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Application of pulsed electric field in reducing internal browning and maintaining the functional potential of 'Pérola' pineapple[1]

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ABSTRACT - The pineapple, the most important fruit in the state of Paraíba, is characterized as an important generator of employment, income and regional development. With the increase in production, challenges arise in reaching distant competitive markets, including exports, making refrigerated storage necessary. However, pineapple, a tropical fruit, is subject to Chilling Injury (CI) when stored under sub-optimal temperatures, below 12 ºC, which provide internal browning (IB) and quality loss. The use of pulsed electric field (PEF), a non-thermal technology, can be an alternative in ensuring the control of IB in pineapple. Therefore, the objective of this work was to evaluate the influence of PEF application on IB, enzyme expression and activity, bioactive compounds and antioxidant activity of 'Pérola' pineapple. Initially, to define the best strategy, 8 kV/cm of PEF was applied to pineapples directly and indirectly (drinking water), in 4 levels of electrical pulses: 0 (control), 5, 20 and 35, proving to be the most efficient the indirect pulses. From these results, a completely randomized design was used, with indirect application of PEF at the four levels in pineapples, with 5 periods of evaluations at refrigeration at 5 ºC, followed by transferring at each period for two more days to the room condition, in 4 replicants (3 fruit / rep). The indirect application of 35 pulses provided a reduction in expression and enzyme activity, IB and maintained the functional potential, being a promising alternative for the storage of pineapple under sub-optimal temperatures.

Key words: Non-thermal Technology. Browning Index. Chilling Injury. Enzyme Expression and Activity. Antioxidant Activity.

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INTRODUCTION

The pineapple plant is native to Uruguay, Brazil, and Paraguay, and is the most important of the commercially cultivated *Bromeliaceae* in the tropics and parts of the subtopics (TFnet, 2016) for ornamental purposes (SILVA *et al*., 2019), but mainly as a fruit of widespread worldwide consumption and trade (SANGPRAYOON *et al.*, 2019). Its infructescence, the pineapple, is highly appreciated for its aroma and flavor, and is recognized for having nutrients that provide energy to the usual diet and exert powerful biological activities which provide health benefits (DANTAS *et al*., 2015).

Brazil has stood out as the second largest pineapple producer in the world with production of 2,694,555 ton/ha in a production area of 68,699 ha; the Northeast region is responsible for 40% of production and yield (FAO, 2021), and the state of Paraíba is currently the second largest producer in the country (IBGE, 2020), with 'Pérola' pineapple being the main cultivar (DANTAS *et al*., 2015). Growing production opens up challenges to reach competitive export markets, demanding development of technologies which ensure maintenance of high quality standards (GUIMARÃES *et al*., 2017).

Reducing storage temperature is the main strategy used to maintain quality and increase postharvest shelf life (SIDDIQ, 2018). However, a temperature reduction can cause chilling damage or Chilling Injury, an important physiological disorder that imposes limitations on the refrigerated storage of subtropical and tropical fruits (YOURYON *et al.*, 2018). Thus, postharvest useful life of pineapple is limited due to its susceptibility to chilling injury when kept at sub-optimal temperatures of below 12 °C (SANGPRYOON *et al*., 2019), which manifests as a symptom of internal pulp browning (internal browning – IB) (RAIMBAULT *et al*., 2011) caused by the loss of lipid membrane integrity (NUKUNTORNPRAKIT *et al*., 2015) and oxidative stress (VALENZUELA *et al*., 2017), causing severe postharvest losses (YOURYON *et al*., 2018).

The development of alternative non-thermal technologies aimed at food preservation has shown increasing interest (VOLLMER *et al*., 2021). In this sense, high-intensity pulsed electric field (PEF) is an emerging non-thermal technology that consists of subjecting liquids, drinks, and foods to high-intensity fields (to the order of 5 to 55 kilovolts per centimeter – $kV.cm^{-1}$) with electrical pulses of short duration (ms or s), repeated many times (constituting the number of pulses) with the purpose of inactivating enzymes and destroying microorganisms (QIAN *et al*., 2016), generally retaining their physical, chemical, and nutritional characteristics. Chilling injury increases the expression and activity of the polyphenol oxidase (PPO) and peroxidase (POD) enzymes (RAIMBAULT *et al*., 2011) and oxidative stress (VALENZUELA *et al*., 2017). In turn, high-intensity PEF reduced the activity of enzymes such as PPO (AGUILÓ-AGUAYO *et al*., 2008) in tomato juice and POD (ZHONG *et al*., 2007), maintaining the bioactive compound and antioxidant activity levels. PEF treatment has the potential to stimulate metabolic activity and accumulate secondary metabolites, and keeps the cell alive as it is a reversible electroporation process (ARSHAD *et al*., 2020). Therefore, PEF can be an efficient alternative in ensuring the maintenance of membrane integrity and controlling IB, the main chilling injury symptom in 'Pérola' pineapples.

