50% of lipids, 80.0% of carbohydrates and 7.8% of fibre (dry weight basis) at their commercial maturity.

### 2.2. Apple Puree Preparation

Fresh apples were washed, cut into 4 pieces using a sterile stainless knife and pre-treated at 100 °C for 2 min to inactivate browning-related enzymes [6]. Next, the pieces were blended using a kitchen robot (Bimby, Vorwerk, Thermomix TM31, Wuppertal, Germany), and fresh lemon juice (2.5% *w/w*) was added as a natural acidulant [7]. The puree was sieved due to the high amount of cored and pieces of peel and to avoid the formation of air bubbles. Then, the puree was immediately processed by the different technologies, as explained in the section below.

### 2.3. Target Microorganism and Inoculation

*Escherichia coli* WDCM00013 (ATCC 25922, a surrogate for the pathogenic *E. coli* O157:H7) was used as target microorganism, taking into account the likely contamination at the industrial level. *E. coli* is one of the most common causal agents in fruit products associated with several outbreaks of foodborne illnesses [13].

Frozen stock culture of *E. coli* was activated following the procedure provided by the supplier BAControl—5 (Alicante, Spain). One tablet was dispensed into a sterile tube containing 20 mL of sterile distilled water. Afterwards, it was let to dissolve for 10 min at room temperature, shaking it gently every 2 min. Before *E. coli* inoculation, AP samples were pasteurized at 72 °C for 15 s to eliminate vegetative microorganisms. Then, AP samples (100 g) were inoculated with 3 mL of the *E. coli* solution to obtain an approximate final concentration of  $\sim 10^8$  cells/g. After that, the already inoculated samples were shaken for 2 min to ensure that the inoculum was uniformly homogenized throughout the sample. Afterward, inoculated samples were aseptically manipulated to be subsequently subjected to HPP, US, PEF, and thermal pasteurization.

### 2.4. Apple Puree Processing

As aforementioned, the present study aimed to evaluate equivalent processing conditions using different technologies to achieve, at least, a 5-log-unit reduction in a surrogated microorganism (*E. coli*), corresponding to the minimum inactivation level required to achieve the pasteurization status [14].

The selection of the processing conditions used in the present study for the different technologies was based on the current literature, except for achieving inactivation of at least 5 log units of *E. coli*. The technologies were then compared. Before and immediately after each treatment, the samples were plated for enumeration of any possible surviving *E. coli* cells.

The samples were differently processed: one batch was used without any treatment and is described as the untreated “control” throughout this work. A second batch was blanched (100 °C for 2 min, to inactivate browning-related enzymes) and will be termed as “BL”. Then, after blanching, the AP processing conditions were performed as follows.

#### 2.4.1. Conventional Thermal Pasteurization (CTP)

Sample processing was performed according to the treatment described by Wibowo and colleagues [15] with some modifications. One hundred grams of AP samples were placed in a glass flask (11 cm height, Ø 7.5 cm) and processed in an autoclave (Autester ST, J.P Selecta S.A, Barcelona, Spain) at TAGUSVALLEY set to 72 °C for 15 s of holding time. The process was monitored using a datalogger (IFC400, MadgeTech, Houston, TX, USA) inserted in one flask with AP made for this propose. After thermal treatment, the flasks were rapidly cooled and stored at  $3 \pm 1$  °C until used.

#### 2.4.2. Ultrasound (US)

US treatments were conducted at TAGUSVALLEY in an ultrasonic device (Sonics & Materials, Inc., Vibra-Cell VCX 1500 HV, Newtown, CT, USA, equipped with a probe of 22 mm diameter, sonotrode H22 and power of 1500 watt maximum) at  $60 \pm 2$  °C. One hundred grams of AP samples were placed in a beaker and the ultrasonic probe between the top and the bottom of the AP (immersed 2 cm). Afterwards, the AP was processed for 3, 6 and 12 min with an amplitude of 100% (specific energy between 625 and 2500 kJ/kg), pulse durations of 2 s on and 2 s off, frequency of 20 kHz and constant manual stirring.

The circulating water (at 4 °C) used to minimize the temperature increase during sonication and the AP temperature were monitored using K-type thermocouples inserted in one flask and in the AP during the treatment. After thermal treatment, the flasks were rapidly cooled and stored at  $3 \pm 1$  °C until used.

#### 2.4.3. Pulsed Electric Fields (PEF)

AP with an electrical conductivity of 5 mS/cm (at 25 °C) was placed in the PEF treatment chamber between electrodes and pasteurized. PEF treatments were performed using the PEF generator model EPULSUS\_LBM3B-15 (EnergyPulse Systems, Lisbon, Portugal) at TAGUSVALLEY in bipolar mode (8 µs pulse) and a frequency of 2 Hz at different electric field strengths (7, 10, and 12.5 kV/cm), numbers of pulses (5, 70 and 90), currents (70, 88 and 115 A) and specific energies (44, 378 and 604 kJ/kg).

The AP temperature was measured at the inlet (26 °C) and outlet of each treatment (max 57.2 °C) by K-type thermocouples. After the treatment, the product was rapidly cooled and stored at  $3 \pm 1$  °C until used.

#### 2.4.4. High-Pressure Processing (HPP)

One hundred grams of AP samples were packed and heat-sealed in 90 µm thick polyamide/polyethylene bags (PA/PE, 4PACK—Soluções para Embalagem, Jorge de Selho, Portugal with a metallic surface on the two sides to keep the product in the dark), using a packaging machine (Henkelman, Marlin 42, Henkelman, The Netherlands) and treated by HPP in an industrial scale unit (Hiperbaric 55, Hiperbaric S.A, Burgos, Spain) at University of Aveiro at 200, 300, 400, and 500 MPa for 1 min at 17 °C. Water was used as a pressurization fluid, at a rate of 3 MPa s<sup>-1</sup>, while decompression took less than 2 s.

### 2.5. Impact of the Processing Technologies' Evaluation

#### 2.5.1. Microbiological Analysis

The present study aimed to evaluate the impact of each processing technology on the inactivation of *E. coli* and indigenous microorganisms, namely the total mesophilic aerobic bacteria (TMAB) and yeasts and moulds (YM). TMAB were quantified according with the ISO 4833-1:2013/Amd 1:2022 [16] and YM followed the *Norma Portuguesa* NP-3277-2:1987 [17], while the *E. coli* quantification was performed according to ISO 16649-2:2001 [18].

The microbiological analyses were performed by serially diluting the AP. TMAB and YM counts were analysed before (raw control AP), after the treatments, and at regular intervals on AP samples stored at  $5 \pm 1$  °C, throughout the 30 days of storage.

A sample of AP (9 g) was diluted in a plastic sterile bag with 90 mL of saline peptone and homogenized in a stomacher (Model 400, Seward, London, UK) for 2 min at 230 rpm. To determine the TAMB counts of AP samples, homogenates were serially diluted and plated by the incorporation method on plate count agar (Biokar Diagnostics, Beauvois, France) followed by incubation at  $30 \pm 1$  °C for 3 days. YM were determined by surface-plating 0.1 mL of the homogenates on Glucose Chloramphenicol Agar (Biokar Diagnostics, Allonne, France) followed by incubation at  $25 \pm 1$  °C for 5 days. Regarding *E. coli*, they were determined by surface-plating 0.1 mL of the homogenate solution on Tryptone-Bile-X-Glucuronate Agar (TBX, Biokar Diagnostics, Allonne, France) followed by incubation at  $44 \pm 1$  °C for 24 h. After incubation, plates were counted, and the results were expressed as a logarithm of colony forming units per gram (log CFU/g).

### 2.5.2. Colour and Antioxidant Analyses

Before and immediately after each treatment, the colour and antioxidant activity of the AP were analysed. For colour parameters, the AP's hue alteration was determined using a colorimeter (Konica Minolta, CR-310, Tokyo, Japan). This apparatus measures the light received and converts it into space coordinates. These values are then translated to a  $L^*a^*b^*$  standard, where  $L^*$  represents lightness,  $a^*$  indicates the hue from green (−) to red (+), and  $b^*$  the hue from blue (−) to yellow (+). To determine the magnitude of colour variation ( $\Delta E^*$ ), between the colour parameter of the samples processed and the colour parameter of the control sample (unprocessed AP)  $L^* 39.5$ ,  $a^* 5.0$  and  $b^* 20.7$ , Equation (1) was applied.

