

Article

Heat Treatment and Wounding as Abiotic Stresses to Enhance the Bioactive Composition of Pineapple By-Products

Diana I. Santos ¹, Diana L. Faria ¹, Sofia C. Lourenço ¹, Vitor D. Alves ¹, Jorge A. Saraiva ², António A. Vicente ³ and Margarida Moldão-Martins ^{1,*}

- ¹ LEAF, Linking Landscape, Environment, Agriculture and Food, School of Agriculture, University of Lisbon, Tapada da Ajuda, 1349-017 Lisbon, Portugal; dianaisantos@isa.ulisboa.pt (D.I.S.); dianafaria88@gmail.com (D.L.F.); sofiaclourenco@isa.ulisboa.pt (S.C.L.); vitoralves@isa.ulisboa.pt (V.D.A.)
- ² LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal; jorgesaraiva@ua.pt
- ³ CEB, Centro de Engenharia Biológica, Departamento de Engenharia Biológica, Campus de Gualtar, Universidade de Minho, 4710-057 Braga, Portugal; avicente@deb.uminho.pt
- * Correspondence: mmoldao@isa.ulisboa.pt; Tel.: +351-213653547

Abstract: Abiotic stress, like heat treatment and wounding, applied to pineapple by-products induce the accumulation of new compounds and add value. In this work the effect of the individual or combined application of wounding and heat treatment stresses on total phenolic content, antioxidant activity through complementary methods (DPPH, FRAP, and ABTS) and enzymatic activity (bromelain, phenylalanine ammonia lyase (PAL) and polyphenol oxidase) were evaluated. Whole and wounded pineapple shell and core were dipped in a hot water bath at 30 ± 1 °C or 40 ± 1 °C for 10 min and stored under refrigeration conditions (4 ± 1 °C) for 24 h or 48 h. Results allowed that pineapple by-products reacted differently to the tested stresses. For the core, the application of wounding and heat treatment (40 °C) before storage (24 h) induced a synergistic effect on the accumulation of phenols (increased 17%) and antioxidant activity (4–22%). For the shell samples, the treatment that most increased the content of phenols (14%) and antioxidant activity (38–45%) was heat treatment at 30 °C and storage for 48 h. Treatments that positively influenced the content of phenols and antioxidant activity of the samples did not affect the activity of bromelain or PAL. This study showed that proper abiotic stresses could increase the functional value of by-products.

Keywords: pineapple by-products; abiotic stress; wounding; thermal treatment; enzyme activity; bioactive compounds

Citation: Santos, D.I.; Faria, D.L.; Lourenço, S.C.; Alves, V.D.; Saraiva, J.A.; Vicente, A.A.; Moldão-Martins, M. Heat Treatment and Wounding as Abiotic Stresses to Enhance the Bioactive Composition of Pineapple By-Products. *Appl. Sci.* **2021**, *11*, 4313. <https://doi.org/10.3390/app11094313>

Academic Editor: Gang Ma

Received: 13 April 2021

Accepted: 6 May 2021

Published: 10 May 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Postharvest abiotic stress influences the quality of fruits and vegetables in terms of their food and nutritional attributes, shelf life, and vulnerability to pathological and physiological disorders. All these factors will thus influence postharvest losses. Postharvest technologies often apply stress conditions to extend the storage and shelf life of fruits and vegetables [1]. Abiotic stress has a positive impact on the quality and nutritional conditions of fresh cut fruits and vegetables due to the abiotic stresses that occur throughout processing, packaging, and storage. The impact of abiotic stress can be assessed through colour change, increased respiration rates, ethylene evolution, loss of taste, and texture (softening of the tissues), weight loss, increase of off-odors, and rupture of the cell membrane [2,3].

Postharvest treatments preserve the quality of fresh produce and control microbial growth through a secondary response that induces plant metabolic activity. Most postharvest treatments involve changing the natural conditions of the fruit and trigger mechanisms that develop, for example, the antioxidant activity of the fruit [4].

Fruits and vegetables immersed in hot-water baths showed improvements in quality, namely the reduction of diseases during the storage period [5]. Heat stress is likely to cause changes in biochemical pathways such as synthesis of heat shock proteins, suspension of protein synthesis, including quality-related enzymes (polyphenol oxidase, peroxidase, phenylalanine ammonia-lyase, and pectin methylesterase), as well as variations in kinetic characteristics of enzymes [5,6].

Wounding in fresh-cut fruits and vegetables induces the synthesis of enzymes of phenylpropanoid metabolism, and consequently the synthesis and accumulation of phenolic compounds [6]. Stress or damage in the plant cell can trigger the oxidation of phenolic compounds due to cell membrane rupture that causes contact between phenolic compounds and oxidative enzyme systems or promote phenolic synthesis to repair wound damage. The phenolic synthesis to repair wound damage is produced by variations in phenylalanine ammonia lyase activity (PAL, EC 4.3.1.5), since it is the metabolic enzyme responsible for the phenylpropanoid pathway [7]. Some studies indicate that PAL enzymatic activity becomes significantly higher during the storage time after an abiotic stress has occurred [8].

Polyphenol oxidase (PPO; EC 1.14.18.1) is an oxidative enzyme existent in pineapple accountable for unwanted sensorial effects, such as darkening and deterioration of taste [9]. PPO catalyzes the hydroxylation of monophenol to diphenol and the oxidation of diphenol to *o*-quinones [10]. PPO is thus responsible for the breakdown of antioxidant compounds such as pigments, phenols, and flavonoids of pineapples, promoting darkening and devaluation of the fruit [11].

The industrial processing of pineapple generates a high quantity of by-products, which represent 42–59% of the pineapple fruit, where the shell is the principal fraction [12]. It is imperative to valorize these by-products and converting them into value-added products with the purpose of decreasing the environmental effect [13].

Pineapple has numerous beneficial properties such as the presence of phenolic compounds that can be a useful source of antioxidant activity [14,15]. Phenolic compounds are known as effective antioxidants and have applications in health since phenolic compounds have an antioxidant function as free radical scavengers and metal chelating agents that are efficient in catalyzing lipid peroxidation [16]. Bromelain is an enzyme present in pineapples and has health benefits that include antitumor properties, digestive assistance, immunity modulation, improved wound healing, and cardiovascular and circulatory improvement [17,18]. The health benefits of these compounds are associated with their ingestion, but very dependent on their bioaccessibility and bioavailability in the body. The fraction of a compound that is released during digestion of the food matrix in the gastrointestinal tract and becomes available for intestinal absorption (i.e., its bioaccessibility) can be assessed by several methods, namely, *in vitro* (static and dynamic) digestion models, intestinal absorption and pre-systemic metabolism assessment [19].

Fruit and vegetable by-products after abiotic stresses have many applications in the food industry. Pineapple by-products subjected to abiotic stress (hydrostatic pressure) were incorporated in marinades for meat application. Meat marinated with pineapple by-products showed a 41% reduction in hardness, compared to meat immersed in brine [20], thus, pointing at the very interesting effects of such by-products in food processing.

The aim of the present work was to study the effect of abiotic stresses by wounding, heat treatment and storage time on the enzymatic activity and on the bioactive components of pineapple by-products (pineapple shell and pineapple core). The pineapple by-products after the application of abiotic stresses can be used directly as raw material or alternatively used as sources of compounds beneficial to health.

2. Materials and Methods

2.1. Sample Preparation

Pineapple by-products (*Ananas comosus* L.) were provided by Campotec S. A., located in Torres Vedras, Portugal. The pineapple by-products (~16 kg of pineapple shell and ~16 kg of pineapple core) were stored under refrigeration (4 ± 1 °C) approximately 18 h before packaging and abiotic stresses application. Half of the amount of by-products were cut into specific dimensions: ~8 kg pineapple core cylinders ($\sim 52.5 \times 30$ mm) and ~8 kg pineapple shells ($\sim 110 \times 40$ mm). The remaining 8 kg of pineapple shell and the remaining 8 kg of pineapple core were wounded separately (700 g/batch) in a Thermomix (TM31, Vorwerk Thermomix) at a speed of 5650 rpm for 25 s. Subsequently, all the by-products were packaged in separate portions (~40 g) in PA/PE-90 (Alempack—Embalagens Flexíveis, Elvas, Portugal) and vacuum sealed (85% of vacuum). The thermal treatments were carried out through the bags with pineapple by-products immersed in a bath of hot water at 30 ± 1 °C or 40 ± 1 °C for 10 min. Heat treatment conditions (temperature and time) were selected based on a previous study [21]. After heat treatment, the samples were immediately placed in a water and ice bath to stop the reactions caused by the heat in the samples. Samples that were not immersed in the hot water bath were used as controls. After the application of heat stress, the pineapple bags were stored for 24 h or 48 h at 4 ± 1 °C so that the enzymes had time to act and, later, they were frozen at -80 °C until the moment of the analyses. The storage time control samples were frozen at -80 °C immediately after the heat treatment. All samples were prepared in triplicate for each treatment.

2.2. Analytical Methods

The pineapple shell and pineapple core samples subjected to wounding, heat treatment and storage time were analyzed by the following methodologies: total phenolic content, antioxidant activity (DPPH, FRAP, and ABTS), enzymatic activity (bromelain, phenylalanine ammonia-lyase, and polyphenol oxidase). All analytical methods were applied to three independent samples. Each independent sample was analyzed in triplicate and the average was used to characterize each treatment condition.

2.2.1. Pineapple Extract Preparation for Total Phenolic Content and Antioxidant Activity

The pineapple extract preparation was performed following the procedure of Heredia and Cisneros-Zevallos (2009) and Swain and Hillis (1959) [22,23], with some modifications. The extraction was carried out in the proportion 1: 10 (w: v) of sample and methanol (100%) followed by an Ultra-Turrax homogenizer (IKA LABORTECHNIK T25 basic, Germany) at 8000 rpm for 2 min. The homogenates were incubated overnight (12–24 h) at 4 °C and then centrifuged at 8000 rpm for 20 min (4 °C). The supernatants were collected and stored at 4 °C, protected from light until analysis.

Total Phenolic Content (TPC)

The total phenolic content (TPC) were determined according to Heredia and Cisneros-Zevallos (2009), and Swain and Hillis (1959) [22,23], with some modifications. The extracts aliquots (150 µL) were diluted with nanopure water (2400 µL), and added 150 µL 0.25 mol L^{-1} Folin–Ciocalteu reagent (Panreac AppliChem, Darmstadt, Germany). The reaction was stopped by adding 1 mol L^{-1} of sodium carbonate (300 µL) and the mixture was incubated (2 h) protected from light; the samples subsequently were read at 725 nm in a spectrophotometer (UNICAM UV/Vis Spectrometer). The total phenolics were quantified by a standard curve of chlorogenic acid equivalents (CAE) and expressed as mg CAE. g^{-1} of dry weight.