The objective of this study was to evaluate the influence of applying high-intensity pulsed electric fields on the internal browning incidence, as well as on the bioactive compound and antioxidant activity of 'Pérola' pineapple in cold storage at 5 °C.

MATERIAL AND METHODS

Pineapples (*Ananas comosus* var. Comosus) were harvested from a commercial plantations in the municipality of Itapororoca – PB/ Brazil, at the commercial maturity stage (green skin color, with the beginning of yellow color at the base and detachment of the meshes. Following the harvest,the pineapples were then transported to the laboratory, and the initial quality wa characterized by the evaluation of 16 fruits selected for uniformity of maturity, which presented on average: mass 1512.26 g, pulp moisture 86.70%, soluble solids 13,07%, titratable acidity (AT – %citric acid) 0.72%, and ripening index (SS/AT ratio) 18.15.

High-intensity pulsed electric field (PEF) treatments were applied to pineapple samples using electrical pulse generating equipment, which consisted of a system composed of a high voltage source and a pulse generator; capacitors, electrical resistances, as well as other electronic components common to several electrical systems, interconnected to an electrical distribution box with simultaneous testing capacity with the same application characteristics. The treatment chamber was conical-shaped, made of transparent plastic material with the following dimensions: height 24 cm x upper diameter 25 cm x lower diameter 18 cm, with a capacity of 8L. This chamber was equipped with spaced stainless steel electrodes, measuring each: 180 mm x width 10 mm x thickness 1 mm, connected to the test leads of the electrical distribution box cables.

PEF applications were carried out directly (PD), with the electrode probes connected directly to the samples, by penetrating 5 mm of the positive pole at the base of the

stalk and the negative pole at the region adjacent to the crown of each sample; and indirectly (PI), using treatment chambers, each containing 3 liters of drinking water with conductivity of 329.8 ± 1 μS.cm^{-1} at 25 °C, covering the sample up to the area adjacent to the crown, using one sample per chamber. The treatments for applying PEF directly (PD) or indirectly (PI) were: C – control (without application of PEF); 5 pulses; 20 pulses and 35 pulses, 4 repetitions per treatment (1 fruit/repetition). The characteristics of the pulses were monopolar and exponential, electric field intensity 8 kV/cm, width ~ 70 μs and frequency 0.97 Hz.

After applying PEF, the pineapples were kept at room conditions (24 ± 2 ºC and 75 ± 3% RH) for 1 hour to allow the metabolic reactions that account for defending the plant tissue against possible damage to occur. Then, the pineapples were stored at 5 ºC with 85% RH for 5, 10, 15 and 20 days and transferred to room conditions for another 2 days, before evaluations.

Upon prior observation that the indirect pulses (IP) application was more efficient in reducing the abundance of enzymes, through the electrophoretic profile analysis, an experiment was carried out with the IP application for the evaluation of biactive compounds, antioxidant activity, enzymatic activity, and incidence of internal browning in pineapples.

Enzymatic assays were performed using the same crude extract from 2 g of pulp, homogenized with 5 mL of 200 mM potassium phosphate buffer solution (pH 6.7) and 0.1 g of polyvinylpyrrolidone (PVP). Then it was centrifuged at 9000 rpm for 25 min at 4 °C, and the supernatant used as an enzyme extract (YANG; ZHENG; CAO YANG., 2009).

Enzyme abundance was determined by SDS-PAGE electrophoresis. An aliquot containing 120 μL of the enzyme extract was solubilized in 0.0625 M Tris-HCl buffer, containing 2% SDS, 2% 2-mercaptoethanol, 10% glycerol and 0.010% bromophenol blue, followed by the application of an aliquot containing 20 μL in 4 g/100 g stacking gel and 12.5 g/100 g in polyacrylamide running gel (10 x 10.5 cm, with 0.75 mm spacers), subjected to a constant current of 25 mA, for approximately 3 hours. After electrophoresis, the gel was fixed using a methanol/ acetic acid, and water fixative solution, followed by staining. In the dye solution, 1% Coomassie Blue R-250 (Sigma Chemical Co.), 40% methanol, 10% acetic acid in distilled water were used. Bleaching was done with a solution containing 10% acetic acid and 20% methanol in distilled water. To estimate the molecular weight, a commercial standard (Sigma Chemical Co.), with a wide range of proteins (Myosin, 200 kDa; β-galactosidase, 120 kDa; Bovine Serum, 91 kDa; Glutamine, 62 kDa; Ovoalbumin, 47 kDa; Carbonic Anhydrase, 37 kDa; Myoglobin, 28 kDa; Lysozyme, 19 kDa; Aprotinin, 9 kDa) was used (LAEMMLI, 1970).