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{0.5} \quad (1)$$

The antioxidant activity of the AP was assessed with two different methods: a method based on the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) and the radical cation of 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS). The DPPH radical scavenging assay was performed as described by Kim and colleagues [19]. Briefly, AP was centrifuged at  $4000 \times g$  for 10 min to separate the pulp from the juice (supernatant). Afterwards, 100 µL of the supernatant was added to 900 µL of a methanolic solution of DPPH ( $1.5 \times 10^{-4}$  M) and the mixture was vortexed and left to react in the dark for 30 min. Then, the absorbance of the reaction mixture was measured at 517 nm, using a microplate reader (Multiskan GO Microplate Spectrophotometer, ThermoScientific, ThermoFisher Scientific, Waltham, MA, USA). The antioxidant activity was obtained from a standard curve of Trolox and expressed as mmol of Trolox equivalent (TE) per 100 g of fresh weight sample. The ABTS radical scavenging assay was also performed as described by Kim and colleagues [19]. Succinctly, the ABTS<sup>+</sup> solution was prepared by reacting 7 mM of aqueous ABTS solution with 2.45 mM of potassium persulfate, at a ratio of 1:1 (V/V). Then, the solution was left for 16 h in the dark at 4 °C. Afterwards, the solution was diluted with a solution of 80% of ethanol/water (V/V) to obtain an absorbance of  $0.700 \pm 0.005$  at 734 nm. Next, 100 µL of the samples to be tested was added to 3.9 mL of ABTS solution, vortexed and incubated at room temperature for 6 min in the dark. After the incubation, the absorbance was measured at 734 nm using the aforementioned microplate reader. The antioxidant activity was obtained from a standard curve of Trolox and expressed as mmol of TE per 100 g of fresh weight sample.

### 2.6. Thirty-Day Storage Test

Samples were collected before, immediately after each treatment (0 days of storage) and every 10 days up throughout the refrigerated storage period (30 days, 5 °C and 70% relative humidity (RH)) to analyse native microorganisms (TMAB and YM) and inoculated *E. coli* loads. In addition, pH, water activity ( $a_w$ ), and total soluble content (TSS) values were measured at 0, 15 and 30 days of storage. The pH was determined by direct immersion of a pH electrode (C931, Consort, Turnhout, Belgium) in the AP, while TSS was measured using a refractometer (HI 96801, Hanna, Limena, Italy) at  $25 \pm 1$  °C and the results were

expressed as °Brix.  $a_w$  was measured using an  $a_w$  meter (Aqualab 4TE, Decagon Devices, Pullman, WA, USA) at  $25 \pm 1$  °C.

### 2.7. Data Evaluation

The results were compared by one and two-way Analysis of Variance (ANOVA). Tukey's test (at a significance level of 5%) was used as a post hoc test using the GraphPad Prism v6. Ink software. The data reported are expressed as the average of triplicate observations  $\pm$  standard deviation. The similarities/differences among treatments between parameters were assessed by Principal Component Analysis (PCA).

## 3. Results

### 3.1. Inactivation of *E. coli* and Selection of Equivalent Processing Conditions for Each Technology

To identify the best processing conditions required for each technology to achieve a 5-log *E. coli* reduction, AP samples were processed by CTP, US, PEF and HPP, with Figure 1 presenting the results.

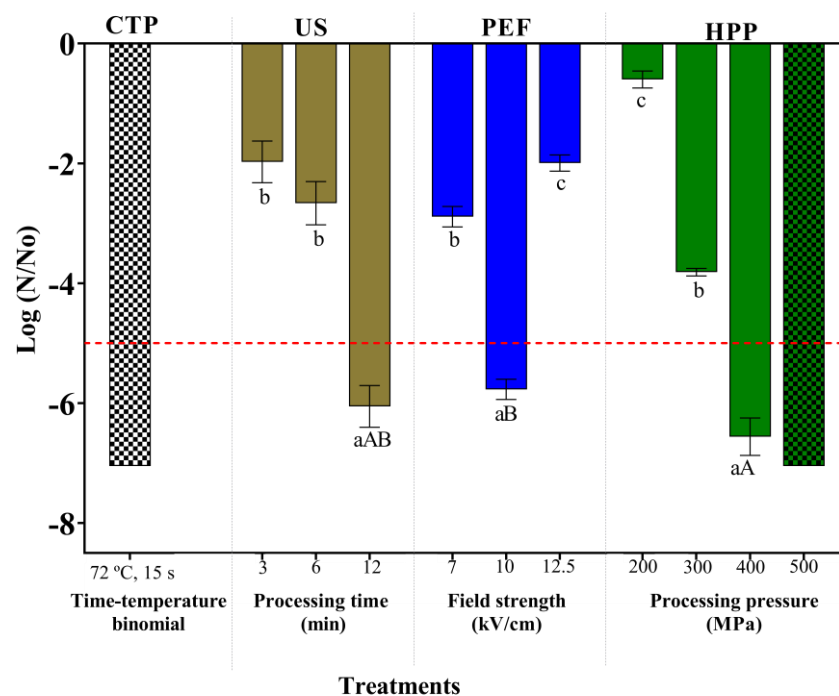


**Figure 1.** *E. coli* inactivation by thermal pasteurization (CTP), ultrasound (US), pulsed electric fields (PEF), and high-pressure processing (HPP) to identify the required treatment conditions to achieve a 5-log *E. coli* load reduction (represented by the dashed red line). Bars with different small letters indicate a significant difference ( $p < 0.05$ ) between the processing conditions for each technology. Bars with different capital letters indicate a significant difference ( $p < 0.05$ ) between the selected processing conditions. The bars with squared texture indicate that the detection limit (1.00 log CFU/g) was surpassed.

The initially inoculated *E. coli* load was  $8.05 \pm 0.50$  log CFU/g and its inactivation in the AP samples was observed for all technologies, in varying degrees: it generally increased with the increment in the processing conditions' intensity (except for PEF), which permitted selecting the processing conditions that met the reduction criteria of 5 log units. These results are shown in Figure 1.

The application of CTP (72 °C for 15 s) led to a decrease of at least 7.01 logarithmic units of *E. coli*, reducing the levels to a point below the detection limit (1.00 log CFU/g). The results demonstrate the impact of the treatment (20 kHz, 2 s on and 2 s off pulses, in a bath at 60 °C) on different processing times (3, 6, and 12 min). A significantly higher level of inactivation ( $6.06 \pm 0.25$  log units) was observed for the 12 min treatment, surpassing the 5-log reduction criteria with statistical significance ( $p < 0.05$ ). The results of PEF-treated samples at different field strength values (7, 10, and 12.5 kV/cm) are presented in Figure 1. Specifically, at a field strength of 10 kV/cm, with treatment conditions of 115 A, 604 kJ/kg, 70 bipolar pulses of 8 µs, and at a temperature of 57.2 °C, a reduction of  $5.77 \pm 0.12$  log units was observed. This reduction was significantly higher ( $p < 0.05$ ) compared to the other field strength values and exceeded the target of at least 5-log-unit inactivation of *E. coli*. Finally, the results likewise pertain to AP samples that were subjected to HPP treatments at various pressure levels (200, 300, 400, and 500 MPa) for a duration of 1 min. The rise in processing pressure led to a corresponding increase in inactivation levels, as anticipated. Both treatments at 400 and 500 MPa achieved reductions exceeding the 5-log target. The treatment at 500 MPa resulted in a decrease of at least 7.01 log units, bringing the levels below the detection limit. The CTP and HPP processing technologies demonstrated the highest levels of inactivation, surpassing the 5-log-unit reduction threshold, particularly at 500 MPa. Based on these results, the selected processing conditions were 12 min for US, 10 kV/cm of field intensity for PEF and a pressure level of 400 MPa for HPP. This pressure level was selected to provide equivalent inactivation results for the technologies. Given the previously mentioned information, the impacts of these equivalent processing technologies on selected physicochemical properties, antioxidant activities after processing and during cold storage were further evaluated.

### 3.2. Impact of the Selected Processing Conditions on the Characteristics of AP

#### 3.2.1. Colour

Colour and the magnitude of its variation (before and immediately after processing) were expressed through the  $L^*a^*b^*$  parameters. This was the physical parameter that showed the most differences amongst technologies, with the results presented in Figure 2.

The results of the  $L^*$  parameter (Figure 2A) revealed that US ( $47.46 \pm 0.66$ ) and CTP ( $43.52 \pm 1.17$ ) treatments significantly increased the  $L^*$  from the untreated AP control ( $39.91 \pm 1.81$ ).

Regarding the  $a^*$  parameter (Figure 2B), there was a decrease in CTP, US, and HPP-treated AP compared to untreated AP. The decrease was particularly significant for US ( $1.64 \pm 0.38$ ) compared to the untreated AP ( $5.80 \pm 0.88$ ). The third parameter,  $b^*$  (Figure 2C), exhibited a reduction in both the BL and treatment groups, in comparison to the control group ( $21.05 \pm 0.44$ ). The most significant impact was observed in the AP group treated with CTP ( $14.34 \pm 0.70$ ).

The  $\Delta E^*$  values for colour variation immediately after processing (Figure 2D) increased from  $3.42 \pm 0.62$  (BL) to  $10.10 \pm 0.20$  (US), with US exhibiting the highest variance. This result is in line with the expected variation in individual colour characteristics. The PEF treatment had the lowest  $\Delta E^*$  value of  $4.67 \pm 0.31$ . Figure 2E clearly demonstrate the variance in colour across the samples. In general, the CTP and PEF-processed AP samples have a brighter colour, whereas the US and HPP-processed AP samples tend to have a darker colour.