Antioxidant Activity (DPPH Assay)

The antioxidant activity was analysed by the scavenging ability of the 2,2-diphenyl-1-picrylhydrazyl (DPPH; Sigma-Aldrich, Darmstadt, Germany) radical following the procedure of Brand-Williams et al. (1995) [24]. The supernatants of extraction (100 μL) and DPPH solution (3.9 mL) were mixed, and the reaction occurred for 40 min. Subsequently, the absorbance was measured in a spectrophotometer at 515 nm. The antioxidant activity was determined using Trolox (Acrós Organics, Geel, Belgium), and the results were expressed by Trolox Equivalent Antioxidant Capacity [TEAC ($\mu\text{mol Trolox. g}^{-1}$ of dry matter)].

Antioxidant Activity (FRAP Assay)

The Ferric Reducing Antioxidant Power (FRAP) assay was performed according to Benzie and Strain (1996) [25] with some modifications. The reaction started with the mixture of the FRAP solution (2.7 mL), extract samples (90 μL), and nanopure water (270 μL), and subsequently warmed in a water bath at 37 °C for 30 min. The absorbance of the colored product (ferrous tripyridyltriazine complex) was measured at 595 nm. The antioxidant capacity was calculated using a standard Trolox (Acrós Organics, Belgium) curve and the results were expressed as TEAC ($\mu\text{mol Trolox. g}^{-1}$ of dry matter).

Antioxidant Activity (ABTS-Assay)

Antioxidant activity was measured using 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS; Sigma-Aldrich, Germany) method as described by Re et al. (1999) and Rufino et al. (2007) [26,27] with some modifications. The reaction was performed by sample aliquots (30 μL) mixed with ABTS solution (2970 μL) for 6 min, and the absorbance at 734 nm was measured. The absorbance of the samples was related to the Trolox (Acrós Organics, Belgium) standard curve and the results were expressed as TEAC ($\mu\text{mol Trolox. g}^{-1}$ of dry matter).

2.2.2. Enzymatic Activity

Bromelain Activity

The bromelain assay was determined according to Chakraborty et al. (2014) [10] with some modifications reported by Santos et al. (2020) [28]. The assay consisted in mixing the enzyme extract (50 μL) and 1150 μL casein 1% (*w/v*) (Sigma-Aldrich, Germany) solution in 0.1 mol L⁻¹ glycine (Sigma-Aldrich, Germany) and 25 mmol L⁻¹ cysteine. The mixture was incubated in a shaking water bath (10 min at 37 °C) and the reaction was stopped by adding 5% (*w/v*) trichloroacetic acid (1.8 mL). The assay mixture was filtrated (0.45 μm) and the absorbance was measured at 280 nm. The bromelain activity was calculated using a tyrosine (Alfa Aesar, Lancashire, United Kingdom) standard curve and expressed as the amount of tyrosine on a dry weight basis ($\mu\text{mol tyrosine. min}^{-1}. \text{g}^{-1}$ of dry weight).

Phenylalanine Ammonia-Lyase (PAL) Activity

The PAL activity determination was performed as described in Alegria et al. (2016) [29] with few modifications. The reaction was performed by addition of borate buffer (2 mL), 100 mmol L⁻¹ L-phenylalanine (Alfa Aesar, United Kingdom) substrate solution (600 μL) and crude enzyme extract (400 μL). Samples' absorbency was read before and after incubation in a water bath (40 °C, 1 h) in a spectrophotometer at 290 nm. PAL activity was expressed as the amount of synthesized t-cinnamic acid on a dry weight basis ($\mu\text{mol t-cinnamic acid. g}^{-1}$ of dry weight. h⁻¹).

Polyphenol Oxidase (PPO) Activity

The extraction of PPO followed a modified method of Zhou et al. (2003) [30] with some modifications. PPO activity was assayed spectrophotometrically by a modified

method based on Babu et al. (2008) [31] with some modifications. The assay mixture consisted of 2.5 mL substrate solution (50 mmol L⁻¹ catechol (Alfa Aesar, United Kingdom) in 0.1 mol L⁻¹ phosphate buffer, pH 6.5) and enzyme extract (500 µL). The rate of catechol oxidation was followed at 420 nm for 1 min and the enzyme activity unit was defined as the amount of enzyme that causes an increase of 0.1 in absorbance per min and per mL ($\Delta\text{Abs. min mL}^{-1}$).

2.3. Statistical Analysis

The experimental data were statistically evaluated using Statistica™ v.8 Software (StatSoft Inc., Tulsa, OK, USA, 2007) [32]. Statistically significant differences ($p < 0.05$) between samples were defined using Tukey's honestly significant difference test.

3. Results and Discussion

3.1. Total Phenolic Content and Antioxidant Activity

3.1.1. Pineapple Shell

According to Figure 1, the wound had no effect on the content of phenolic content (TPC) in the pineapple shell, showing only a slight increase when combined with the treatment temperature. The wounded samples showed a significant ($p < 0.05$) reduction in TPC compared to the raw material (95.89 ± 0.84 mg CAE. g⁻¹ dry matter), with losses between 10 and 17% depending on the storage time. The wound samples with heat treatment at 30 °C and without storage time (0 h) showed (97.86 ± 0.67 mg CAE. g⁻¹ dry matter) an increase of 2% compared to the raw material (95.89 ± 0.84 mg CAE. g⁻¹ dry matter). The wounded samples had the lowest TPC values, regardless of the storage time. In the case of no wound samples, the storage time of 24 h (104.08 ± 0.84 mg CAE. g⁻¹ dry matter) showed a 9% increase in TPC values ($p < 0.05$) compared to the raw material (95.89 ± 0.84 mg CAE. g⁻¹ dry matter). The abiotic stress conditions that increase significantly TPC are whole samples ($p < 0.05$), subjected to heat treatment of 30 °C and with a storage time of 48 h. These samples (Figure 1) showed an increase (109.40 ± 0.70 mg CAE. g⁻¹ dry matter) of 14% compared to the raw material (95.89 ± 0.84 mg CAE. g⁻¹ dry matter). The samples with heat treatment at 30 °C showed an increase of 8% with an increase in the storage time between 0 h (101.63 ± 0.82 mg CAE. g⁻¹ dry matter) and 48 h (109.40 ± 0.70 mg CAE. g⁻¹ dry matter). Heat treatments with a temperature of 40 °C (87.46 – 95.49 mg CAE. g⁻¹ dry matter) are not beneficial for TPC, when compared to treatments at a temperature of 30 °C (93.17 – 109.40 mg CAE. g⁻¹ dry matter).

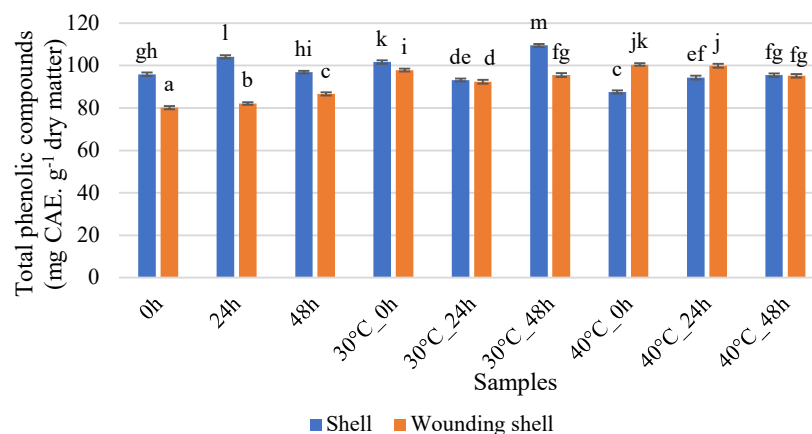
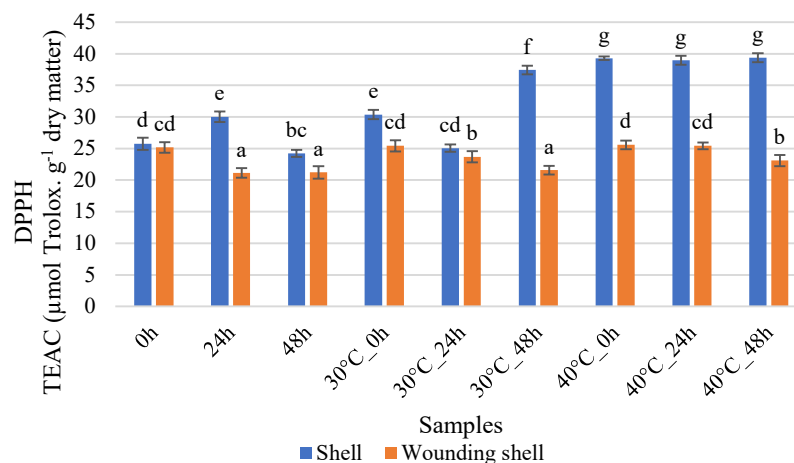


Figure 1. Effect of abiotic stresses, heat treatment and wounding, on total phenolic content of pineapple shell. Error bars stand for \pm standard deviation ($n = 9$). Different letters express significant differences between samples.

In general, wounding has a negative effect on the antioxidant activity when compared to the values obtained for the raw material (Figure 2). By the DPPH method, the storage time that seems to maximize the antioxidant activity is 48 h, with an increase of 45% in the whole sample with heat treatment at 30 °C ($37.40 \pm 0.67 \mu\text{mol Trolox. g}^{-1}$ dry matter) and an increase of 53% in the whole sample heat treated with 40 °C ($39.36 \pm 0.71 \mu\text{mol Trolox. g}^{-1}$ dry matter), when compared to raw material ($25.73 \pm 0.97 \mu\text{mol Trolox. g}^{-1}$ dry matter). The samples also showed an increase in the antioxidant activity values by both FRAP and ABTS methods. For the storage time of 48 h, the samples with heat treatment at 30 °C increased their antioxidant activity in 40% as evaluated by FRAP ($35.80 \pm 0.77 \mu\text{mol Trolox. g}^{-1}$ dry matter) and in 38% as evaluated by ABTS, while the corresponding increases in the antioxidant activity of the samples with thermal treatment at 40 °C were of 75% ($44.77 \pm 0.85 \mu\text{mol Trolox. g}^{-1}$ dry matter) and 56% ($45.17 \pm 0.94 \mu\text{mol Trolox. g}^{-1}$ dry matter), respectively, compared to the raw material (FRAP: $25.59 \pm 0.76 \mu\text{mol Trolox. g}^{-1}$ dry matter; ABTS: $29.05 \pm 0.83 \mu\text{mol Trolox. g}^{-1}$ dry matter). The storage time (24 h) in the whole samples, compared to the raw material (DPPH: $25.73 \pm 0.97 \mu\text{mol Trolox. g}^{-1}$ dry matter; FRAP: $25.59 \pm 0.76 \mu\text{mol Trolox. g}^{-1}$ dry matter; ABTS: $29.05 \pm 0.83 \mu\text{mol Trolox. g}^{-1}$ dry matter), showed increases of 17%, 18%, and 20% in the antioxidant activity when evaluated by the methods of DPPH ($30.01 \pm 0.84 \mu\text{mol Trolox. g}^{-1}$ dry matter), FRAP ($30.26 \pm 0.60 \mu\text{mol Trolox. g}^{-1}$ dry matter), and ABTS ($34.80 \pm 0.44 \mu\text{mol Trolox. g}^{-1}$ dry matter), respectively ($p < 0.05$). In the case of storage time 48 h, there was a decrease in antioxidant activity by all methods (Figure 2). Globally, sample treatments at a temperature of 40 °C were more efficient in increasing antioxidant activity than treatments with temperatures of 30 °C, regardless of the method used to determine antioxidant activity (Figure 2). The treatments at 40 °C in whole pineapple shell did not present significant differences in antioxidant activity measured by DPPH ($38.95\text{--}39.36 \mu\text{mol Trolox. g}^{-1}$ dry matter), although there has been a significant increase ($p < 0.05$) in the antioxidant activity by the FRAP ($44.72\text{--}44.77 \mu\text{mol Trolox. g}^{-1}$ dry matter) and ABTS ($45.17\text{--}46.02 \mu\text{mol Trolox. g}^{-1}$ dry matter) methods, between the storage time 0 h (DPPH: $39.29 \pm 0.27 \mu\text{mol Trolox. g}^{-1}$ dry matter; FRAP: $39.53 \pm 0.78 \mu\text{mol Trolox. g}^{-1}$ dry matter; ABTS: $41.94 \pm 0.85 \mu\text{mol Trolox. g}^{-1}$ dry matter) and the longest storage times (24 h and 48 h). In wounding shell samples, the heat treatments at 40 °C did not show significant differences between 0 h (DPPH: $25.57 \pm 0.69 \mu\text{mol Trolox. g}^{-1}$ dry matter; FRAP: $33.43 \pm 0.54 \mu\text{mol Trolox. g}^{-1}$ dry matter; ABTS: $31.37 \pm 0.88 \mu\text{mol Trolox. g}^{-1}$ dry matter) and 24 h storage time, and decrease for 48 h storage time (DPPH: $23.08 \pm 0.87 \mu\text{mol Trolox. g}^{-1}$ dry matter; FRAP: $30.97 \pm 0.58 \mu\text{mol Trolox. g}^{-1}$ dry matter; ABTS: $29.43 \pm 0.80 \mu\text{mol Trolox. g}^{-1}$ dry matter) in antioxidant activity by any of the methods studied ($p < 0.05$).