The polyphenol oxidase (POD, EC 1.14.18.1) activity was determined according to Wissemann and Lee (1980), using 0.3 mL aliquots of the enzymatic extract. 1.85 mL of 0.2 M phosphate buffer (pH 6.8) was added, containing 0.1 M KCl and 0.1 M catechol, with the procedure carried out at ± 4 ºC. It was incubated for 30 minutes at 30 ºC, and the reaction was stopped by adding 0.8 mL of 2 M HClO. The samples were vacuum filtered using Whatman #1 paper and absorbance readings were taken within a maximum of 30 minutes at 395 nm in a GenesesTM 10s UV VIS spectrophotometer, after stopping the reaction. For calibration, a blank was used as a control, replacing the enzyme extract with distilled water. The results were expressed in μmol of catechol. min^{-1}.mg^{-1} of pulp.

Peroxidase activity (POD, EC 1.11.1.7) was measured using the reaction mixture (1.5 mL) composed of 1.2 mL of 100 mM potassium phosphate buffer (pH 7.0), 0.1 mL of 0.5 M hydrogen peroxide, 0.1 mL of 3% guaiacol, and 0.1 mL of the enzyme extract. The activity was determined based on the oxidation of guaiacol using H_2O_2 and an extinction coefficient of 26.6 mM^{-1} cm^{-1} (WU *et al*., 2010). The increase in absorbance was monitored for 5 min at 470 nm. The results were expressed in μmol of guaiacol.min^{-1}.mg^{-1} of pulp.

The color index (ΔE_{Lab}) was measured using a portable colorimeter (Minolta CR-10, Osaka, Japan). The color changes were read directly on the pineapples using the *L**, *a**, and *b** scale, (CIELAB) where *L** is the luminosity, *a** is the red/ green intensity and *b** is the yellow/blue intensity. The ΔE_{Lab} was calculated according to Allegra *et al.* (2017), using the equation: $\Delta E_{Lab} = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2}$, considering the difference between the color measured in the first period in relation to the other storage periods. Soluble solids were determined in the homogenized pulp, through direct Reading at 20 ºC, using a portable digital refractometer (Milwaukee, model - MA871: 0 – 85%), with the results expressed as a percentage (DANTAS *et al*., 2015). The spectrophotometric browning index (OD_{420}.g^{-1}) of the core was obtained according to Raimbault *et al*. (2011). Tissues adjacent to the core were homogenized with 65% (v/v) ethanol and then left at room condition for 1 h. Absorbance was measured at 420 nm in the filtered extract. The results were expressed as OD420.g^{-1} fresh weight (FW). Absorbance was measured at 420 nm in the filtered extract. The results were expressed as OD_{420}.g^{-1} fresh weight (FW). Ascorbic acid content was determined according to Strohecker and Henning (1967), which 1g of sample was homogenized with 50 mL of 0.5% oxalic acid, titrating with 0.02% 2,6-dichlorophenolindophenol-sodium (DFI) solution until a light pink color.

To determine total extractable polyphenols (TEP) and antioxidant activity using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method, a phenolic extract obtained according to Larrauri, Rupérez and Saura-Calixto (1997) was used. 5 g of ultrafrozen (-80 ºC) pulp were used, with 4 mL of 50% methanol added, leaving to rest for 1 hour for extraction and followed by refrigerated centrifugation for 15 minutes at 15,000 rpm. 4 mL of 70% acetone was added to the residue, allowed to extract for 1 hour, and centrifuged for 15 minutes at 15,000 rpm. The supernatant was removed and placed together with the first supernatant, completing the volume to 11 mL with distilled water. The entire procedure was performed in the absence of light.

The TEP contents were assessed according to Larrauri, Rupérez and Saura-Calixto (1997), taking an aliquot of 0.3 mL of the phenolic extract, completing the volume to 1 mL with distilled water, this aliquot being defined based on a standard curve of gallic acid (0 to 50 μg. mL^{-1}), considering the absorbance linearity range of the standard curve (Figure 2). To this dilution, 1 mL of Folin-Ciocalteau reagent, 2 mL of 20% sodium carbonate and 2 mL of distilled water were added. After shaking, the extract solution was incubated for 30 minutes in the absence of light. For control, the extract volume was replaced with distilled water. The reading was carried out at 700 nm using a spectrophotometer (GenesesTM 10s UV VIS).

To quantify total extractable polyphenols (TEP), expressed in mg of gallic acid.100 g^{-1} of pulp, the following equation was used:

$$TEP\ \left(mg.100g^{-1}\right) = X * \frac{FD1 * FD2}{1000} * 100 \qquad (1)$$

Where:

X = Calculated from the gallic acid standard curve equation (μg. mL^{-1}), using the sample absorbance as the Y axis value;

FD1 (g. mL-1) = Extract final volume (11 mL) / Pulp Fresh Weight (g);

FD2 = Final volume of the mixture in the reaction tube (extract + distilled water) – 1 mL/ aliquot vol. of the extract used.