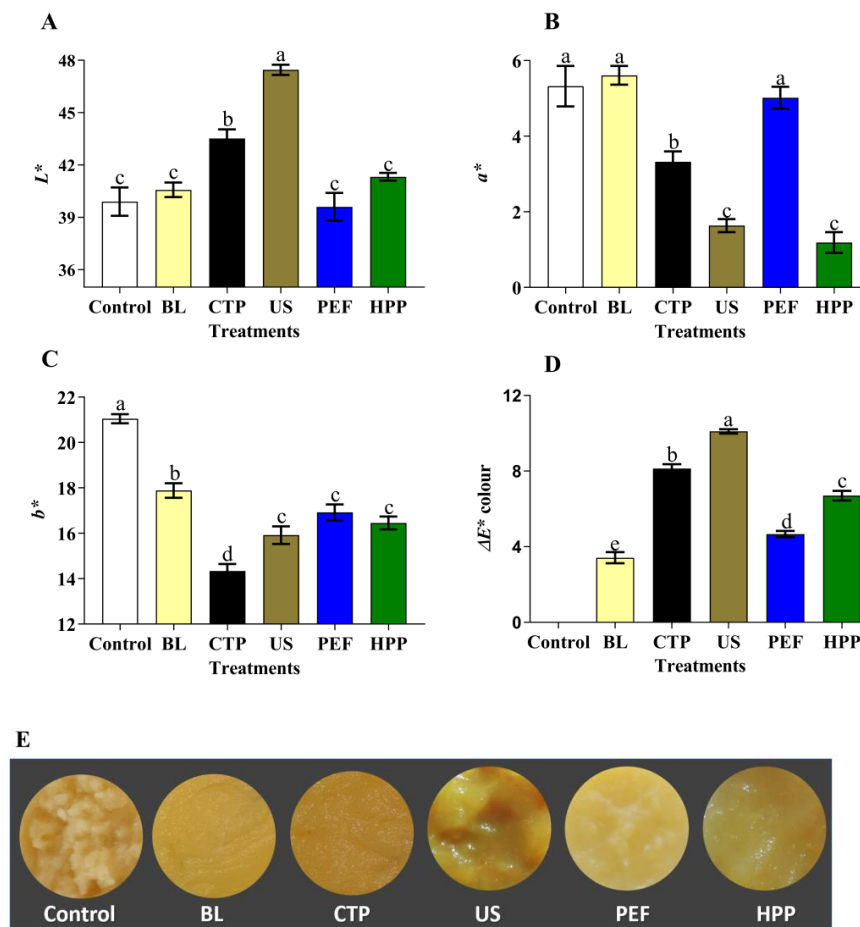


**Figure 2.** Instrumental colour parameters of AP before and immediately after treatment at the selected conditions for each technology. (A)  $L^*$  is lightness, (B)  $a^*$  varies from green to red, (C)  $b^*$  varies from blue to yellow, (D) total colour difference/variation ( $\Delta E^*$ ) and (E) visual appearance of final product. Different letters above the bars on the same graph represent significant statistical differences ( $p < 0.05$ ) between treatments.

### 3.2.2. Antioxidant Activity

The AP's antioxidant activity was assessed both before and immediately after the treatments using the DPPH and ABTS assays. The results are presented in Figure 3. Both assays showed comparable inhibitory patterns, as the treated AP samples exhibited a statistically significant increase in inhibition relative to the untreated or blanched AP. The DPPH antioxidant experiment revealed that BL significantly enhanced the antioxidant activity of AP by 33.4%, increasing it from  $0.195 \pm 0.016$  mmol TE/100 g to  $0.260 \pm 0.027$  mmol TE/100 g, as seen in Figure 3A. In general, all of the processing methods that were examined significantly improved the antioxidant activity. The results showed that CTP, US, and PEF had similar effects with statistical significance ( $p < 0.05$ ). Significantly, the DPPH scavenging activity of HPP-pasteurized samples increased by 66.7% ( $p < 0.05$ ) compared to BL samples, making it the highest among the technologies examined in this work. The increase in antioxidant activity resulting from HPP may be attributed to the extraction of antioxidant compounds from the AP plant cells, which were then dissolved in the AP [20]. The increase in PEF-mediated DPPH radical scavenging activity in other technologies may be attributed to the electroporation of plant cells, which leads to the extraction of antioxidants from the vacuoles [21]. On the other hand, in the case of US processing, the increase in scavenging activity may be linked to the cavitation phenomenon, which causes the disruption of plant cells and leakage of cell contents [22].

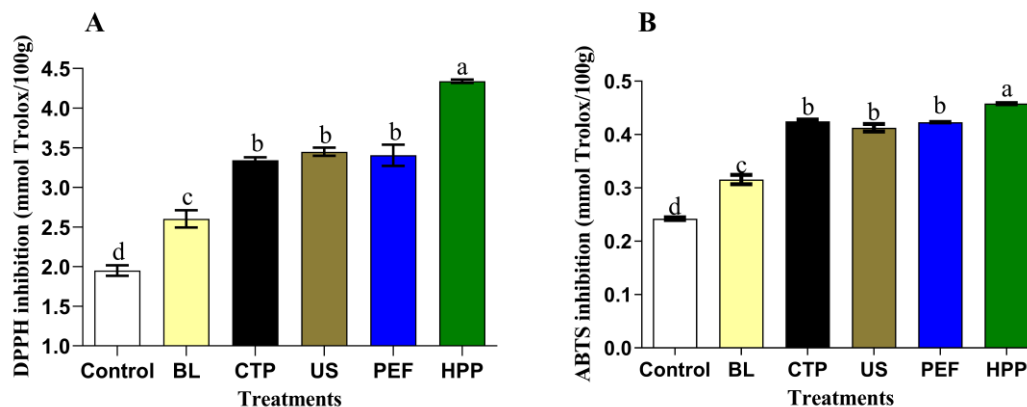


**Figure 3.** Antioxidant activities of the AP before and immediately after treatment at the selected conditions for each technology, using the DPPH (A) and ABTS (B) assays. Bars with different letters on the same graph indicate a significant difference ( $p < 0.05$ ) between each technology.

Regarding the ABTS assay, a similar scenario was found to that observed for the DPPH scavenging assay. The BL process per se increased the ABTS scavenging activity by 30.3% compared to the raw AP (Figure 3B). A general increase in the ABTS radical scavenging activity was observed, regardless of the pasteurization methodology, yet with different degrees of magnitude. In fact, the highest increase in antioxidant activity was observed for samples processed by HPP (58.8% compared to BL samples), while a smaller increase was observed for samples processed by CTP, US, and PEF, as seen in Figure 3, with an overall increase of 34.6, 34.2 and 45.3%, respectively, when compared to BL samples.

The antioxidant activity of pasteurized foods can indeed be affected by the presence of air in the food product, such as when it is stored in a container with empty space or vacuum sealed. The antioxidant activity of AP was quantified after exposing it to a temperature of 90 °C for 30 min, with and without the presence of oxygen. Compared to the fresh AP, the samples that were heated in the presence of oxygen exhibited a notable reduction in DPPH scavenging activity, decreasing from 0.71 to 0.68 g/kg TE (fresh weight). Similarly, in the ABTS assay, the activity decreased from 0.27 to 0.26 g/kg TE. Under anaerobic conditions, the outcomes were comparable to those of the recently obtained AP [23]. This outcome is predictable and can be ascribed to the probability of the generation of reactive oxygen species that can oxidize the antioxidants, so limiting their capacity to counteract free radicals. Consequently, the free radicals have the ability to further damage the constituents of food, resulting in a decrease in antioxidant capacity [24]. Odriozola-Serrano and colleagues (2022) [25] achieved a comparable outcome, observing a reduction in the DPPH scavenging activity of apple juice when subjected to thermal pasteurization (90 °C, 60 s) and high-intensity PEF (35 kV, 1700  $\mu$ s, 180 Hz) (0.41 and 0.45, respectively). Both of those circumstances contradict the conclusions of the current study, as it reported an overall increase in antioxidant activity across all processing conditions. The observed differences may arise from the specific processing conditions, apple variety, and level of ripeness.

### 3.3. Thirty-Day Storage Test

#### 3.3.1. Microbial Stability

To assess the microbial stability of the AP, a 30-day storage evaluation was performed, and the effectiveness of the treatments (at the previously selected processing conditions) for the inoculated *E. coli* and indigenous microorganisms (TMAB and YM) was measured for AP samples processed and stored for 30 days at 5 °C. Analyses were performed every 10 days and the TMAB and YM results are summarized in Figure 4.