(a)

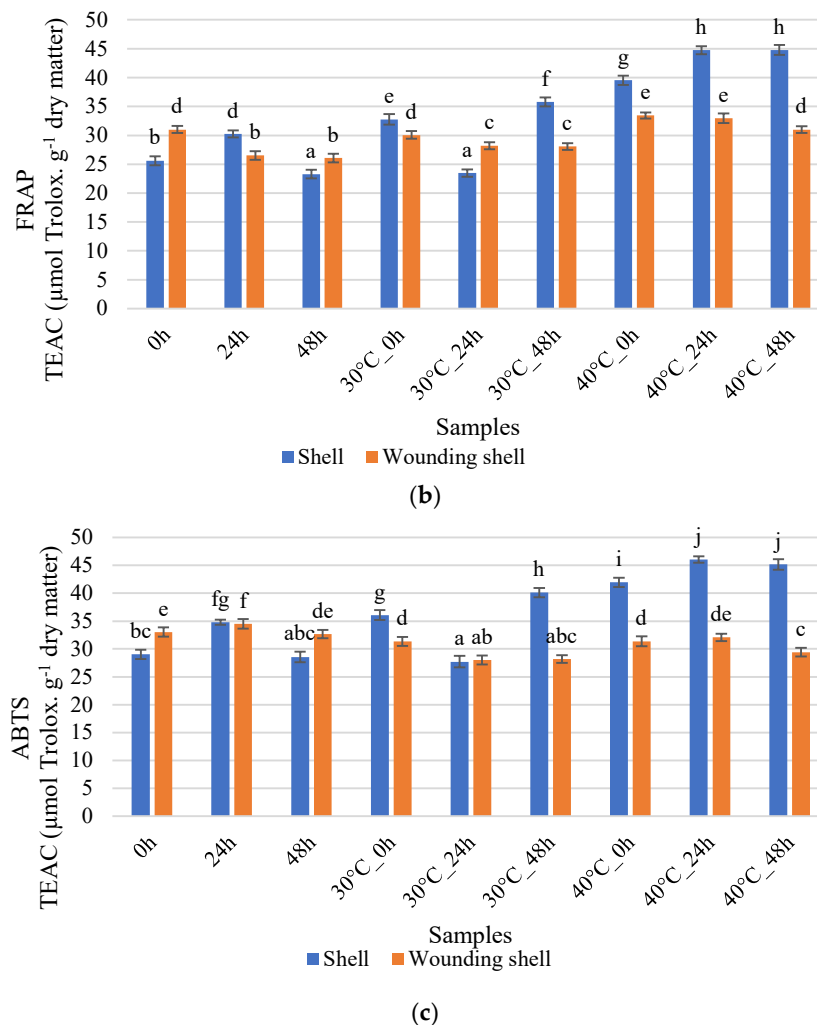


Figure 2. Effect of abiotic stresses, heat treatment and wounding, on antioxidant activity of pineapple shell: (a) DPPH (2,2-diphenyl-1-picrylhydrazyl); (b) FRAP (Ferric Reducing Antioxidant Power); and (c) ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)). Error bars stand for \pm standard deviation ($n = 9$). Different letters express significant differences between samples.

Jacobo-Velázquez et al. (2011) [33] have also studied the effect of wounding and heat treatment as abiotic stresses on other fruits and vegetables. Carrots (*Daucus carota*), when subjected to stress from wounding and hyperoxia, function as bio-factories of caffeoylquinic acids. Carrots with stress caused by wounding and stored for 48 h at 20 °C showed an increase of $\pm 287\%$ in the total phenolic content. The accumulation of stress-induced total phenolic content in carrots is influenced by the signalling molecules of reactive oxygen species. The application of wounds promoted an increase in total phenolic content and also of antioxidant activity in carrots stored at 20 °C for 48 h, and the accumulation of total phenolic content was intensified with an increase in the intensity of the wound. The shredded carrots stored under air showed a higher increase in the value of antioxidant activity ($\sim 240\%$) than samples before storage. Surjadinata and Cisneros-Zevallos (2012) [7] studied the effect of wounding on phenolic compounds in carrots and found that more intense damages with 4 days of storage at 15 °C increased the phenolic content by 2.5 times compared to whole samples. The most severe wound in the tissues produced a more accentuated response, which developed a higher synthesis of phenolic compounds and antioxidants. Wounding is thus an economical process that can be used to enrich fresh products with phenolic compounds and antioxidants [3].

The content of phenolic compounds in whole strawberries without hull was 13% greater than in halved strawberry samples. Whole strawberries without hull and halved strawberries showed no differences in the content of phenolic compounds [34]. Reyes and Cisneros-Zevallos (2003) [35] studied purple-fleshed potatoes (cv. All Blue) and also evaluated the effect of various abiotic stresses on the induction of phenolic compounds and antioxidant activity. The total phenolic compounds in the purple-fleshed potatoes increased by ~60%, as well as the antioxidant capacity increased by ~85%.

Contrary to that obtained by other authors, wounds in pineapple shell by-products maintained or decreased the content of phenolic compounds. The synthesis of total phenolic compounds and the consequent antioxidant activity in pineapple by-products may have been limited by the storage temperature since the refrigeration temperature (5 °C) is not close to the optimum temperature of enzymatic activity.

Not all studies have seen an increase in antioxidant activity after wounding. A study of several fruits such as pineapple, mango, cantaloupe, watermelon, strawberry, and kiwi fruit compared the effect of fresh cut with the whole fruit and concluded that the cut induced losses of antioxidant carotenoids (0–25%) during 9 days storage in air at 5 °C [36]. In the present study, the wound pineapple shell samples showed a decrease in antioxidant activity only by the DPPH method, although the storage time in present study (24 h or 48 h) was shorter than the storage time (9 days) studied by Gil, Aguayo, and Kader (2006) [36].

Our results show that heat treatment, as well as storage time, influence the synthesis of bioactive compounds. A study carried out with mangoes observed an increase in polyphenols and carotenoids, as well as an increase in antioxidant capacity in samples treated with hot water (immersion at 50 °C for 60 min and successive storage at 5 °C and 20 °C) compared to untreated fruits. The determining factor in changes in polyphenolic content and antioxidant capacity was the storage temperature and not the effect of the treatment with immersion in hot water [37]. The heat treatment (46 or 50 °C for 30 or 75 min and stored at 6 °C for 9 d) also prolonged the shelf-life and promoted an increase in bioactive compounds in another study developed with mangoes [38].

The total phenolic compounds and the antioxidant capacity in fresh-cut pineapple showed higher values under low O₂ (12% O₂ in combination with 1% CO₂) than under high O₂ (38% O₂) atmosphere [39]. In our work, the pineapple shell samples were packed with 85% of vacuum; therefore, the oxygen inside the package (about 3% O₂) is less than the oxygen (21% O₂) present in a package with atmospheric pressure.

Postharvest stress can stimulate some enzymes and/or non-enzymatic antioxidant systems of fruits, maintaining fruit quality and improving antioxidant capacity [40]. On the other hand, postharvest techniques cause damage and increase the oxidative stress that is reduced or limited by atmospheres with low oxygen content [41]. Another possible factor that may have inhibited the synthesis and accumulation of phenolic compounds was the composition of the atmosphere (absence of oxygen) since samples of pineapple shell by-products were vacuum-packed and there was virtually no oxygen available for oxidation reactions.

3.1.2. Pineapple Core

The TPC (Figure 3) showed a significant ($p < 0.05$) increase (17%) in the sample wounded with heat treatment at 40 °C and storage time of 24 h (95.34 ± 0.82 mg CAE. g⁻¹ dry matter), compared to the raw material (81.53 ± 0.81 mg CAE. g⁻¹ dry matter). With the longest storage time, the TPC decreased, with a reduction of 7% for storage time 24 h (76.02 ± 0.60 mg CAE. g⁻¹ dry matter) and 27% for storage time 48 h (59.59 ± 0.69 mg CAE. g⁻¹ dry matter). Wounding (63.93 ± 0.59 mg CAE. g⁻¹ dry matter) reduced significantly ($p < 0.05$) the TPC in 22% compared to the raw material (81.53 ± 0.81 mg CAE. g⁻¹ dry matter), although the reduction is only of 3% in the case of wounded samples with a storage time of 48 h (78.92 ± 0.61 mg CAE. g⁻¹ dry matter).