Total antioxidant activity (AAT), through DPPH• free radical scavenging, was determined according to Rufino *et al*. (2010). From the phenolic extract, three dilutions (200, 600, and 1000 μL. mL^{-1}) were prepared in three replications of each dilution, based on a DPPH• standard curve (final concentration ranging from 0 to 60 μM, diluted in metanol P.A). From each dilution, a 100 μL aliquot of the control solution was used (50% methyl alcohol + 70% acetone + distilled water – 4:4:2). To calibrate the spectrophotometer (GenesesTM 10s UV VIS) at a wavelength of 515 nm, P.A. methanol was used. The dilutions were left at room condições, in the absence of light for 70 minutes, based on stabilization of absorbance. To calculate the ATT, the equation curve was determined, based on the absorbance of the three dilutions, then replacing in the equation the absorbance equivalent to 50% of the DPPH concentration (initial absorbance of the control/2), finding the amount of sample necessary to reduce the initial concentration of the DPPH• radical by 50% (EC50). The results were expressed as EC50 in g pulp / g DPPH.

The experiment was conducted in a completely randomized design, with the application of four levels of pulses, in 5 evaluation periods on pineapples kept under refrigeration at 5 oC, which were transferred each period for another two days to the room condition, in 4 replications (3 fruits/rep). The data were subjected to analysis of variance and the means compared using the Tukey test at 5% probability.

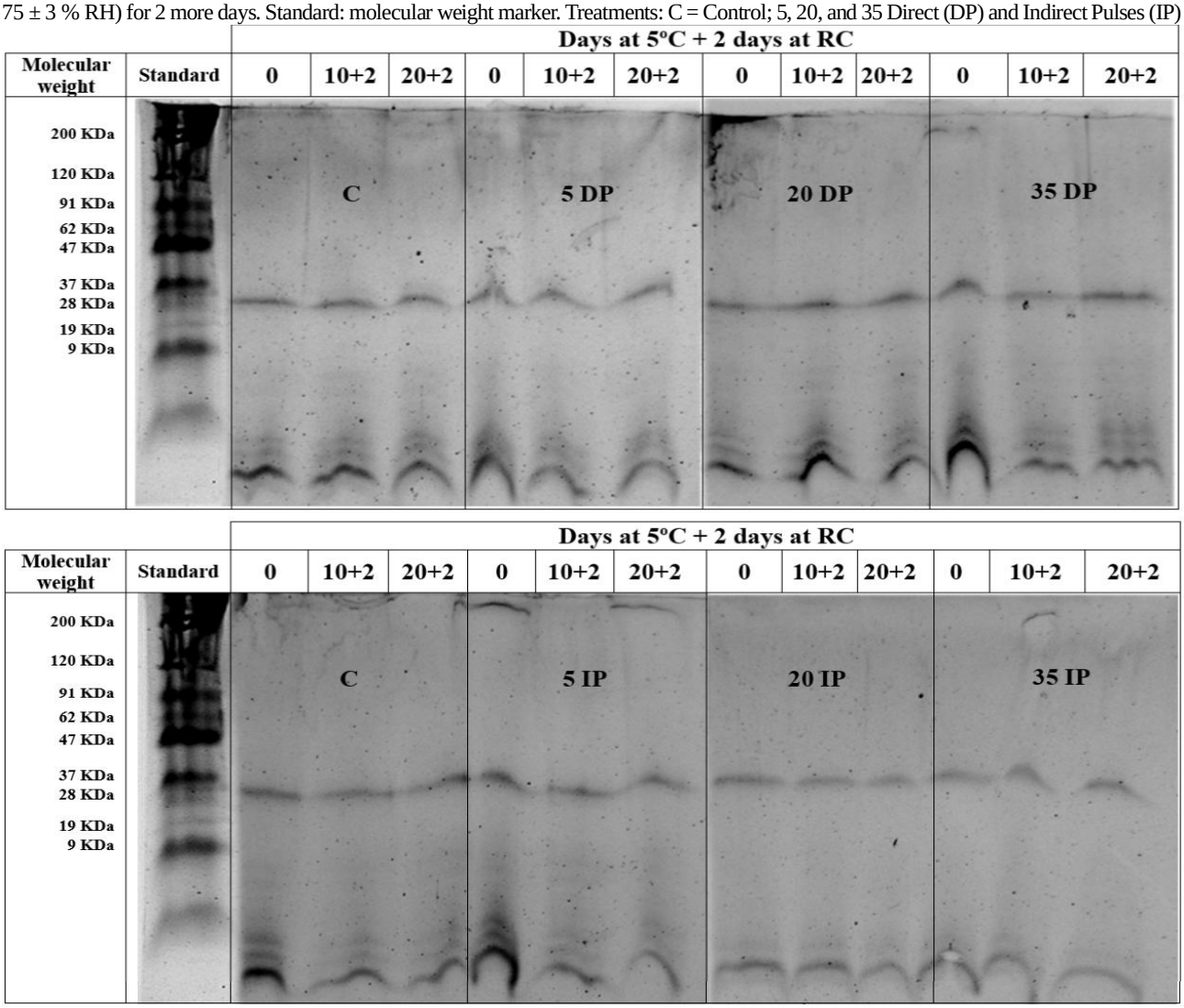
RESULTS AND DISCUSSION

The protein patterns of 'Pérola' pineapple, separated in bands by polyacrylamide gel electrophoresis (SDS-PAGE) are presented in Figure 1. Based on the molecular weight (MW), it was found that the MW in the protein fraction, as a function of the molecular marker, was approximately 28 KDa, corresponding to peroxidase (POD) expression. Close values were also reported by Forsyth and Robinson (1998) in kale, which the molecular weights for POD ranged between 26.8 and 48.3 KDa. Based on these results, lower POD abundance was observed in pineapple treated with 35 indirect pulses (IP) at the end of storage when compared to those applied with the same number of direct pulses (DP), which may serve as an indication of lower IB incidence.