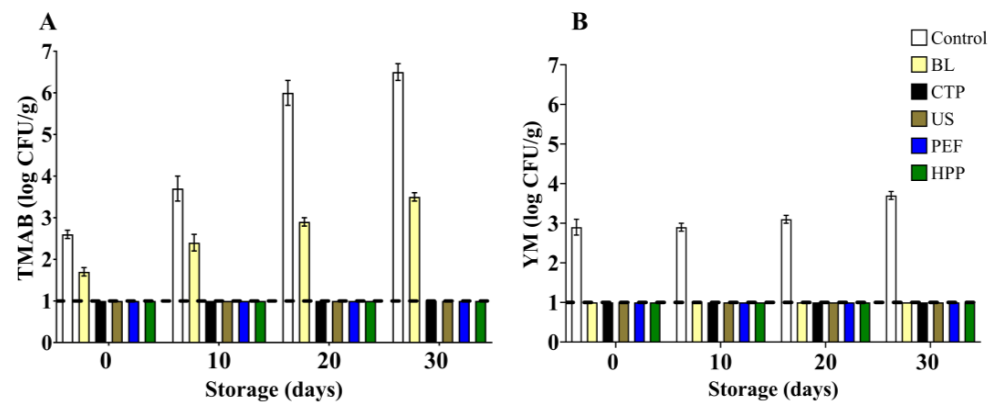


**Figure 4.** Evaluation of total mesophilic aerobic bacteria (TMAB) (A) and yeast and mould (YM) (B) counts in the AP samples before, immediately after each treatment at the selected conditions for each technology (0 storage day), and throughout 30 days of storage (5 °C). Bars under the dashed line represent counts below the detection limit of 1 log CFU/g.

As confirmed, *E. coli* was not detected in the treated AP samples immediately after each processing condition, as they resulted in a reduction of at least 5 logs. Furthermore, during the 30 days storage period, all treatments successfully prevented the growth of *E. coli*. Thus, the treated AP samples did not show any *E. coli* CFU during the cold storage study in all the treatments studied (all treatments studied reduced *E. coli* load below the minimum detection limit; 1.00 log CFU/g). These results revealed a clear positive effect of these treatments on microbial load reduction after processing and throughout 30 days of cold storage.

As expected, the control AP samples showed microbial growth over the 30-day storage period. The growth was around 3.9 log units for TMAB and 0.8 log units for YM. The BL technique successfully deactivated YM, and no instances of this microbiological group were found over the 30-day period. However, a small amount of TMAB remained after BL and was able to grow, reaching a level of  $3.5 \pm 0.1$  log CFU/g. The BL values for TMAB and YM indicated that pre-treatment alone had a minor impact on the microbiological safety of the AP. However, this method alone is insufficient to completely inactivate certain vegetative bacteria [26].

As expected, the selected processing conditions for all technologies were effective in ensuring AP's microbial safety, as counts for the TMAB and YM were undetectable right after processing and throughout the 30 days of storage evaluation.

### 3.3.2. Physicochemical Quality

The AP control sample (unprocessed) was only included at the beginning of storage (day 0), since the microbial load increased afterwards, as aforementioned in Section 3.3.1; thus, no further microbiological analyses were performed. Treated samples of AP (at selected processing conditions) were analysed in relevant physicochemical parameters to measure the impact of the treatments. The results are shown in Figure 5.

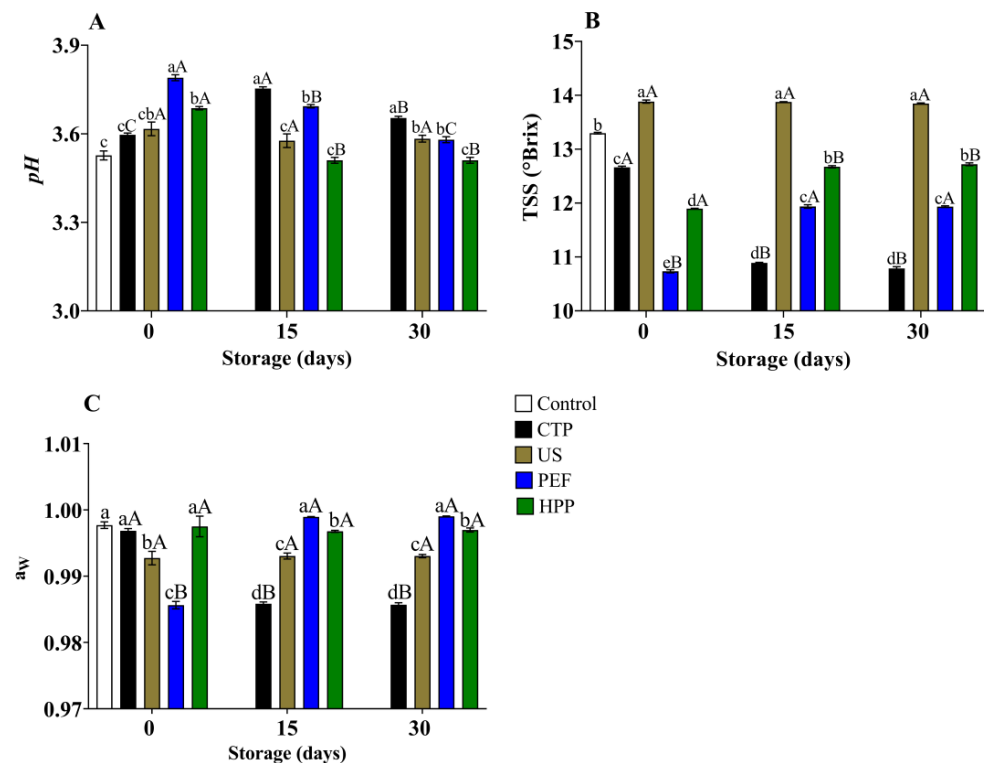


**Figure 5.** Physicochemical results of the AP before, immediately after each treatment at the selected conditions for each technology (0 storage day), and throughout 30 days of cold storage (5 °C). (A) pH, (B) total soluble solids, and (C)  $a_w$ . Bars with different lower-case letters indicate a significant difference between treatments at the same time point. Bars with different capital letters indicate a significant difference in the same treatment in different time points ( $p < 0.05$ ).

The pH values before and after processing, as well as during the 30-day storage study, are shown in Figure 5A. The control AP had a pH value of  $3.53 \pm 0.01$ , which did not change significantly after BL treatment ( $3.52 \pm 0.03$ ). However, the pH increased after the US, PEF, and HPP treatments, with the PEF treatment resulting in the highest increase to a pH of  $3.79 \pm 0.01$ . In the case of TSS (Figure 5B), there was a noticeable overall drop in all processing conditions, especially for PEF-treated AP samples, which decreased from  $13.27 \pm 0.09$  (control) to  $10.77 \pm 0.24$ . An exception was noted only for AP treated with the US, where the TSS increased significantly ( $p < 0.05$ ) to  $13.87 \pm 0.05$ . The  $a_w$  values obtained after processing showed minimal variation relative to the  $a_w$  of the control ( $0.9977 \pm 0.0004$ ), except for US and PEF, where statistically significant variations ( $p < 0.05$ ) were observed (Figure 5C). The AP treated with PEF exhibited the most significant variations, with a drop in  $a_w$  of  $0.9856 \pm 0.0004$ . However, these modifications, albeit tiny, are still quite mild, amounting to less than 1.3% when compared to the control AP.

Throughout the 30-day storage test, there were no significant fluctuations in the pH of the AP; however, there were minor alterations observed. The pH values for HPP ranged from  $3.51 \pm 0.01$  to  $3.81 \pm 0.01$  for US. Comparable patterns were noted for  $a_w$  levels, which likewise encountered localized small fluctuations. The TSS values exhibited varying patterns over the 30-day refrigerated storage period, depending on the specific pasteurization process used. The total soluble solids (TSS) decreased ( $p < 0.05$ ) after 30 days of storage at 5 °C for CTP (from  $13.30 \pm 0.02$  (control) to  $10.79 \pm 0.03$ ). However, no changes in TSS were found for the US samples throughout storage. Observations were made about the increase in TSS after 15 days in relation to HPP and PEF. The observed results can be attributed to the damage caused to the plant cells in the aerial part by the processing methods, such as electroporation and the creation of fractures. This damage leads to

the release of previously trapped chemicals that have been dissolved in the surrounding media [27].

On the other hand, the PCA performed explained the variability of the dataset among the samples and evaluation days. The loading plot shows the distribution of chemical properties (Figure 6A) while the score plot of the experimental samples (Figure 6B) reveals four clusters which explain that the assessed chemical properties had a marked impact on the quality of AP samples. The results indicated that 89.74% of the total variance was explained through this analysis (eigenvalues > 1), namely, CP1 represented 70.69% of the relationship between the samples and chemical features, whereas CP2 explained 19.05% of the total variation.