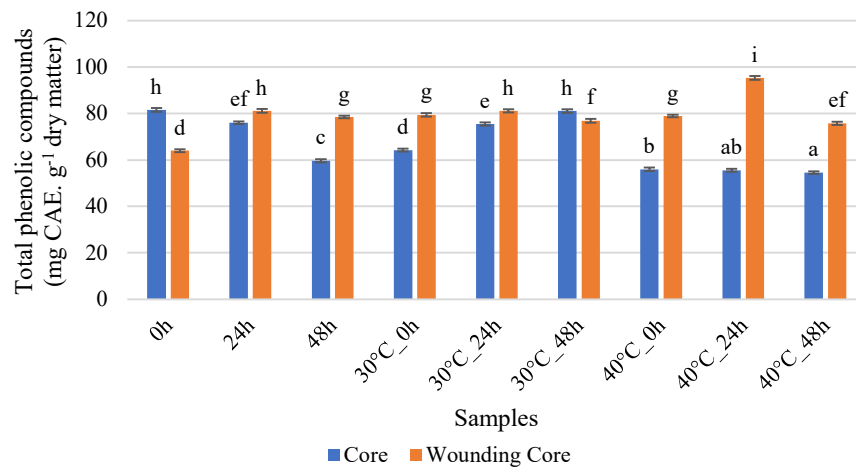
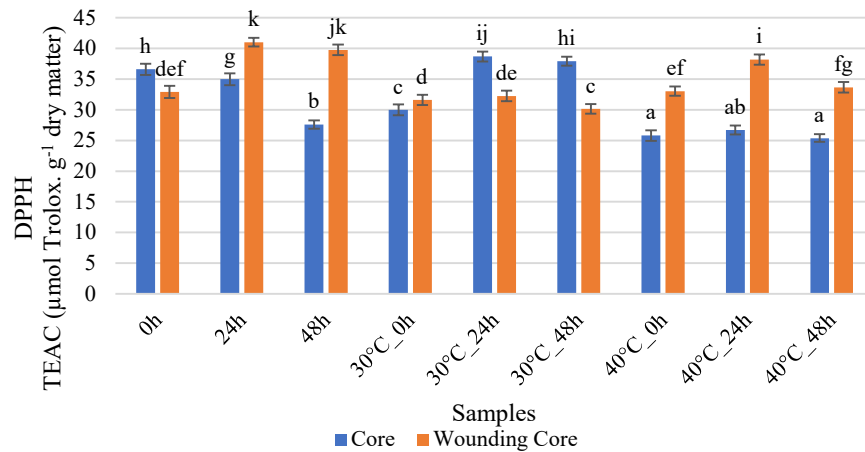
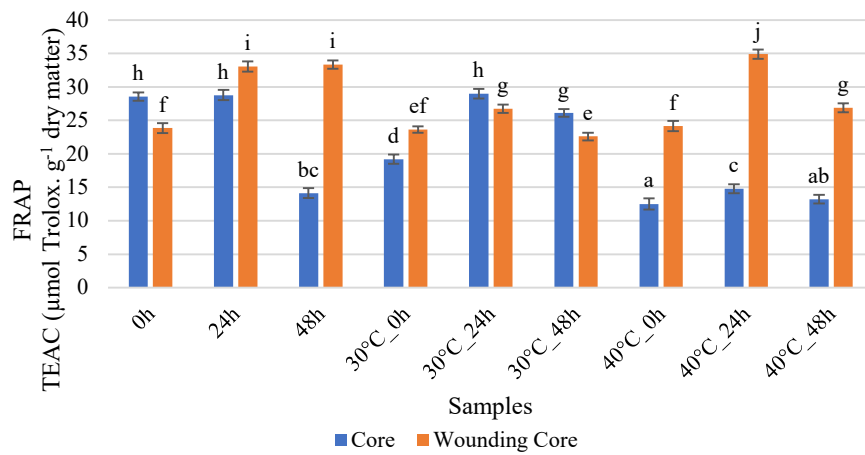


Figure 3. Effect of abiotic stresses, heat treatment and wounding, on total phenolic content of pineapple core. Error bars stand for \pm standard deviation ($n = 9$). Different letters express significant differences between samples.

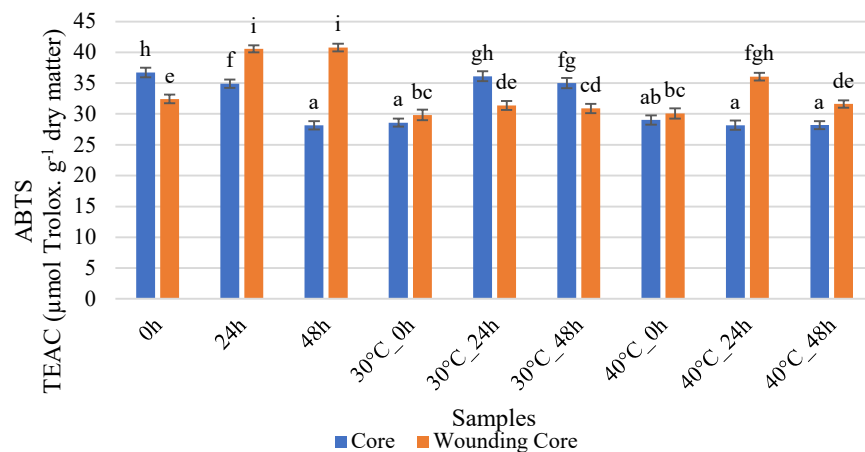
The abiotic stress (Figure 4) caused by the wound along with the storage time promoted a significant increase in antioxidant activity ($p < 0.05$). The samples with wound and storage time 24 h showed an increase in antioxidant activity of 12%, 16%, and 11% by the DPPH ($41.04 \pm 0.71 \mu\text{mol Trolox. g}^{-1}$ dry matter), FRAP ($33.06 \pm 0.77 \mu\text{mol Trolox. g}^{-1}$ dry matter) and ABTS ($40.55 \pm 0.58 \mu\text{mol Trolox. g}^{-1}$ dry matter) methods, respectively, compared to the raw material (DPPH: $36.62 \pm 0.91 \mu\text{mol Trolox. g}^{-1}$ dry matter; FRAP: $28.55 \pm 0.63 \mu\text{mol Trolox. g}^{-1}$ dry matter; ABTS: $36.62 \pm 0.80 \mu\text{mol Trolox. g}^{-1}$ dry matter). The samples with wound and storage time 48 h showed a significant increase of 9% by the DPPH ($39.76 \pm 0.86 \mu\text{mol Trolox. g}^{-1}$ dry matter) method, 17% by the FRAP ($33.33 \pm 0.62 \mu\text{mol Trolox. g}^{-1}$ dry matter) method and 11% by the ABTS ($40.77 \pm 0.63 \mu\text{mol Trolox. g}^{-1}$ dry matter) method, compared to the raw material, respectively. The samples wounded with storage time 24 h and 48 h showed no significant differences in antioxidant activity by any of the methods studied (DPPH, FRAP, and ABTS). The maximum antioxidant activity obtained by the FRAP method was observed for the wounded sample, with heat treatment at 40 °C and storage time 24 h ($34.90 \pm 0.70 \mu\text{mol Trolox. g}^{-1}$ dry matter), which registered an increase of 22% ($p < 0.05$), compared to the raw material ($28.55 \pm 0.63 \mu\text{mol Trolox. g}^{-1}$ dry matter). As observed in TPC, the increase in storage time maintained or reduced the antioxidant activity (Figure 4). The storage time of 24 h maintained the antioxidant activity as measured by the FRAP ($28.77 \pm 0.77 \mu\text{mol Trolox. g}^{-1}$ dry matter) method and reduced the antioxidant activity by 5% when evaluated by DPPH ($35.00 \pm 0.96 \mu\text{mol Trolox. g}^{-1}$ dry matter) and ABTS ($34.90 \pm 0.67 \mu\text{mol Trolox. g}^{-1}$ dry matter), compared to the raw material, respectively. In the case of storage time 48 h, the antioxidant activity decreased significantly ($p < 0.05$) 25%, 51%, and 23% by the DPPH ($27.57 \pm 0.68 \mu\text{mol Trolox. g}^{-1}$ dry matter), FRAP ($14.12 \pm 0.75 \mu\text{mol Trolox. g}^{-1}$ dry matter) and ABTS ($28.15 \pm 0.66 \mu\text{mol Trolox. g}^{-1}$ dry matter) methods, respectively, compared to the raw material (DPPH: $36.62 \pm 0.91 \mu\text{mol Trolox. g}^{-1}$ dry matter; FRAP: $28.55 \pm 0.63 \mu\text{mol Trolox. g}^{-1}$ dry matter; ABTS: $36.62 \pm 0.80 \mu\text{mol Trolox. g}^{-1}$ dry matter).



(a)



(b)



(c)

Figure 4. Effect of abiotic stresses, heat treatment and wounding, on antioxidant activity of pineapple core: (a) DPPH (2,2-diphenyl-1-picrylhydrazyl); (b) FRAP (Ferric Reducing Antioxidant Power); and (c) ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)). Error bars stand for \pm standard deviation ($n = 9$). Different letters express significant differences between samples.

Wound-induced signalling starts by damage, then the signalling migrates to the adjacent undamaged tissue and can change the metabolic activity promoting phenolics production as observed in fresh-cut lettuce (*Lactuca sativa* L.) samples [42]. Plant tissue responds to damage by increasing respiration rate and ethylene production [43]. A study carried out with freshly cut carrot samples found that the increase in respiration occurs due to the synthesis of enzymes involved in the respiratory pathway [44]. Phenolic compounds also increase due to wound induction in the enzyme responsible for phenolic biosynthesis, phenylalanine ammonium lyase (EC 4.3.1.5). Phenolic compounds can be oxidized to quinones by another polyphenol oxidase enzyme (EC 1.10.3.1), which polymerizes and produce dark compounds as seen in wound lettuce samples [45].

The values of phenolic content from wounded pineapple core samples do not evidence the effect of damages on changing respiratory activity, metabolic activity, and the synthesis of the new bioactive compounds. The enzymatic activity promoted by the wounds was quantified in order to obtain more information about the phenomena that occurred in the samples of pineapple by-products.

The total antioxidant capacity of fruits and vegetables may increase due to wounding, since the wounds induce the synthesis of phenolics and not the increase in antioxidant compounds such as ascorbic acid [46]. Wounding is one of the stresses most applied to freshly processed products. The shape of the cut is of great importance in the quality of freshly cut fruits and vegetables. The trapezoidal cut of melon was considered the best option for the panelists due to the balance between texture and translucency. On the other hand, the melon cylinders were firmer and had greater translucency during 10 days of storage [47]. The slices of papaya (*Carica papaya* L.) presented 2 more days at 5 °C and 1 more day at 10 °C of shelf-life compared to the papaya cubes [48]. Contrary to the results obtained for total phenolic content, in the present work the antioxidant activity of pineapple core samples was favored by the wound, although the longer storage time (48 h) did not show increases in antioxidant activity compared to the shorter storage time (24 h).

The phenolic compounds of fruits can be modified by oxidative reactions during processing and storage, namely by the antioxidant activity of phenols and as substrates for oxidative browning reactions. Phenolics can be reactive oxygen species and can act as substrates for polyphenol oxidases and as antioxidants for free radicals, which are the main oxidants in food [49]. Hot water treatment (45 °C for 10 min and storage at 4 and 25 °C) applied to kiwis showed advantages at the microbiological level and accumulation of reactive oxygen species. This heat treatment also promoted a significant increase in the activity of antioxidant enzymes and the content of total phenolic content [50].

In the present study, the content of phenolic content in the pineapple core samples was improved with the application of thermal treatments (40 °C for 10 min, storage during 24 h) alike what was observed for the test performed with kiwi fruit reported above. The antioxidant activity in the pineapple core was maximized by the treatment (30 °C for 10 min, storage during 24 h), although wounding caused a greater impact on this increase.