Visual perception quantified in IB percentages is presented in Figure 2. The application of increasing levels of electrical pulses (5, 20, and 35 pulses) resulted in reduced internal browning (IB) incidence at the end of storage (30.11%, 24.18%, and 15.53%, respectively) in relation to the control, which presented 37.70% IB at the end of storage. These results indicate a clear reduction in internal browning by applying PEF, especially in fruits treated with 35 pulses compared to the control.

Pineapples treated with 5 and 25 IP showed fluctuations in PPO activity (Fig. 5). This behavior was also reported by Yeoh and Ali (2017) using ultrasound in fresh-cut pineapple, and López-Gámez *et al*. (2020) in carrots using a pulsed electric field. Increasing activities were observed in control pineapples and those treated with 35 IP, although lower than those of the other treatments. However, control pineapples had higher PPO activity than the other treatments at the end of storage.

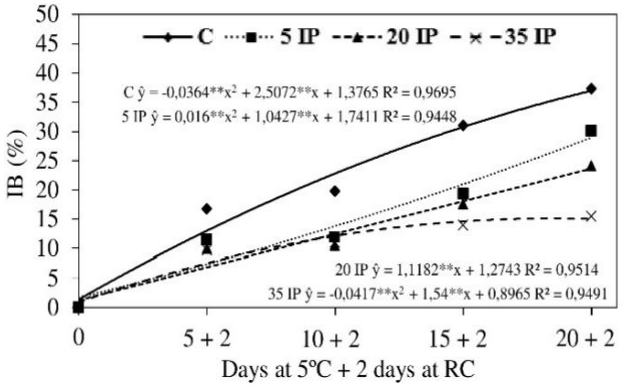
Figure 1 - Polyacrylamide gel electrophoresis (SDS) in 'Pérola' pineapple in pulp treated with direct pulses (DP) and indirect pulses (IP) of an electric field (8.0 kV/cm) and stored at 5 ºC and 85% RH during 10 and 20 days and transferred to room conditions (RC) (24 ± 2 ºC and 75 ± 3 % RH) for 2 more days. Standard: molecular weight marker. Treatments: C = Control; 5, 20, and 35 Direct (DP) and Indirect Pulses (IP)



Enzyme inactivation by pulsed electric field (PEF) occurs due to changes in the tertiary and/or secondary structure of the protein, since the enzyme is exposed to an electrical force when it is subjected to PEF due to charged groups present in different positions, which promote altered electrical forces which can lead to changes in their structural conformation, causing their denaturation (ZHONG *et al.*, 2007).

As with PPO activity, pineapples also showed fluctuations in peroxidase (POD) activity (Figure 3). Control pineapples showed a reduction in POD activity until the second storage period, with activity then increasing until the end of storage, and being higher than the other treatments. Pineapples treated with 5 IP showed a reduction in POD activity in the first storage period, and an increase after the fourth storage period. In turn, pineapples treated with 35 IP showed a peak in POD activity in the third storage period, although they showed the lowest

Figure 2 - Internal Browning - IB (%) of 'Pérola' pineapple treated with indirect pulses (IP) of an electric field (8.0 kV/cm) and stored at 5 ºC and 85% RH during 5, 10, 15, and 20 days and transferred to room conditions (RC) (24 ± 2 ºC and 75 ± 3 % RH) for 2 days. Treatments: C = Control; 5, 20, and 35 Indirect Pulses (IP)



POD activity at the end of storage. The greater or lesser resistance of an enzyme to PEF will depend on its number of hydrogen bonds, the amino acid composition, which gives it greater or lesser hydrophobicity, the presence of metals in its structure and its volume (QIAN *et al*., 2016). Zhong *et al.* (2007) reported that the POD enzyme is more resistant to inactivation by PEF than PPO, which, at least in part, explains the increasing activity at the end of storage period for lower levels of IP applications.

The core (central axis) color index (ΔE_{Lab}) in 'Perola' pineapple did not differ according to the level of IP applied, which constitutes an indicator that IP application does not affect the Perola' pineapple color index, but it differed among the sections (apex, center, and base) of the fruit core (Figure 4 A). In this sense, the pineapples presented a yellower color at the apex of the central axis than at the center and base during storage. However, the yellow color at the apex tended to decrease in intensity from the first to the fourth storage period. The central area of pineapple cores showed decreased yellow color from the second period until the end of storage. The yellow color showed an increase in intensity at the apex and base of the central axis after the fourth storage period.