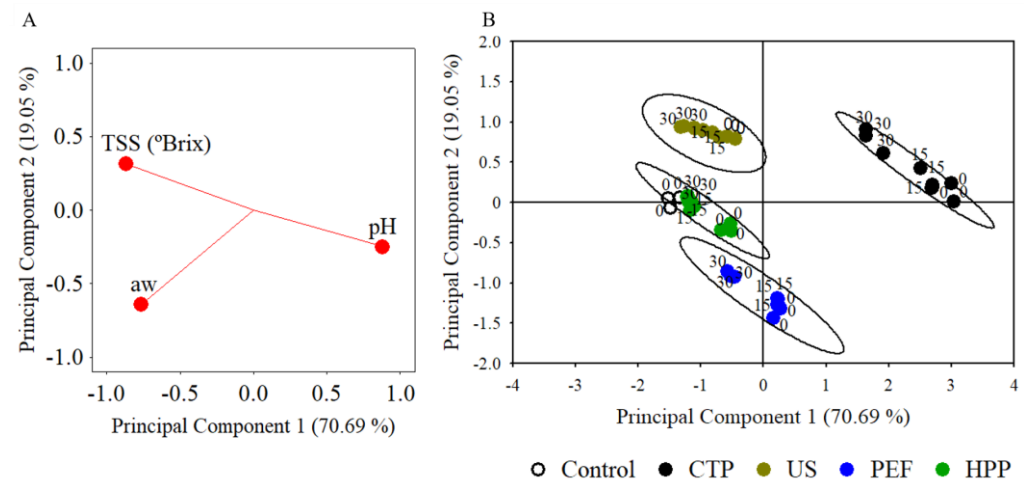


**Figure 6.** Loading plots (A) and PCA score (B) for differentiation of untreated and treated AP with equivalent processing conditions selected for each technology, based on chemical characteristics during storage.

As presented in Figure 6B, except for CTP, most of the treatments are located on the central axis like the positions of the control method. The simultaneous evaluation of Figure 6 plots indicates that treated samples located close to each other in the score plot present similar attributes, in terms of the chemical properties, given the corresponding region of the loading plot. It indicated that nonthermal technologies retained the most similarity of these parameters when compared to the control treatment at the beginning of the cold storage study (day 0). The CTP sample was separated on the positive side of Prin 1 due to the higher difference to the control treatment, which could be related to the impact of the temperature used on these properties. Likewise, HPP samples collected after 30 days were the most similar to the control samples.

#### 4. Discussion

The contamination of fresh produce is common during harvesting, handling, and processing of fruits and vegetables, causing great concern among fruit processors. Even though several strategies are used to minimize contamination, such as hygienic handling and washing processes, it remains a challenge to minimize the introduction of both spoilage and pathogenic microorganisms [28]. Additionally, in spite of the conventional thermal processes' ability to ensure a desirable level of food safety, the quality of the resultant food products, like pulps and purees, is sometimes compromised. Thus, proper screening and selection of processing conditions is of utmost importance to balance food quality and safety [29].

In this sense, emergent processing technologies that allow us to reduce or even disregard the use of heat have been widely studied as a potential replacement for the conventional thermal pasteurization processes [30]. Thus, it is pertinent to evaluate the potential of

emergent processing technologies to meet the pasteurization status of foods and evaluate the impact of the selected processing conditions on the overall quality of foods.

#### 4.1. Inactivation of *E. coli* and Selection of Equivalent Processing Conditions for Each Technology

*E. coli* O157:H7 is a pathogenic bacterium commonly present in apple and apple processing environments. It is known to have the ability to tolerate acidic conditions and thrive in refrigerated conditions [31]. The chosen pasteurization methods were able to effectively inactivate *E. coli* by at least 5 log units, which is the minimal level of inactivation necessary to attain pasteurization status. Although apple products are typically pasteurized at higher temperatures and for longer durations to eliminate thermophilic bacteria like *Alicyclobacillus* spp., the flash pasteurization method employed in this study successfully inactivated *E. coli* by more than 5 log units.

This could allow us to reduce the processing temperature and time of apple products, if they are kept under refrigeration conditions, as some thermoresistant bacteria and spores could develop [32].

Concerning the use of ultrasound (US) for pasteurization purposes, specifically in relation to targeting *E. coli*, there are reports indicating that this technique is capable of inactivating at least 5 log units. However, it is important to note that this effectiveness is achieved when accompanied by moderate temperatures. Ugarte-Romero and colleagues demonstrated a significant decrease in *E. coli* K12 loads in apple cider treated with ultrasound (20 kHz and 0.46 W/mL of acoustic energy density). The reduction was at least 5 logs and occurred after 5, 10, and 15 min of processing at temperatures of 60, 55, and 50 °C, respectively [33]. In a separate investigation, Baboli and colleagues [34] examined the viability of utilizing ultrasound (US) with specific parameters (5 s on, 30 s off, 24 kHz, at 60 °C and 45% amplitude) for the pasteurization of apple juice and pulp. The findings indicated that this method also met the requirement of achieving a 5-log reduction, which was not attainable through conventional pasteurization at 60 °C. This study showcases the feasibility of integrating US (ultrasound) technology with moderate temperatures (60 °C) for the purpose of pasteurizing food. This combination enables shorter processing times and reduces the amount of heat exposure, resulting in pasteurized foods that possess enhanced nutritional and sensory qualities.

At selected processing conditions, PEF was the processing technology used in this study with the lowest impact on *E. coli* loads (regardless of achieving the 5-log threshold), and the electric field strength selected was in the mid-range of the ones tested (i.e., the electric field strength varied between 7 and 12.5 kV, and the highest inactivation level was observed for a field strength of 10 kV). The reader may find this result rather curious, as usually more intense processing conditions yield higher inactivation rates, but this did not happen in the present study. It is possible that a synergistic effect between the applied electric field strength and the processing time led to an overall temperature rise of about 31 °C (from an initial temperature 26 to a final temperature 57 °C after processing) and greater inactivation effects of PEF, as observed by Yan and colleagues (2021), who reported that the inactivation of *E. coli* was proportional to the increase in the set processing temperature, i.e., higher inactivation levels were observed at higher temperatures when compared to conventional PEF at room temperature [35].

The inactivation of *E. coli* O157:H7 on Granny Smith apple peels subjected to microwave for 35 s at 652 W that achieved an average peak temperature of 75.3 °C was examined. These operational parameters are akin to the time–temperature profiles utilized in the CTP process. The results revealed a reduction in microbial load by 1.01 log CFU/g from an initial concentration of 7.35 log CFU/g, indicating the significantly diminished efficacy of microbial inactivation compared to the reduction of 7.52 log CFU/g reported in the current investigation [36].

#### 4.2. Impact of Each Equivalent Processing Condition on Physicochemical Parameters

As mentioned before, AP samples were tested at different processing conditions and physicochemical parameters were analysed for the untreated (control) and treated AP. These results can be found in Figure 2 (for the colour) and Figure 6 (for the other parameters).

When comparing the  $L^*a^*b^*$  parameters for the HPP treatment to the thermal-based treatments used (BL and CTP), the HPP values either do not show a significant difference or are smaller, apart from a significant effect on the  $b^*$  variable, when the HPP value was bigger than the CTP, but not BL. These results are comparable to those obtained by Rinaldi and colleagues that found lower  $a^*$  and  $b^*$  values for HPP-treated AP (600 MPa, 3 min) than the conventional thermal treatment (35 s at 107 °C) [37]. However, these results differ from those of another study where the addition of inulin (8%  $w/w$ ) to AP, together with thermal processing (a time temperature equivalent of  $P_{90} \geq 10$  min to accomplish a procedure similar to a 6-log reduction of vegetative cells), resulted in lower  $L^*a^*b^*$  values compared to HPP AP [38]. However, in contrast to the current study where the rise in the  $L^*$  parameter did not have statistical significance, another study (using Golden Delicious AP) observed a significant increase in that variable (by 4.65) [39].

Other study that measured the effects of heating for 30 min at 90 °C with or without oxygen noted a decrease in the  $L^*$  variable for both samples of Fuji AP, a decrease in the  $a^*$  variable for heating in the presence of oxygen and an increase in the  $a^*$  variable when samples were heated without oxygen, in comparison to the fresh AP values. These results differ from those seen in the present study for the  $L^*$  variable (control value of 39.91) but in relation to the CTP value of the  $a^*$  variable, a decrease was also observed, when compared to the untreated AP sample [23]. In another work, a significant increase in the  $L^*$  parameter was found for a mild thermal treatment (99 °C, 1 min) and a standard (92 °C, 5 min) thermal treatment of 5.52 and 6.68, respectively, when compared to the control ( $L^*$  of 43.40) [39].

Other thermal treatments like ohmic and microwave have also been tested for AP. A similar trend was observed by Nistor and colleagues, which measured the effects of an ohmic treatment on the colour of Golden Delicious AP and observed a decrease in the  $L^*$  values but no statistically significant change was observed for the  $a^*$  and  $b^*$  variables [40]. Other recent ohmic work showed differences between the thermal treatments (conventional and ohmic) and the control, as the thermally treated AP showed lower  $L^*$  and higher  $a^*$  values but for the  $b^*$  parameter, the conventional thermal treatment (35 s at 107 °C) remained similar to the control, as opposed to the decrease in value observed for the ohmic treatment (flow rate of 2000 L/h, at 107 °C) [37].