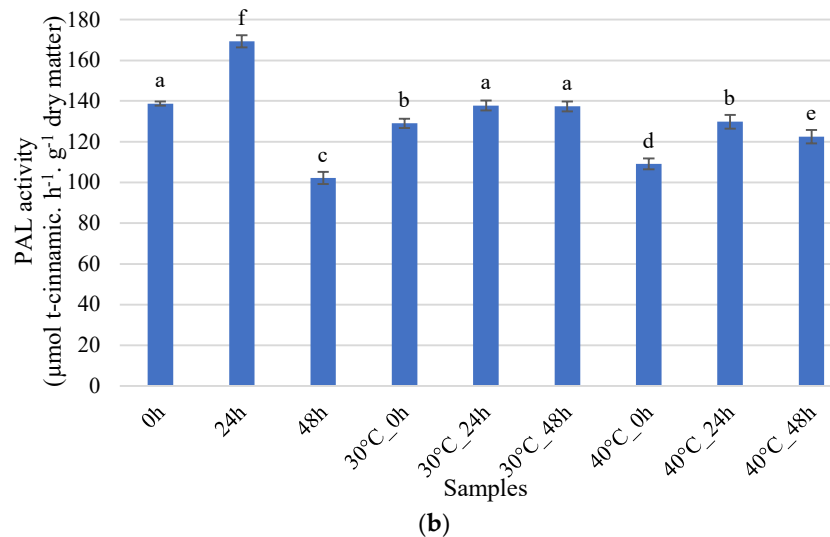
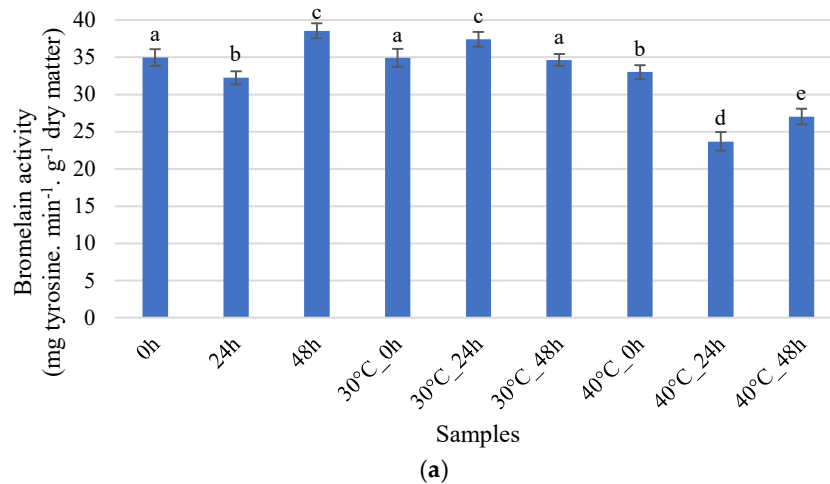
Kim, Lounds-Singleton, and Talcott (2009) [51] observed polyphenolic and antioxidant changes in mangoes after immersion in hot water (46.1 °C, 70–110 min, 4 days of storage at 25 °C). The polyphenolic gallic acid and gallotannins, as well as total soluble phenolics, decreased due to long treatments in hot water, while the antioxidant capacity was maintained after the treatment. All treatments reduced total soluble phenolics and antioxidant capacity over 4 days of storage, regardless of treatment time. In general, the heat treatments applied to the pineapple core decreased or maintained the content of phenolic compounds and the antioxidant activity, as well as the results obtained in the study carried out with mangoes.

3.2. Enzymatic Activity

3.2.1. Pineapple Shell

The values obtained for TPC and antioxidant activity in the pineapple shell indicate that the assay should proceed only with whole samples, excluding wound samples in the analysis of enzymatic activity.

Bromelain activity (Figure 5a) in pineapple shell was not favorably influenced by heat treatments at 40 °C (23.70–33.00 mg tyrosine.min⁻¹.g⁻¹ dry matter). The thermal treatment at 30 °C showed similar values to the raw material (34.97 ± 1.11 mg tyrosine.min⁻¹ g⁻¹ dry matter) for a storage time of 0 h (34.90 ± 1.23 mg tyrosine.min⁻¹ g⁻¹ dry matter) and 48 h (34.64 ± 0.79 mg tyrosine.min⁻¹ g⁻¹ dry matter), and showed a significant increase ($p < 0.05$) of 7% in the enzymatic activity of bromelain for treatments with a temperature of 30 °C and storage time of 24 h (37.42 ± 0.98 mg tyrosine.min⁻¹ g⁻¹ dry matter). The isolated effect of storage time for 24 h (32.23 ± 0.87 mg tyrosine.min⁻¹ g⁻¹ dry matter) significantly decreased (8%) bromelain activity, and storage time at 48 h (38.55 ± 1.00 mg tyrosine.min⁻¹ g⁻¹ dry matter) significantly increased (10%) bromelain activity ($p < 0.05$), compared to the raw material (34.97 ± 1.11 mg tyrosine.min⁻¹ g⁻¹ dry matter). The enzymatic activity of bromelain was improved by the lower temperature (30 °C) combined with the shorter (37.42 ± 0.98 mg tyrosine.min⁻¹ g⁻¹ dry matter) storage time (24 h) or by the longer storage time (48 h) without the effect of heat treatment (38.55 ± 1.00 mg tyrosine.min⁻¹ g⁻¹ dry matter).



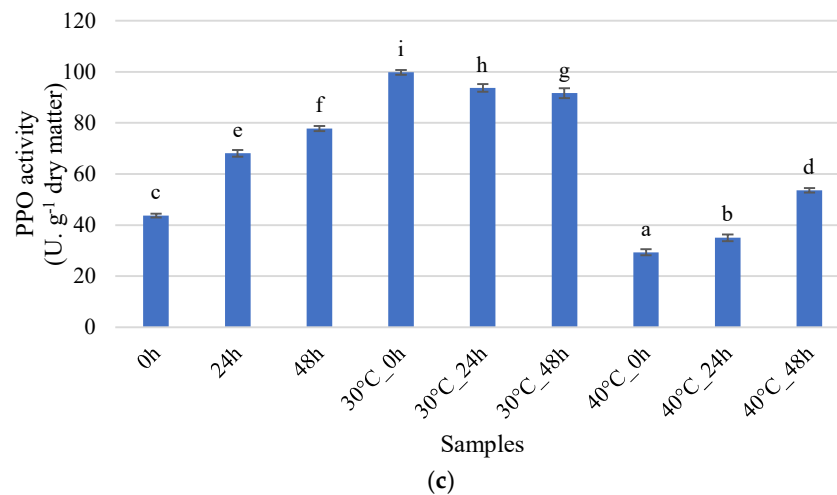


Figure 5. Effect of heat treatment, on the enzymatic activity of pineapple shell: (a) bromelain activity; (b) phenylalanine ammonia-lyase activity (PAL); and (c) polyphenol oxidase activity (PPO). Error bars stand for \pm standard deviation ($n = 9$). Different letters express significant differences between samples.

The behaviour of bromelain under the effect of postharvest abiotic stresses needs to be further studied since there are only a few works in this area. One of them concluded that, in order to maintain the beneficial effects of pineapples for health, namely, the activity of bromelain, the treatment temperature that allows obtaining maximum functionality varies between 35 and 50 °C and the pH between 4.5 to 8.5 [52]. The pineapple shell samples evaluated in the present work showed slight decreases when subjected to thermal treatments with temperatures of 40 °C and maintained or showed a slight increase with the temperature of 30 °C. The effect of the storage time seems to be more evident than the heat treatment.

Bromelain is very stable at low temperature, with incubation at 40 °C showing no loss of activity in treatments up to 60 min, while treatments at 50 °C showed 83% of the initial activity [53]. The results of enzymatic activity obtained in the present work for the pineapple shell agree with those obtained by other authors. The heat treatment at 40 °C showed enzymatic activity between 68 and 94% when compared to the initial activity.

The enzymatic activity (Figure 5b) of phenylalanine ammonia-lyase (PAL) was maintained or was reduced with the application of abiotic stresses at 30 °C (129.10–137.83 $\mu\text{mol t-cinnamic.h}^{-1} \text{g}^{-1}$ dry matter) and 40 °C (109.17–129.83 $\mu\text{mol t-cinnamic.h}^{-1} \text{g}^{-1}$ dry matter), as compared to the raw material (138.77 \pm 0.97 $\mu\text{mol t-cinnamic.h}^{-1} \text{g}^{-1}$ dry matter). The 24 h storage time (169.31 \pm 2.99 $\mu\text{mol t-cinnamic.h}^{-1} \text{g}^{-1}$ dry matter) showed PAL enzyme activity 22% higher ($p < 0.05$) than the raw material (138.77 \pm 0.97 $\mu\text{mol t-cinnamic.h}^{-1} \text{g}^{-1}$ dry matter), which suggests that the isolated effect of the storage time has an important effect on PAL enzymatic activity (Figure 5b). PAL's enzymatic activity is related to the synthesis of TPC, but this behaviour was not observed in all studied samples. The whole pineapple shell sample with storage time 24 h (169.31 \pm 2.99 $\mu\text{mol t-cinnamic.h}^{-1} \text{g}^{-1}$ dry matter) showed an increase of 22% in the enzymatic activity of PAL, and consequently an increase of 9 % in the TPC values (104.08 \pm 0.74 mg CAE.g⁻¹ dry matter), compared to the raw material (PAL: 138.77 \pm 0.97 $\mu\text{mol t-cinnamic.h}^{-1} \text{g}^{-1}$ dry matter; TPC: 95.89 \pm 0.84 mg CAE. g⁻¹ dry matter). The sample with heat treatment of 30 °C and storage time of 48 h (137.34 \pm 2.46 $\mu\text{mol t-cinnamic.h}^{-1} \text{g}^{-1}$ dry matter) showed non-significant differences concerning the raw material (138.77 \pm 0.97 $\mu\text{mol t-cinnamic.h}^{-1} \text{g}^{-1}$ dry matter) in the enzymatic activity of PAL, although it showed a significant ($p < 0.05$) increase of 14% in the TPC values (109.40 \pm 0.70 mg CAE. g⁻¹ dry matter), comparatively with the raw material (95.89 \pm 0.84 mg CAE. g⁻¹ dry matter).

The biosynthesis of several secondary products derived from phenylpropanoids is triggered by the activity of PAL as a first step in the metabolism of phenylpropanoids to catalyze the deamination of L-phenylalanine in trans-cinnamic acid [54]. The accumulation of secondary metabolites is crucial in the defense mechanisms of plants, such as flavonoids, lignins, coumarins, and other phenolic compounds [22]. A kinetic model was developed to describe the transformations that occur in phenolic compounds during the storage of fresh products through the reactions responsible for the de novo biosynthesis of phenols by the enzymatic activity of PAL and oxidation catalyzed by the enzyme PPO. The model explains the development of phenolic content as a function of time and described the effect of variables on phenolic synthesis and oxidation. An increase of 5 °C in the storage temperature in fresh-cut purslane samples did not show modifications in the metabolism of phenylpropanoids, although it did show a significant increase in the oxidation rate. The apples and broccoli samples indicated differences in the initial phenolic synthesis and content [55].