The soluble solids (SS) content of control pineapples showed an increase after the first evaluation period, followed by a decline until the end of storage (Figure 3B), as an indicator of senescense. In turn, pineapples treated with 35 IP presented higher SS values than those of the other treatments between the third and fifth storage period, indicating a reduction of the metabolic rate. Pineapples treated with PEF presented SS contents above 12%, constituting the minimum content established by the Pineapple Codex Alimentarius (TFnet, 2016) which serves as a quality attribute indicator. Therefore, the SS content was maintained or increased during storage under the study conditions, which implies that the application of PEF maintains fruit metabolic rate and quality. SS are often used as an indicator of pineapple quality and ripeness level (DANTAS *et al*., 2015), requiring a minimum threshold of 12% for consumer acceptance of pineapple (GUIMARÃES *et al.*, 2017).

The browning index (BI) showed a significant reduction ($p < 0.05$) between treatments (Figure 3B) as the number of pulses applied was increased, so pineapples treated with 35 IP showed the lowest BI, therefore, the lower levels of 'Perola' pineapple internal browning. The gradual BI reduction between treatments may be associated with the increase in reversible electroporation caused by the increase in the number of electrical pulses, resulting in cellular recovery and decreased expression of oxidative enzyme genes (MANNOZZI *et al*., 2019) in treated pineapples. The results of Sangprayoon *et al*. (2019) using salicylic acid or methyljasmonate plant regulators corroborate the internal browning behavior of this study, although with higher values and a less practical approach than the one presented herein.

An increase in ascorbic acid (AA) content was observed until the third storage period for control, 5 IP and 35 IP pineapples (Figure 5A). However, AA levels during storage of pineapples with applications of 35 IP and 5 IP were higher than the control ($p < 0.05$), showing that the application of PEF maintains the levels of the important antioxidant. The AA content in pineapples treated with 20 IP varied during storage, although the AA content was higher than those in control pineapples and 5 IP. Qian *et al*. (2016) applied PEF to fresh apples to inactivate PPO and also found an increase in AA levels. Ascorbic acid is produced in the secondary metabolism of plants and is an antioxidant which prevents oxidation, meaning the loss of electrons, with its synthesis

Figure 3 - Activity of polyphenoloxidase (PPO) (A) and peroxidase (POD) (B) of 'Pérola' pineapple treated with indirect pulses (IP) of an electric field (8.0 kV/cm) and stored at 5 ºC and 85% RH during 5, 10, 15, and 20 days and transferred to room conditions (RC) (24 ± 2 ºC and 75 ± 3 % RH) for 2 more days. Treatments: C = Control; 5, 20, and 35 Indirect Pulses (IP)

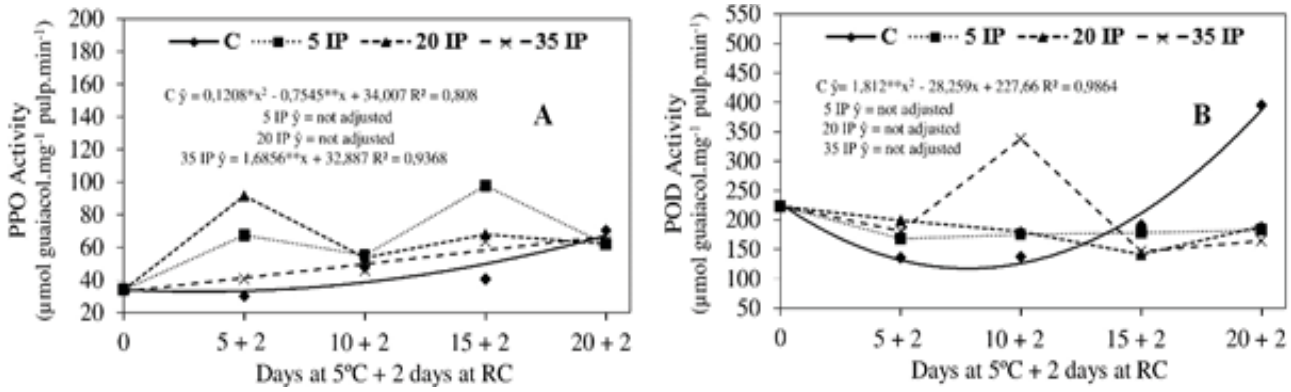


Figure 4 - Color index (ΔE_{Lab}) of the core sections (A), Soluble solids – SS (%) (B) and Browning index - BI (DO_{420} g^{-1} weight fresh) (C) of 'Pérola' pineapple treated with indirect pulses (IP) of an electric field (8.0 kV/cm) and stored at 5 ºC and 85% RH during 5, 10, 15, and 20 days and transferred to room conditions (RC) (24 ± 2 ºC and 75 ± 2 % RH) for 2 more days. Treatments: C = Control; 5, 20, and 35 Indirect Pulses (IP)