In addition, two studies analysed microwave treatments on different apple varieties and observed similar outcomes. The first study, using Idared and Shampion AP, found significant reductions in the  $L^*$  and  $b^*$  variables after a 2 min microwave treatment at 80 °C, compared to the control group that was heated for 4 min at the same temperature. However, for the  $a^*$  variable, while Idared AP showed a decrease, Shampion AP exhibited an increase in value [41]. The second study, using Granny Smith AP, applied a shorter treatment (35 s, 652 W with a maximum temperature of 75.3 °C) and observed changes in colour, including a decrease in  $L^*$ , an increase in  $a^*$ , and a non-significant decrease in  $b^*$ . These results differ from those obtained in the present study using Reineta Parda AP [36]. The observed variations may be attributed to changes in apple cultivars, differences in heat treatments, and the comparatively milder thermal treatments used in this study in terms of both duration and temperature, as compared to previous research.

The  $\Delta E^*$  obtained in the previously mentioned work by Rinaldi and colleagues [37] was at the lowest for the HPP-treated AP samples, followed by the ohmic and the conventional thermal treatment, which is similar to what was observed by Salazar-Orbea and colleagues in their work with Golden Delicious AP treated by HPP (6 bar, 1 min at 4 °C), a mild thermal treatment (99 °C, 1 min) and a standard treatment (92 °C, 5 min). The authors noted that the  $\Delta E^*$  was the lowest for HPP, followed by the mild and the standard thermal treatments, respectively [39]. These results partially coincide with the findings of the present study, where HPP-treated AP showed a lower  $\Delta E^*$  for HPP when compared to

the CTP-treated AP but the BL-treated AP was the lowest of all the treatments, perhaps due to its effect on inactivating enzymes like polyphenol oxidase, which are involved in enzymatic browning starting immediately after the mechanical damage induced to the cells during homogenization [42].

Regarding the effects PEF on colour attributes, Geicu et al. [43] observed that AP produced from the Golden Delicious variety, subjected to PEF treatment at 20 to 30 kV and a frequency of 800 Hz, featuring pulse fronts between 50 and 200 ns and a pulse duration of 1 ms, exhibited no immediate alterations in colour post-treatment. This outcome is consistent with untreated control samples, corroborating the findings of the current research. Similar findings were reported by Wibowo and colleagues [15] for apple juice processed by PEF (12.3 kV/cm, 94 Hz, 132.5 kJ/L and 12.5 kV/cm, 62 Hz and 76.4 kJ/L) who compared the processing conditions with thermal pasteurization (72 °C/15 s and 80 °C for 30 s) and HPP (400 and 600 MPa for 3 min). In terms of impact on quality attributes such as colour, cloud stability, taste, and aroma, severe thermal processing (85 °C/15 s) produced the brightest colour compared to the other processing conditions [15].

The stability of colour attributes in fruit juices and pulps is heavily influenced by various factors, including the ripening stage of the fruit, pH levels, processing temperature (which affects both Maillard and caramelization reactions), indigenous enzymes (such as polyphenol-oxidase and peroxidase), and exposure to oxygen. Therefore, it is crucial to take into account these elements when establishing processing conditions for fruit juices and pulps. However, the impact of these factors can be managed through the use of additions, such as ascorbic acid (often referred to as vitamin C) or other substances [44].

When considering the AP pH values reported in the literature, the pH value of the control Reineta Parda AP ( $3.53 \pm 0.01$ ) was higher than those reported for Granny Smith AP (3.2 [36] and 3.4 [45]), similar to Braeburn and Gold Delicious AP (3.6 and 3.7, respectively [45]) and lower than an industrial AP (11 °Brix) and a thermal treated AP (3.86 [46] and 3.85–3.90 [37], respectively).

Except for the PEF-treated AP samples (10.77 °Brix), the TSS values obtained fit well within reported AP TSS values that range from 11 °Brix (industrial AP) [46] to 17.4 °Brix (Golden Delicious AP) [47] for different apple cultivars, including Braeburn, Golden Delicious, Golden Smoothee, Granny Smith and Royal Gala [36,37,45,47]. A study tested the physicochemical properties of Bramley's seedling AP enriched with two prebiotics (8% *w/w*) (inulin and fructooligosaccharides) immediately after processing (thermal and HPP) and during storage (30 days at 4 °C). After 30 days, no change in pH was observed for the treated and untreated samples for AP containing either prebiotic. However, for TSS, some differences were noticed. TSS was affected by the type of processing in the case of the samples with inulin, as the thermally processed samples had significantly higher TSS values than the HPP samples (500 MPa for 90 s at  $\approx 20$  °C). In addition, a decrease in the TSS of HPP-treated samples after 30 days of storage was observed [38].

#### 4.3. Impact of Each Equivalent Processing Condition on the Antioxidant Activity

Antioxidant compounds play a major role protecting cells from reactive oxygen species, which can contribute to the development of chronic diseases or other disorders. Apples are a reliable source of antioxidant compounds such as quercetin, catechin, chlorogenic acid, and phloridzin that have been associated with several health benefits (lower changes in heart diseases, cancer, diabetes, etc.) [48]. However, it is noteworthy that the antioxidant content of apples may vary depending on the apple cultivar, growing conditions, and, ultimately, processing methods.

The antioxidant capacity of apple puree, as determined through DPPH and ABTS assays, has been addressed in several papers. The antioxidant activity of Reineta Parda AP produced in this study was  $0.195 \pm 0.02$  mmol/100 g Trolox, surpassing that of the Idared variety (0.16 mmol Trolox/100 g) [41], yet not reaching the levels found in the Champion variety (0.40 mmol Trolox/100 g) [41]. Such variability in antioxidant activity could be attributed to multiple factors including the diversity and quantity of phytochemicals

present, differences in total phenolic content, and the distinct structural and functional properties of these phytochemicals across various apple varieties [49]. Apples are notably rich in polyphenolic compounds such as flavonoids and phenolic acids, renowned for their antioxidative properties. Nonetheless, the polyphenol profile, including the content and composition, can vary significantly among different apple varieties, influenced by factors such as the cultivar, stage of ripeness, and environmental conditions. The structural variations in flavonoids are also known to affect their antioxidative potency [50].

Regarding the ABTS assay, the antioxidant capacity of Reineta Parda apple puree was measured at  $0.242 \pm 0.006$  mmol Trolox/100 g, markedly lower than the values recorded for purees from Granny Smith (7.35 mmol Trolox/100 g) [51], Gold Rush (12.78 mmol Trolox/100 g) [51], Idared (0.54 mmol Trolox/100 g) [41], and Shampion (1.27 mmol Trolox/100 g) [41] varieties. This discrepancy could be ascribed to the same factors mentioned earlier, highlighting the significant impact of phytochemical diversity, phenolic content, and the specific properties of these compounds on the antioxidant potential of different apple cultivars.

For both DPPH and ABTS, the increase in the antioxidant activity of AP right after BL is remarkable, and it increased even more after each processing condition. This may be attributed to an increased extraction (due to the disruption of cell walls and release of cellular content to the external media) and solubilization of compounds with antioxidant capacity, which will ultimately lead to an increment in the antioxidant activity.

These results highlight that these processing technologies not only can be used to ensure food safety, but also to enhance the bioavailability of antioxidant compounds, which are known to have key health-promotion abilities and could allow the formulation of functional foods [52]. Additionally, as AP can be used as an ingredient for other food products, enhancing its antioxidant activity with these emergent technologies can considerably contribute to the development of other apple-based functional foods, like smoothies, yoghurts, fruit jams, etc.

#### 4.4. Thirty-Day Storage Test

Integrating novel processing techniques with extracts possessing antimicrobial and nutraceutical properties presents a promising strategy to enhance the shelf-life of processed foods. Additionally, this approach may allow for the moderation of processing intensities, potentially yielding improvements in the quality of the end products [53].

After a quick microwave treatment (35 s, 652 W, 75.3 °C of maximum temperature), a study with Granny Smith AP stored the samples up to 14 days at 5 °C. *E. coli* O157:H7 and total aerobic mesophilic microorganisms (TAM) were quantified during this period. The results were distinct: for *E. coli* O157:H7, a progressive decrease was observed, reaching a final load of 1.28 log CFU/g from the 6.34 log CFU/g obtained after the microwave treatment. In relation to TAM, counts remained stable until day 8, followed by a slight increase, quantified at the 14th day, reaching 2.16 log CFU/g (started at 1.70 log CFU/g) [36]. These results differ from those obtained in the present study for *E. coli* (here used as a surrogate for *E. coli* O157:H7), which after the treatments remained with counts below the detection limit during the 30 days of storage; and for TMAB, for which growth was determined in the untreated and the blanched AP samples, unlike the other treatments, whose counts remained undetectable in the 30-day storage evaluation.