The stress caused by temperature did not alter the accumulation of phenolic compounds significantly compared to the control in purple-flesh potatoes, although the damage promoted an increase in the enzymatic activity of PAL and the accumulation of phenolic compounds [35]. Contrary to the previous study, the temperature applied to the pineapple shell samples did not affect the enzymatic activity of PAL, but it did affect the synthesis of phenolic compounds. The de novo biosynthesis of phenols in pineapple peel samples by the enzymatic activity of PAL and oxidation catalyzed by PPO may have been limited by packaging conditions (vacuum).

The enzymatic activity of PAL increased with increasing storage temperature is defined as the ideal temperature for enzymatic activity at 37 °C [56]. Storage conditions can limit PAL activity since the refrigeration temperature (5 °C) has been carried out. This reason may explain the fact that the enzyme activity is reduced and the synthesis of phenolic content also.

In the pineapple shell, the storage time (48 h) and the moderate temperature had no effect on the development of phenolic content and antioxidant activity. These treatments did not prove to be advantageous and it would be more interesting, from an economic point of view, to use pineapple residues or extracted compounds in the first 24 h after the pineapple fruit processing by the industry.

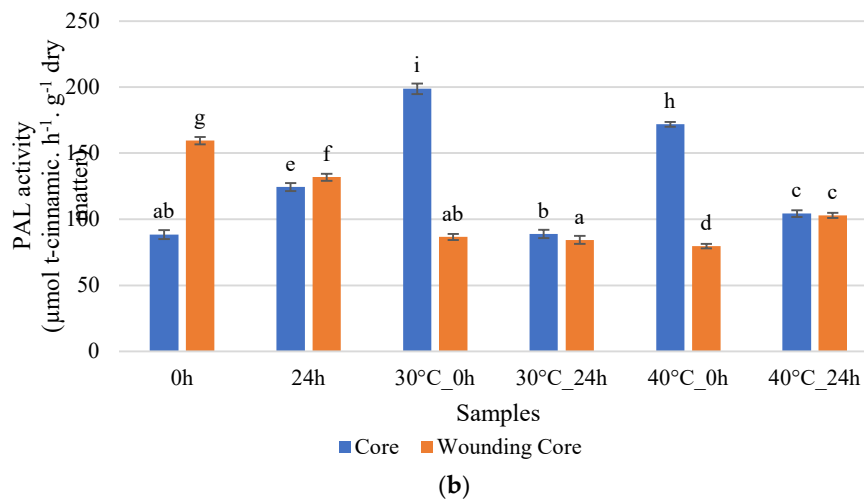
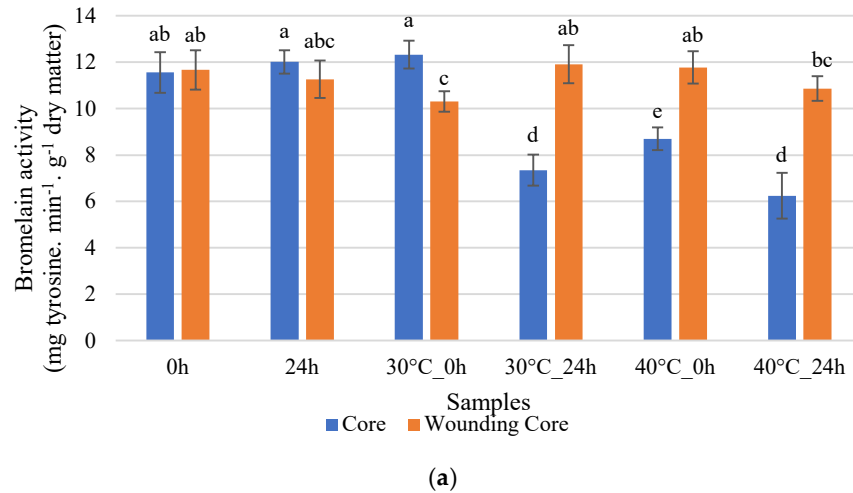
The enzymatic activity of polyphenol oxidase (PPO) in pineapple shell was increased by abiotic stress at 30 °C (91.69–99.78 U. g⁻¹ dry matter), regardless of the storage time (Figure 5c). The heat treatment at 30 °C without storage time (0 h–99.78 ± 0.95 U. g⁻¹ dry matter) increased the enzymatic activity of PPO 128%, compared to the raw material (43.74 ± 0.73 U. g⁻¹ dry matter). The enzymatic activity of PPO showed higher values for the heat treatment of 30 °C, with an increase in enzymatic activity as the storage time decreases. The storage time promoted PPO activity (Figure 5c) by 56% and 78%, respectively, for 24 h (68.09 ± 1.37 U. g⁻¹ dry matter) and 48 h (77.82 ± 1.00 U. g⁻¹ dry matter). Abiotic stress with a temperature of 30 °C promoted a significant increase ($p < 0.05$) of the enzymatic activity of PPO, while heat treatment with a temperature of 40 °C with storage time (0 h–29.37 ± 1.15 U. g⁻¹ dry matter and 24 h–35.03 ± 1.33 U. g⁻¹ dry matter) significantly reduced the activity of PPO ($p < 0.05$), compared to the raw material (43.74 ± 0.73 U. g⁻¹ dry matter).

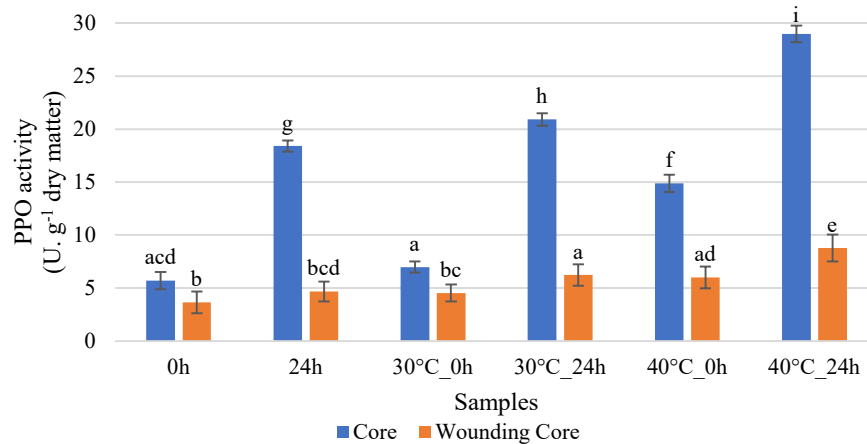
PPO activity has also been evaluated in other fruits. The enzymatic activity of PPO in apples and pears showed greater activity between 30 and 40 °C [57]. Similarly, to the results obtained for apples and pears, the pineapple shell samples used in the present work showed higher values of PPO enzymatic activity in treatments at 30 °C (91.69–99.78 U. g⁻¹ dry matter) than in treatments at 40 °C (29.37–53.67 U. g⁻¹ dry matter).

Pineapple PPO has less cresolase activity than other plants, but it catalyzes the oxidation of diphenol to *o*-quinone in the presence of molecular oxygen [58]. The pineapple shell samples were packed under vacuum, which limits the availability of oxygen and can reduce the occurrence of oxidation reactions.

3.2.2. Pineapple Core

As the storage time of 48 h did not differ in comparison with the storage time of 24 h in TPC and antioxidant activity, the enzymatic determination included only the samples with a storage time of 24 h. The shorter storage time would be beneficial at the industrial level, as it speeds up the production process and reduces costs with the refrigeration system. Wounding and storage time did not influence the enzymatic activity of bromelain since the results (10.31–11.92 mg tyrosine.min⁻¹ g⁻¹ dry matter) did not show significant differences (Figure 6a). In general, heat treatments maintained or decreased the enzymatic activity of bromelain. The whole pineapple core samples did not show significant differences between the thermal treatments at 30 °C (7.35 ± 0.67 mg tyrosine.min⁻¹ g⁻¹ dry matter) and 40 °C (6.25 ± 0.98 mg tyrosine.min⁻¹ g⁻¹ dry matter) with a storage time of 24 h, although the samples showed a reduction in the enzymatic activity of bromelain 36% and 46%, respectively, compared (Figure 6a) with the raw material (11.56 ± 0.88 mg tyrosine.min⁻¹ g⁻¹ dry matter). The maximum value of enzymatic activity (12.33 ± 0.60 mg tyrosine.min⁻¹ g⁻¹ dry matter) was obtained for the sample with heat treatment at 30 °C, without other abiotic stress (wounding or storage time), although there are no significant differences compared to the raw material (11.56 ± 0.88 mg tyrosine.min⁻¹ g⁻¹ dry matter).





(c)

Figure 6. Effect of abiotic stresses, heat treatment and wounding, on the enzymatic activity of pineapple core: (a) bromelain activity; (b) phenylalanine ammonia-lyase activity (PAL); and (c) polyphenol oxidase activity (PPO). Error bars stand for \pm standard deviation ($n = 9$). Different letters express significant differences between samples.

A study carried out Poh and Abdul Majid (2011) [59] with pineapple juice observed that the enzymatic activity of bromelain gradually decreased between 25 and 95 °C, although it presented activity stable to heating up to 85 °C. The addition of ethanolic extract of cashew leaves with polyphenols helped stabilizing bromelain activity due to the bromelain-polyphenol complex, that showed good resistance to heat treatment. In the present work, wounding in the pineapple core samples may have released phenolic compounds from the cell compartments, binding with bromelain. This bond may be responsible for the increase in temperature resistance since the stresses did not promote a measurable increase in the enzymatic activity of bromelain.

Pardo et al. (2000) [60] mentioned that bromelain did not lose activity after 120 min incubated at 37 °C and maintained 80% of activity when incubated at 45 °C. Additionally, if the heating is more intense (60 min at 75 °C), bromelain is almost completely inactivated. Vallés, Furtado, and Cantera (2007) [61] also found that there was no loss of bromelain enzyme activity when incubated at 37 °C for 180 min or at 55 °C for 60 min, but at a higher temperature (60 °C, 30 min) bromelain still held 80% of its initial activity. In agreement with the results obtained in the above-mentioned studies, in the present work and for the same temperature range the by-products of pineapple core also maintained the initial enzymatic activity, although in some cases there was a decrease (6–32%).