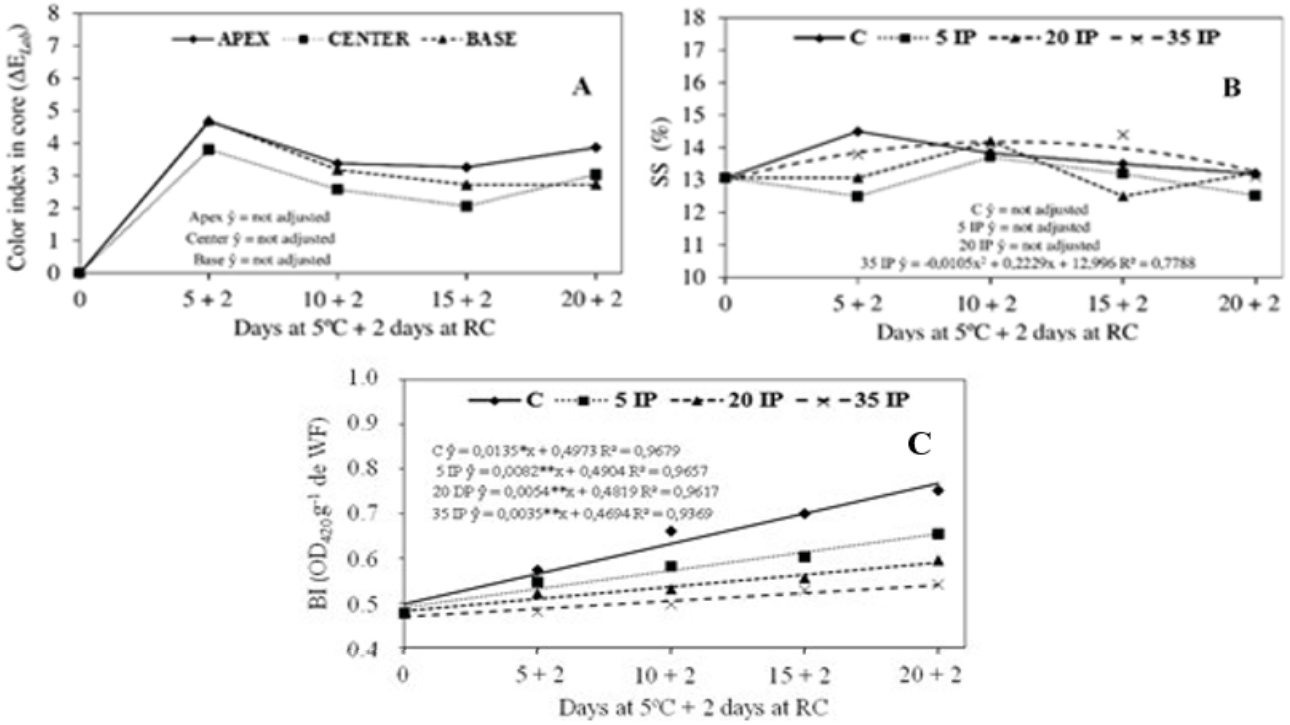
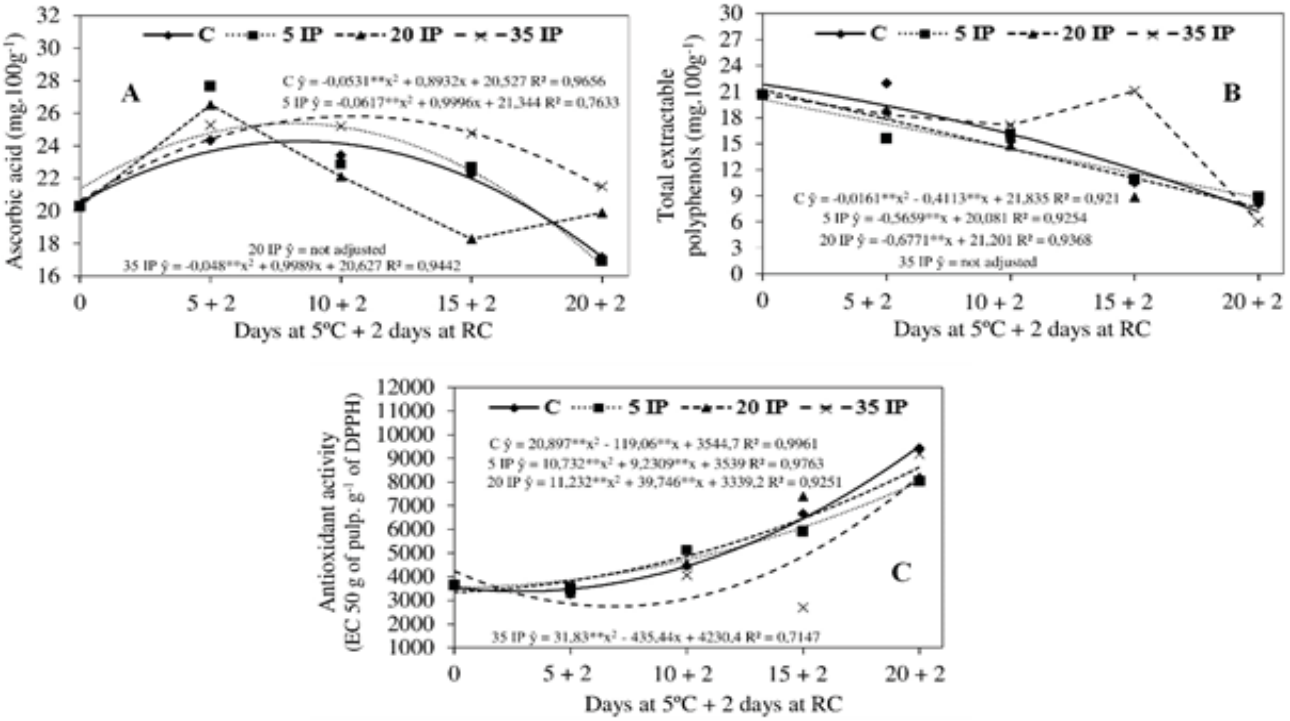