## 5. Conclusions

HPP, US and PEF treatments can effectively pasteurize Reineta Parda AP, guaranteeing its refrigerated shelf-life for at least 30 days while maintaining its quality and safety. HPP at 400 MPa for 1 min, US for 12 min, and PEF at 10 kV/cm resulted in reductions in *E. coli* in AP by at least 5 log units, meeting the Food and Drug Administration requirements for fruit processing, and the pasteurization status. The nonthermal treatments (HPP and PEF) retained better antioxidant activity than the CTP. Therefore, HPP and PEF can be used as a potential alternative for Reineta Parda AP pasteurization, resulting in a minimally

processed, value-added product that can guarantee the product's safe consumption while keeping fresh-like properties and enhanced antioxidant activity, which could be used, for example, for marketing and branding purposes.

Based on the encouraging results of this study, additional research is required into the industrial implementation of these technologies either alone or used in combination with each other, as well as an effective and deep analysis of the energetic costs associated with each processing technology. Moreover, this work not only provides insights into the use of these technologies in AP, but also underscores their practical significance, offering valuable guidance for stakeholders involved in the production and commercialization of other types of food products.

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**Data Availability Statement:** The raw data supporting the conclusions of this article will be made available by the authors on request.

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## References

1. Food and Agriculture Organization of the United Nations. FAOSTAT. 2023. Available online: <https://www.fao.org/faostat/en/#home> (accessed on 7 July 2023).
2. Fărcaș, A.C.; Socaci, S.A.; Chiș, M.S.; Dulf, F.V.; Podea, P.; Tofană, M. Analysis of Fatty Acids, Amino Acids and Volatile Profile of Apple By-Products by Gas Chromatography-Mass Spectrometry. *Molecules* **2022**, *27*, 1987. [CrossRef] [PubMed]
3. Maria Helene Giovanetti, C.; Alessandro, N.; Carmen Lúcia de Oliveira, P.; Gilvan, W. Characterization of Apple Pectin—A Chromatographic Approach. In *Chromatography*; Leonardo de Azevedo, C., Ed.; IntechOpen: Rijeka, Croatia, 2012; p. 14.
4. Lan, W.; Bureau, S.; Chen, S.; Leca, A.; Renard, C.M.G.C.; Jaillais, B. Visible, near- and mid-infrared spectroscopy coupled with an innovative chemometric strategy to control apple puree quality. *Food Control* **2021**, *120*, 107546. [CrossRef]
5. Techakanon, C.; Sirimuangmoon, C. The Effect of Pasteurization and Shelf Life on the Physicochemical, Microbiological, Antioxidant, and Sensory Properties of Rose Apple Cider during Cold Storage. *Beverages* **2020**, *6*, 43. [CrossRef]
6. Xiao, H.-W.; Pan, Z.; Deng, L.-Z.; El-Mashad, H.M.; Yang, X.-H.; Mujumdar, A.S.; Gao, Z.-J.; Zhang, Q. Recent developments and trends in thermal blanching—A comprehensive review. *Inf. Process. Agric.* **2017**, *4*, 101–127. [CrossRef]
7. Landl, A.; Abadias, M.; Sárraga, C.; Viñas, I.; Picouet, P.A. Effect of high pressure processing on the quality of acidified Granny Smith apple purée product. *Innov. Food Sci. Emerg. Technol.* **2010**, *11*, 557–564. [CrossRef]
8. Bevilacqua, A.; Petrucci, L.; Perricone, M.; Speranza, B.; Campaniello, D.; Sinigaglia, M.; Corbo, M.R. Nonthermal Technologies for Fruit and Vegetable Juices and Beverages: Overview and Advances. *Compr. Rev. Food Sci. Food Saf.* **2018**, *17*, 2–62. [CrossRef] [PubMed]
9. Zhang, Z.-H.; Wang, L.-H.; Zeng, X.-A.; Han, Z.; Brennan, C.S. Non-thermal technologies and its current and future application in the food industry: A review. *Int. J. Food Sci. Tech.* **2019**, *54*, 1–13. [CrossRef]

10. Tian, X.; Yang, Y.; Zhang, W.; Wang, Y. Effect of High Hydrostatic Pressure and Thermal Processing on the Shelf Life and Quality Attributes of Apple-Kiwi-Carrot Puree Blend. *J. Food Qual.* **2023**, *2023*, 1631285. [\[CrossRef\]](#)
11. Sulaiman, A.; Farid, M.; Silva, F.V. Strawberry puree processed by thermal, high pressure, or power ultrasound: Process energy requirements and quality modeling during storage. *Food Sci. Technol. Int.* **2017**, *23*, 293–309. [\[CrossRef\]](#)
12. Geveke, D.J.; Aubuchon, I.; Zhang, H.Q.; Boyd, G.; Sites, J.E.; Bigley, A.B.W. Validation of a pulsed electric field process to pasteurize strawberry purée. *J. Food Eng.* **2015**, *166*, 384–389. [\[CrossRef\]](#)
13. Yildiz, S.; Pokhrel, P.R.; Unluturk, S.; Barbosa-Cánovas, G.V. Identification of equivalent processing conditions for pasteurization of strawberry juice by high pressure, ultrasound, and pulsed electric fields processing. *Innov. Food Sci. Emerg.* **2019**, *57*, 102195. [\[CrossRef\]](#)
14. National Advisory Committee on Microbiological Criteria for Foods. Requisite Scientific Parameters for Establishing the Equivalence of Alternative Methods of Pasteurization†. *J. Food Prot.* **2006**, *69*, 1190–1216. [\[CrossRef\]](#)
15. Wibowo, S.; Essel, E.A.; De Man, S.; Bernaert, N.; Van Droogenbroeck, B.; Grauwet, T.; Van Loey, A.; Hendrickx, M. Comparing the impact of high pressure, pulsed electric field and thermal pasteurization on quality attributes of cloudy apple juice using targeted and untargeted analyses. *Innov. Food Sci. Emerg. Technol.* **2019**, *54*, 64–77. [\[CrossRef\]](#)
16. ISO 4833-1:2013/Amd 1:2022; Microbiology of the Food Chain—Horizontal Method for the Enumeration of Microorganisms. Part 1: Colony Count at 30 °C by the Pour Plate Technique. International Organization for Standardization: Geneva, Switzerland, 2022.
17. NP 3277-2:1987; Microbiologia Alimentar Contagem de Bolores e Leveduras. Parte 2: Incubação a 37 °C. Norma Portuguesa: Caparica, Portugal, 1988.
18. ISO 16649-2:2001; Microbiology of Food and Animal Feeding Stuffs—Horizontal Method for the Enumeration of Beta-glucuronidase-positive *Escherichia coli*. Part 2: Colo-ny-count Technique at 44 Degrees C Using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide. International Organization for Standardization: Geneva, Switzerland, 2001.
19. Kim, A.-N.; Kim, H.-J.; Kerr, W.L.; Choi, S.-G. The effect of grinding at various vacuum levels on the color, phenolics, and antioxidant properties of apple. *Food Chem.* **2017**, *216*, 234–242. [\[CrossRef\]](#) [\[PubMed\]](#)
20. Fernández-Jalao, I.; Sánchez-Moreno, C.; De Ancos, B. Effect of high-pressure processing on flavonoids, hydroxycinnamic acids, dihydrochalcones and antioxidant activity of apple ‘Golden Delicious’ from different geographical origin. *Innov. Food Sci. Emerg. Technol.* **2019**, *51*, 20–31. [\[CrossRef\]](#)
21. Ranjha, M.M.A.N.; Kanwal, R.; Shafique, B.; Arshad, R.N.; Irfan, S.; Kieliszek, M.; Kowalczewski, P.L.; Irfan, M.; Khalid, M.Z.; Roobab, U.; et al. A Critical Review on Pulsed Electric Field: A Novel Technology for the Extraction of Phytoconstituents. *Molecules* **2021**, *26*, 4893. [\[CrossRef\]](#) [\[PubMed\]](#)
22. Abid, M.; Jabbar, S.; Wu, T.; Hashim, M.M.; Hu, B.; Lei, S.; Zhang, X.; Zeng, X. Effect of ultrasound on different quality parameters of apple juice. *Ultrason. Sonochem.* **2013**, *20*, 1182–1187. [\[CrossRef\]](#) [\[PubMed\]](#)
23. Kim, A.N.; Lee, K.Y.; Rahman, M.S.; Kim, H.J.; Kerr, W.L.; Choi, S.G. Thermal treatment of apple puree under oxygen-free condition: Effect on phenolic compounds, ascorbic acid, antioxidant activities, color, and enzyme activities. *Food Biosci.* **2021**, *39*, 100802. [\[CrossRef\]](#)
24. Munteanu, I.G.; Apetrei, C. Analytical Methods Used in Determining Antioxidant Activity: A Review. *Int. J. Mol. Sci.* **2021**, *22*, 3380. [\[CrossRef\]](#)
25. Odriozola-Serrano, I.; Bellí, G.; Puigpinós, J.; Herrero, E.; Martín-Belloso, O. Screening the Antioxidant Activity of Thermal or Non-Thermally Treated Fruit Juices by In Vitro and In Vivo Assays. *Beverages* **2022**, *8*, 36. [\[CrossRef\]](#)
26. Wicaksono, W.A.; Buko, A.; Kusstatscher, P.; Sinkkonen, A.; Laitinen, O.H.; Virtanen, S.M.; Hyöty, H.; Cernava, T.; Berg, G. Modulation of the food microbiome by apple fruit processing. *Food Microbiol.* **2022**, *108*, 104103. [\[CrossRef\]](#)
27. Xiang, B.; Sundararajan, S.; Mis Solval, K.; Espinoza-Rodezno, L.; Aryana, K.; Sathivel, S. Effects of Pulsed Electric Fields on Physicochemical Properties and Microbial Inactivation of Carrot Juice. *J. Food Process. Pres.* **2014**, *38*, 1556–1564. [\[CrossRef\]](#)
28. Fishburn, J.D.; Tang, Y.; Frank, J. Efficacy of various consumer-friendly produce washing technologies in reducing pathogens on fresh produce. *Food Prot. Trends* **2012**, *32*, 456–466.
29. Ling, B.; Tang, J.; Kong, F.Y.; Mitcham, E.J.; Wang, S. Kinetics of Food Quality Changes during Thermal Processing: A Review. *Food Bioprocess Technol.* **2015**, *8*, 343–358. [\[CrossRef\]](#)
30. Rocha, C.d.S.; Magnani, M.; Ramos, G.L.d.P.A.; Bezerril, F.F.; Freitas, M.Q.; Cruz, A.G.; Pimentel, T.C. Emerging technologies in food processing: Impacts on sensory characteristics and consumer perception. *Curr. Opin. Food Sci.* **2022**, *47*, 100892. [\[CrossRef\]](#)
31. Price, S.B.; Wright, J.C.; DeGraves, F.J.; Castanie-Cornet, M.P.; Foster, J.W. Acid resistance systems required for survival of *Escherichia coli* O157:H7 in the bovine gastrointestinal tract and in apple cider are different. *Appl. Environ. Microbiol.* **2004**, *70*, 4792–4799. [\[CrossRef\]](#) [\[PubMed\]](#)
32. Zhao, D.; Barrientos, J.U.; Wang, Q.; Markland, S.M.; Churey, J.J.; Padilla-Zakour, O.I.; Worobo, R.W.; Kniel, K.E.; Moraru, C.I. Efficient Reduction of Pathogenic and Spoilage Microorganisms from Apple Cider by Combining Microfiltration with UV Treatment. *J. Food Prot.* **2015**, *78*, 716–722. [\[CrossRef\]](#) [\[PubMed\]](#)
33. Ugarte-Romero, E.; Feng, H.; Martin, S.E.; Cadwallader, K.R.; Robinson, S.J. Inactivation of *Escherichia coli* with Power Ultrasound in Apple Cider. *J. Food Sci.* **2006**, *71*, E102–E108. [\[CrossRef\]](#)
34. Baboli, Z.M.; Williams, L.; Chen, G. Rapid Pasteurization of Apple Juice Using a New Ultrasonic Reactor. *Foods* **2020**, *9*, 801. [\[CrossRef\]](#) [\[PubMed\]](#)