Whole pineapple core samples with 24 h storage time ($124.38 \pm 3.04 \mu\text{mol t-cinnamic.h}^{-1} \text{g}^{-1}$ dry matter) showed a significant increase (41%) in PAL enzyme activity while samples crushed with 24 h storage time ($131.78 \pm 2.59 \mu\text{mol t-cinnamic h}^{-1} \text{g}^{-1}$ dry matter) showed a significant ($p < 0.05$) reduction (17%) in PAL enzyme activity, when compared to raw material ($88.42 \pm 3.42 \mu\text{mol t-cinnamic h}^{-1} \text{g}^{-1}$ dry matter). Storage time increased the enzymatic activity of PAL (Figure 6b) in whole (88.42 – $124.38 \mu\text{mol t-cinnamic h}^{-1} \text{g}^{-1}$ dry matter) samples and reduced it in wounded samples (159.56 – $131.78 \mu\text{mol t-cinnamic h}^{-1} \text{g}^{-1}$ dry matter). The wounded samples (without storage time) showed ($159.56 \pm 2.81 \mu\text{mol t-cinnamic h}^{-1} \text{g}^{-1}$ dry matter) an increase significantly ($p < 0.05$) of 80% in the enzymatic activity of PAL when compared to the raw material ($88.42 \pm 3.42 \mu\text{mol t-cinnamic h}^{-1} \text{g}^{-1}$ dry matter). The wounded samples with a storage time of 24 h ($131.78 \pm 2.59 \mu\text{mol t-cinnamic h}^{-1} \text{g}^{-1}$ dry matter) showed an increase of 6% in PAL activity when compared to the whole samples with the same storage time ($124.38 \pm 3.04 \mu\text{mol t-cinnamic h}^{-1} \text{g}^{-1}$ dry matter), thus showing an effect of storage time greater than the effect of abiotic stress caused by the wound (Figure 6b). The heat treatment promoted increases in the enzymatic activity of PAL of 94% and 125% for the temperatures of 40 °C (171.88 ± 1.83

$\mu\text{mol t-cinnamic h}^{-1} \text{ g}^{-1}$ dry matter) and $30\text{ }^{\circ}\text{C}$ ($198.77 \pm 3.94 \mu\text{mol t-cinnamic h}^{-1} \text{ g}^{-1}$ dry matter), respectively, when compared to the raw material ($88.42 \pm 3.42 \mu\text{mol t-cinnamic h}^{-1} \text{ g}^{-1}$ dry matter). The temperature was the abiotic stress applied to the pineapple core that was most efficient in increasing the enzymatic activity of PAL, and this effect surpassed that of the other applied stresses (wounding and storage time). However, the effect of wounding alone also showed very interesting results in the enzymatic activation of PAL (Figure 6b). The increase in the enzymatic activity of PAL did not show a direct relationship with the increase in TPC, contrary to what would be expected and has been observed by other authors. A possible explanation for this occurrence may be the virtual absence of oxygen in the packaging (vacuum-packed pineapple core). Oxygen limitation may have inhibited the oxidation of compounds and reduced the synthesis of phenolic compounds.

Stress triggers the rupture of the cell membrane, that in turn causes the oxidation of phenolic compounds due to the combination of phenolics with oxidative enzymes, predominantly PPO, and stimulates the degradation of the antioxidant. A second response is developed to repair the damage caused by the wound and involves the synthesis of monomeric or polymeric phenolics through changes in the activity of PAL, which is the main metabolic enzyme in the phenylpropanoid pathway and is responsible for enhancing antioxidant synthesis [34,62].

Some works have studied the effect of abiotic stresses due to wounding, temperature and storage time in fruits. Jacobo-Velázquez et al., (2011) [33] demonstrated that the maximum activity of PAL in control carrot pieces occurred after 24 h of storage, that is, enzymatic activation does not happen immediately after the wounding and needs time to occur. This stress caused by the wound in the carrots promotes the accumulation of phenolic compounds. The activation of PAL gene expression induced by wounding in the expression of and the consequent accumulation of phenolic compounds is associated with signalling molecules (reactive oxygen species). In Reyes and Cisneros-Zevallos (2003) [35], wounding caused an initial PAL activity increase of ~73 and 14-fold in the flesh and peel of purple-flesh potatoes, respectively. The greater increase in PAL activity promoted a greater increase in total phenolic compounds concentration in purple-flesh potatoes. The correlation between antioxidant capacity and phenolic content is evident through the results obtained for the purple-fleshed potatoes which were wounded. The phenolic content showed increases of 65% and 51% in flesh and peel, and the antioxidant capacity increased 107% and 49% in flesh and peel, respectively.

Van de Velde et al. (2018) [34] carried out a work with fresh strawberries to study the changes in bioactive compounds, enzymatic activity in phenylpropanoid metabolism and quality attributes depending on the effect of wounding, storage temperature and time. The activation of PAL and the resulting synthesis of phenolic compounds was influenced by the intensity of the wound, with an increase of 22% additional phenolics content in quartered strawberries than in whole stored strawberries ($2\text{ }^{\circ}\text{C}$ for 15 days). The enzymatic activity of PAL in strawberries increased with the intensity of the wounding, the intensity of the damage being proportional to the activity of the PAL and the content of phenolic compounds.

Amodio et al. (2014) [55] have also reported comparable results where wounding of lemon samples improved the phenolic content, possibly due to PAL activity. Phenolics' changes during the processing and storage of mandarin samples were also described. Wounded lettuce leaves showed an increase in PAL activity of around eight times and a maximum activity 24 h after damaging [63]. In agreement with these studies, in the present work the pineapple core samples' wounding alone, and wounding combined with the 24 h storage time promoted considerable increases in PAL activity of 80% and 49%, respectively.

Liu et al. (2012) [64] showed that the stresses caused by heat treatments also affect the enzymatic activity of PAL in peaches. Hot water treatments ($40\text{ }^{\circ}\text{C}$ for 5 and 10 min) were applied to peach fruits and there was an accumulation of reactive oxygen species

and induction of the expression of defense-related genes, with greater enzymatic activity, namely in terms of PAL. In the present work, the pineapple core samples that showed higher values of PAL activity were the heat treated (30 °C and 40 °C) whole samples without storage time (0 h).

The effect of the abiotic stress caused by wounding maintained or reduced the enzymatic activity of PPO (Figure 6c). The samples wounded with heat treatment at 40 °C and storage time 24 h (8.77 ± 1.27 U. g⁻¹ dry matter) showed a significant increase ($p < 0.05$) of 54%, compared to the raw material (5.69 ± 0.82 U. g⁻¹ dry matter). This change seems to be promoted by heat treatment and storage time, without interference from wounding. The storage time (24 h) had a positive influence ($p < 0.05$) in PPO activity (18.39 ± 0.54 U. g⁻¹ dry matter), which registered a 223% increase when compared to the raw material (5.69 ± 0.82 U. g⁻¹ dry matter). The synergy between storage time (24 h) and the thermal treatments (30 °C— 20.89 ± 0.59 U. g⁻¹ dry matter and 40 °C— 28.97 ± 0.78 U. g⁻¹ dry matter) resulted in higher values of enzymatic activity of PPO than the ones registered for samples treated with storage time alone (18.39 ± 0.54 U. g⁻¹ dry matter). The pineapple core samples with 24 h storage time and heat treatment showed significant increases ($p < 0.05$) in PPO activity of 14% and 58%, respectively, for the temperatures of 30 °C (20.89 ± 0.59 U. g⁻¹ dry matter) and 40 °C (28.97 ± 0.78 U. g⁻¹ dry matter), when compared to samples subjected to the abiotic stress of storage time for 24 h alone (18.39 ± 0.54 U. g⁻¹ dry matter). The heat treatment thus promoted an increase in the enzymatic activity of PPO.

In plant cells, the phenolic compounds and PPO are in different compartments and do not interact with each other in healthy, integer tissues. It is only when cell walls are broken (e.g., when the fruit is peeled, cut, or crushed) that contact between enzyme and substrate (phenolic compounds) can occur [65]. In general, wounding did not affect the activity of PPO in pineapple core samples. A possible explanation could be the already mentioned oxygen limitation that did not allow the compounds to oxidize. On the other hand, other authors have studied the effect of crushing or wounding on other fruits and vegetables and registered increases in PPO activity. E.g., the samples of quartered strawberries showed greater changes in PPO activity with changes in temperature than whole without hull and halved strawberries [34]. The process of fresh cutting products also increased the activity of both PAL and PPO in response to wounding in samples of broccoli, lettuce leaves, and sliced potato strips [65–67].

In the present work, the heat treatment that maximized the enzymatic activity of PPO in pineapple core samples was the one performed at 40 °C. The same behaviour was observed in mango fruits, in which the optimum temperature for the enzymatic activity of PPO was also 40 °C [68].

4. Conclusions

The present study shows that biosynthesis of health-promoting anti-oxidant phenolic compounds may be induced by wounding and thermal treatments of pineapple by-products. The amount of phenolics is dependent on the type of tissue. Results obtained demonstrated that wounding and thermal treatments (40 °C during 10 min) induced the biosynthesis of phenols and enhance antioxidant activity during a short storage (24 h at 5 °C) of pineapple core by-products. In the samples of pineapple shell, wounding was not advantageous, but a milder heat treatment (30 °C) and a 48 h storage promoted the synthesis of phenolics and antioxidant activity.

The enhancement of the biological properties of pineapple by-products using abiotic stresses allows creating value producing co-products, which may be implemented in the minimal processing industrial units. The reuse of pineapple by-products exceeds the objective of waste management and recycling, aiming at a broader application of restructuring processes and technologies, creating value through a business model, and optimizing the exploitation of resources. To optimize these abiotic treatments to maximize the extraction of antioxidants or bromelain and understand the effect of tissue on responses, further studies are needed.

Author Contributions: Conceptualization, D.I.S., V.D.A., and M.M.-M.; methodology, D.I.S., V.D.A., and M.M.-M.; formal analysis, D.I.S. and D.L.F.; investigation, D.I.S., D.L.F., and M.M.-M.; writing—original draft preparation, D.I.S., D.L.F., and S.C.L.; writing—review and editing, D.I.S., D.L.F., S.C.L., V.D.A., J.A.S., A.A.V., and M.M.-M.; supervision, V.D.A., J.A.S., A.A.V., and M.M.-M.; funding acquisition, V.D.A., J.A.S., A.A.V., and M.M.-M. All authors have read and agreed to the published version of the manuscript.

Funding: The first author acknowledges the financial support from Fundação para a Ciência e a Tecnologia (FCT), Portugal, through Doctoral fellowship (SFRH/BD/109124/2015). This work was supported by the national funding of FCT, under the scope of the strategic funding to the research units LEAF, Linking Landscape, Environment, Agriculture, and Food, School of Agriculture, University of Lisbon, 1349-017 Lisbon (UIDP/04129/2020), Associate Laboratory LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, 3810-193 Aveiro (UIDB/50006/2020) and CEB, Centre of Biological Engineering, Department of Biological Engineering, University of Minho, 4710-057 Braga (UIDP/04469/2020) through national funds and where applicable co-financed by the FEDER, within the PT2020 Partnership Agreement.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors acknowledge industrial partner Campotec, located in Torres Vedras, for supplying the pineapple by-products used in this study.

Conflicts of Interest: The authors declare no conflict of interest.