Figure 5 - Ascorbic acid (mg.100g^{-1}) (A), Total extractable polyphenols (mg.100 g^{-1}) (B), and Antioxidant activity (EC 50 g of pulp. g^{-1} of DPPH) (C) of 'Pérola' pineapple treated with indirect pulses (IP) of an electric field (8.0 kV/cm) and stored at 5 ºC and 85% RH during 5, 10, 15, and 20 days and transferred to room conditions (RC) (24 ± 2 ºC and 75 ± 3 % RH) for 2 more days. Treatments: C = Control; 5, 20, and 35 Indirect Pulses (IP)



being activated under certain conditions of environmental stress (FENECH *et al*., 2019), thus indicating that PEF applications provided reversible electroporation under the conditions studied (FERREIRA *et al*., 2019) and stimulated ascorbic acid synthesis.

Pineapples showed a decrease in total extractable polyphenol (TEP) levels during storage (Figure 5B). However, the fruits treated with 35 IP showed an increase in TEP after 15 days at 5 °C, followed by 2 days in room conditions. López-Gámez *et al.* (2020) also reported an increase in the total polyphenol content in carrots after treatment with PEF (voltage: 3.5 kV/cm, frequency: 0.1 Hz, number of pulses: 5 s, and pulse width: 4 µs), but declining at the end of storage. In contrast, Youryon *et al*. (2018) applied calcium chloride and calcium gluconate to pineapple via the stalk and reported that the total polyphenol content of the control significantly increased at the end of storage, while it remained constant in both calcium-treated pineapples ($p < 0.05$). These authors reported that the increase in total polyphenol content in control pineapples was related to an increase in PPO activity, resulting in high IB incidence compared to Ca-infiltrated pineapples. Phenolic compounds are synthesized in the secondary metabolism of vegetables (SIDDIQ, 2018) and are associated with the plant-environment binomial for their production in response to abiotic factors, as is the case with applying PEF, playing an important role in the production of polyphenols and ascorbic acid (for example) (MANNOZZI *et al*., 2019).

The total antioxidant activity (TAA) was determined by the DPPH radical assay, with results expressed in EC_{50} ($g.g^{-1}$), which corresponds to the amount in grams of pulp in the extract necessary to reduce the DPPH radical by 50%; thus, the lower the EC_{50}, the better the antioxidant capacity of the extract (Figure 5C). The control, 5 IP and 20 IP pineapples showed lower antioxidant activity during storage in view of the higher pulp consumption. In turn, pineapples treated with 35 IP showed higher TAA compared to the other treatments during storage considering the lower consumption of pulp to reduce the DPPH radical. Treated pineapples, especially those with 35 IP, showed an increase in the ascorbic acid and polyphenol synthesis during storage, indicating protection of the system against harmful events (JACOBO-VELÁZQUEZ *et al*., 2017; LÓPEZ-GÁMEZ, *et al*., 2020) caused by chilling injury from excessive oxidative processes or reactions (VALENZUELA *et al.*, 2017). Altogether, PEF seams to provide antioxidant protection to pineapple tissues.

CONCLUSIONS

The direct (DP) and indirect (IP) pulsed electric field (PEF) application provided a reduction in internal browning (IB) in pineapples, with the IP application being more efficient in reducing the IB incidence in 'Pérola' pineapple. The indirect application of 35 pulses with an intensity of 8 kV/cm provided a reduction in enzyme expression and activity, a reduction in the browning index, maintained quality and increased the functional potential of pineapples. As a result, PEF application is characterized as a promising emerging technology for pre-treating pineapples for subsequent storage under refrigeration and controlling the adverse effects of chilling injury.

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