35. Yan, Z.; Yin, L.; Hao, C.; Liu, K.; Qiu, J. Synergistic effect of pulsed electric fields and temperature on the inactivation of microorganisms. *AMB Exp.* **2021**, *11*, 47. [\[CrossRef\]](#)
36. Picouet, P.A.; Landl, A.; Abadias, M.; Castellari, M.; Viñas, I. Minimal processing of a Granny Smith apple purée by microwave heating. *Innov. Food Sci. Emerg. Technol.* **2009**, *10*, 545–550. [\[CrossRef\]](#)
37. Rinaldi, M.; Langialonga, P.; Dhenge, R.; Aldini, A.; Chiavaro, E. Quality traits of apple puree treated with conventional, ohmic heating and high-pressure processing. *Eur. Food Res. Technol.* **2021**, *247*, 1679–1688. [\[CrossRef\]](#)
38. Keenan, D.F.; Brunton, N.; Butler, F.; Wouters, R.; Gormley, R. Evaluation of thermal and high hydrostatic pressure processed apple purees enriched with prebiotic inclusions. *Innov. Food Sci. Emerg.* **2011**, *12*, 261–268. [\[CrossRef\]](#)
39. Salazar-Orbea, G.L.; García-Villalba, R.; Bernal, M.J.; Hernández, A.; Tomás-Barberán, F.A.; Sánchez-Siles, L.M. Stability of phenolic compounds in apple and strawberry: Effect of different processing techniques in industrial set up. *Food Chem.* **2023**, *401*, 134099. [\[CrossRef\]](#) [\[PubMed\]](#)
40. Nistor, O.V.; Stănciuc, N.; Andronoiu, D.G.; Mocanu, G.D.Ț.; Botez, E. Ohmic treatment of apple puree (Golden Delicious variety) in relation to product quality. *Food Technol. Biotechnol.* **2015**, *24*, 51–59. [\[CrossRef\]](#)
41. Oszmiański, J.; Wolniak, M.; Wojdyło, A.; Wawer, I. Influence of apple purée preparation and storage on polyphenol contents and antioxidant activity. *Food Chem.* **2008**, *107*, 1473–1484. [\[CrossRef\]](#)
42. Lara-Valderrama, G.; Nagaya, M.; Uemura, K. Quality characterization of apple puree processed by high frequency pulse continuous heating. *Food Chem. Adv.* **2023**, *2*, 100228. [\[CrossRef\]](#)
43. Geicu, M.; Popa, M.; Mitelut, A.; Niculiță, P.; Cramariuc, R. Study of Color Evolution during Shelf Life of Apple Puree Treated with Pulsed Electric Field. In Proceedings of the 2nd International Symposium “New research in Biotechnology”, USAMV, Bucharest, Romania, 19–20 November 2009; pp. 212–218.
44. Aghajanzadeh, S.; Ziaifar, A.M.; Verkerk, R. Effect of thermal and non-thermal treatments on the color of citrus juice: A review. *Food Rev. Int.* **2021**, *39*, 3555–3577. [\[CrossRef\]](#)
45. Lan, W.; Baeten, V.; Jaillais, B.; Renard, C.M.G.C.; Arnould, Q.; Chen, S.; Leca, A.; Bureau, S. Comparison of near-infrared, mid-infrared, Raman spectroscopy and near-infrared hyperspectral imaging to determine chemical, structural and rheological properties of apple purees. *J. Food Eng.* **2022**, *323*, 111002. [\[CrossRef\]](#)
46. Ibarz, A.; Pagán, J.; Garza, S. Kinetic models of non-enzymatic browning in apple puree. *J. Sci. Food Agric.* **2000**, *80*, 1162–1168. [\[CrossRef\]](#)
47. Lan, W.; Jaillais, B.; Leca, A.; Renard, C.M.G.C.; Bureau, S. A new application of NIR spectroscopy to describe and predict purees quality from the non-destructive apple measurements. *Food Chem.* **2020**, *310*, 125944. [\[CrossRef\]](#)
48. Pellegrino, D. Antioxidants and Cardiovascular Risk Factors. *Diseases* **2016**, *4*, 11. [\[CrossRef\]](#) [\[PubMed\]](#)
49. Tsao, R. Chemistry and biochemistry of dietary polyphenols. *Nutrients* **2010**, *2*, 1231–1246. [\[CrossRef\]](#) [\[PubMed\]](#)
50. Bahukhandi, A.; Dhyani, P.; Bhatt, I.D.; Rawal, R.S. Variation in Polyphenolics and Antioxidant Activity of Traditional Apple Cultivars from West Himalaya, Uttarakhand. *Hortic. Plant J.* **2018**, *4*, 151–157. [\[CrossRef\]](#)
51. Lončarić, A.; Kopjar, M.; Piližota, V. Improving the quality of apple purée. *J. Food Sci. Technol.* **2017**, *54*, 3201–3207. [\[CrossRef\]](#) [\[PubMed\]](#)
52. Poljsak, B.; Kovač, V.; Milisav, I. Antioxidants, Food Processing and Health. *Antioxidants* **2021**, *10*, 433. [\[CrossRef\]](#)
53. Li, F.; Chen, G.; Zhang, B.; Fu, X. Current applications and new opportunities for the thermal and non-thermal processing technologies to generate berry product or extracts with high nutraceutical contents. *Food Res. Int.* **2017**, *100*, 19–30. [\[CrossRef\]](#)

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