References

- Pedreschi, R.; Lurie, S. Advances and current challenges in understanding postharvest abiotic stresses in perishables. *Postharvest Biol. Technol.* **2015**, *107*, 77–89.
- Hodges, D.M.; Toivonen, P.M.A. Quality of fresh-cut fruits and vegetables as affected by exposure to abiotic stress. *Postharvest Biol. Technol.* **2008**, *48*, 155–162.
- Cisneros-Zevallos, L. The Use of Controlled Postharvest Abiotic Stresses as a Tool for Enhancing the Nutraceutical Content and Adding-Value of Fresh Fruits and Vegetables. *J. Food Sci.* **2003**, *68*, 1560–1565.
- Gonzalez-Aguilar, G.A.; Villa-Rodriguez, J.A.; Ayala-Zavala, J.F.; Yahia, E.M. Improvement of the antioxidant status of tropical fruits as a secondary response to some postharvest treatments. *Trends Food Sci. Technol.* **2010**, *21*, 475–482.
- Alegria, C.; Pinheiro, J.; Duthoit, M.; Gonçalves, E.M.; Moldão-Martins, M.; Abreu, M. Fresh-cut carrot (cv. Nantes) quality as affected by abiotic stress (heat shock and UV-C irradiation) pre-treatments. *LWT Food Sci. Technol.* **2012**, *48*, 197–203.
- Loaiza-Velarde, J.G.; Mangrich, M.E.; Campos-Vargas, R.; Saltveit, M.E. Heat shock reduces browning of fresh-cut celery petioles. *Postharvest Biol. Technol.* **2003**, *27*, 305–311.
- Surjadinata, B.B.; Cisneros-Zevallos, L. Biosynthesis of phenolic antioxidants in carrot tissue increases with wounding intensity. *Food Chem.* **2012**, *134*, 615–624.
- Aghdam, M.S.; Asghari, M.; Farmani, B.; Mohayjeji, M.; Moradbeygi, H. Impact of postharvest brassinosteroids treatment on PAL activity in tomato fruit in response to chilling stress. *Sci. Hortic.* **2012**, *144*, 116–120.
- Chutintrasri, B.; Noomhorm, A. Thermal inactivation of polyphenoloxidase in pineapple puree. *LWT Food Sci. Technol.* **2006**, *39*, 492–495.
- Chakraborty, S.; Rao, P.S.; Mishra, H.N. Effect of pH on Enzyme inactivation kinetics in high-pressure processed pineapple (*Ananas comosus* L.) puree using response surface methodology. *Food Bioprocess Technol.* **2014**, *7*, 3629–3645.
- Chakraborty, S.; Rao, P.S.; Mishra, H.N. Kinetic modeling of polyphenoloxidase and peroxidase inactivation in pineapple (*Ananas comosus* L.) puree during high-pressure and thermal treatments. *Innov. Food Sci. Emerg. Technol.* **2015**, *27*, 57–68.
- Ketnawa, S.; Chaiwut, P.; Rawdkuen, S. Pineapple wastes: A potential source for bromelain extraction. *Food Bioprod. Process.* **2012**, *90*, 385–391.
- Freitas, A.; Moldão-Martins, M.; Costa, H.S.; Albuquerque, T.G.; Valente, A.; Sanches-Silva, A. Effect of UV-C radiation on bioactive compounds of pineapple (*Ananas comosus* L. Merr.) by-products. *J. Sci. Food Agric.* **2015**, *95*, 44–52.
- Lourenço, S.C.; Fraqueza, M.J.; Fernandes, M.H.; Moldão-Martins, M.; Alves, V.D. Application of edible alginate films with pineapple peel active compounds on beef meat preservation. *Antioxidants* **2020**, *9*, 1–15.
- Hossain, M.A.; Rahman, S.M.M.M. Total phenolics, flavonoids and antioxidant activity of tropical fruit pineapple. *Food Res. Int.* **2011**, *44*, 672–676.
- Correia, R.T.P.; McCue, P.; Magalhães, M.M.A.; Macêdo, G.R.; Shetty, K. Production of phenolic antioxidants by the solid-state bioconversion of pineapple waste mixed with soy flour using *Rhizopus oligosporus*. *Process Biochem.* **2004**, *39*, 2167–2172.

17. Hale, L.P.; Greer, P.K.; Trinh, C.T.; James, C.L. Proteinase activity and stability of natural bromelain preparations. *Int. Immunopharmacol.* **2005**, *5*, 783–793.
18. Maurer, H.R. Bromelain: Biochemistry, pharmacology and medical use. *Cell. Mol. Life Sci.* **2001**, *58*, 1234–1245.
19. Santos, D.I.; Saraiva, J.M.A.; Vicente, A.A.; Moldão-Martins, M. Methods for determining bioavailability and bioaccessibility of bioactive compounds and nutrients. In *Innovative Thermal and Non-Thermal Processing, Bioaccessibility and Bioavailability of Nutrients and Bioactive Compounds*; Elsevier: Amsterdam, The Netherlands, 2019; pp. 23–54. ISBN 9780128141748.
20. Santos, D.I.; Fraqueza, M.J.; Pissarra, H.; Saraiva, J.A.; Vicente, A.A.; Moldão-Martins, M. Optimization of the Effect of Pineapple By-Products Enhanced in Bromelain by Hydrostatic Pressure on the Texture and Overall Quality of Silverside Beef Cut. *Foods* **2020**, *9*, 1752.
21. Santos, D.I.; Correia, M.J.N.; Mateus, M.M.; Saraiva, J.A.; Vicente, A.A.; Moldão, M. Fourier transform infrared (FT-IR) spectroscopy as a possible rapid tool to evaluate abiotic stress effects on pineapple by-products. *Appl. Sci.* **2019**, *9*, 4141.
22. Heredia, J.B.; Cisneros-Zevallos, L. The effect of exogenous ethylene and methyl jasmonate on pal activity, phenolic profiles and antioxidant capacity of carrots (*Daucus carota*) under different wounding intensities. *Postharvest Biol. Technol.* **2009**, *51*, 242–249.
23. Swain, T.; Hillis, W.E. The phenolic constituents of *Prunus domestica*. I.—The quantitative analysis of phenolic constituents. *J. Sci. Food Agric.* **1959**, *10*, 63–68.
24. Brand-Williams, W.; Cuvelier, M.E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *LWT Food Sci. Technol.* **1995**, *28*, 25–30.
25. Benzie, I.F.F.; Strain, J.J. The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: The FRAP assay. *Anal. Biochem.* **1996**, *239*, 70–76.
26. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* **1999**, *26*, 1231–1237.
27. Rufino, M.S.M.; Alves, R.E.; Sousa De Brito, E.; Maia De Morais, S.; Goes Sampaio, C.; Pérez-Jiménez, J.; Saura-Calixto, F.D. *Metodologia Científica: Determinação da Atividade Antioxidante Total em Frutas pela Captura do Radical Livre ABTS +*; Embrapa: Brasília, Brazil, 2007.
28. Santos, D.I.; Pinto, C.A.; Corrêa-Filho, L.C.; Saraiva, J.A.; Vicente, A.A.; Moldão-Martins, M. Effect of moderate hydrostatic pressures on the enzymatic activity and bioactive composition of pineapple by-products. *J. Food Process Eng.* **2020**, doi:10.1111/jfpe.13537.
29. Alegria, C.; Gonçalves, E.M.; Moldão-Martins, M.; Cisneros-Zevallos, L.; Abreu, M. Peel removal improves quality without antioxidant loss, through wound-induced phenolic biosynthesis in shredded carrot. *Postharvest Biol. Technol.* **2016**, *120*, 232–239.
30. Zhou, Y.; Dahler, J.M.; Underhill, S.J.; Wills, R.B.. Enzymes associated with blackheart development in pineapple fruit. *Food Chem.* **2003**, *80*, 565–572.
31. Babu, B.R.; Rastogi, N.K.; Raghavarao, K.S.M.S. Liquid-liquid extraction of bromelain and polyphenol oxidase using aqueous two-phase system. *Chem. Eng. Process. Process Intensif.* **2008**, *47*, 83–89.
32. StatSoft STATISTICA (Data Analysis Software System) 2007.
33. Jacobo-Velázquez, D.A.; Martínez-Hernández, G.B.; Del C. Rodríguez, S.; Cao, C.M.; Cisneros-Zevallos, L. Plants as biofactories: Physiological role of reactive oxygen species on the accumulation of phenolic antioxidants in carrot tissue under wounding and hyperoxia stress. *J. Agric. Food Chem.* **2011**, *59*, 6583–6593.
34. Van de Velde, F.; Fenoglio, C.; Piagentini, A.M.; Pirovani, M.E. Modeling the Impact of the Type of Cutting and Storage Temperature on the Bioactive Compound Content, Phenylpropanoid Metabolism Enzymes and Quality Attributes of Fresh-Cut Strawberries. *Food Bioprocess Technol.* **2018**, *11*, 96–109.
35. Reyes, L.F.; Cisneros-Zevallos, L. Wounding stress increases the phenolic content and antioxidant capacity of purple-flesh potatoes (*Solanum tuberosum* L.). *J. Agric. Food Chem.* **2003**, *51*, 5296–5300.
36. Gil, M.I.; Aguayo, E.; Kader, A.A. Quality changes and nutrient retention in fresh-cut versus whole fruits during storage. *J. Agric. Food Chem.* **2006**, *54*, 4284–4296.
37. Talcott, S.T.; Moore, J.P.; Lounds-Singleton, A.J.; Percival, S.S. Ripening associated phytochemical changes in mangos (*Mangifera indica*) following thermal quarantine and low-temperature storage. *J. Food Sci.* **2005**, *70*, C337–C341.
38. Djioua, T.; Charles, F.; Lopez-Lauri, F.; Filgueiras, H.; Coudret, A.; Jr, M.F.; Ducamp-Collin, M.N.; Sallanon, H. Improving the storage of minimally processed mangoes (*Mangifera indica* L.) by hot water treatments. *Postharvest Biol. Technol.* **2009**, *52*, 221–226.
39. Montero-Calderón, M.; Rojas-Graü, M.A.; Aguiló-Aguayo, I.; Soliva-Fortuny, R.; Martín-Belloso, O. Influence of modified atmosphere packaging on volatile compounds and physicochemical and antioxidant attributes of fresh-cut pineapple (*Ananas comosus*). *J. Agric. Food Chem.* **2010**, *58*, 5042–5049.
40. Lim, Y.Y.; Lim, T.T.; Tee, J.J. Antioxidant properties of several tropical fruits: A comparative study. *Food Chem.* **2007**, *103*, 1003–1008.
41. Toivonen, P.M.A. Postharvest storage procedures and oxidative stress. *HortScience* **2004**, *39*, 938–942.
42. Choi, Y.J.; Tomás-Barberán, F.A.; Saltveit, M.E. Wound-induced phenolic accumulation and browning in lettuce (*Lactuca sativa* L.) leaf tissue is reduced by exposure to n-alcohols. *Postharvest Biol. Technol.* **2005**, *37*, 47–55.
43. Saltveit, M.E.; Choi, Y.J.; Tomás-Barberán, F.A. Involvement of components of the phospholipid-signaling pathway in wound-induced phenylpropanoid metabolism in lettuce (*Lactuca sativa*) leaf tissue. *Physiol. Plant.* **2005**, *125*, 345–355.
44. Surjadinata, B.B.; Cisneros-Zevallos, L. Modeling Wound-induced Respiration of Fresh-cut Carrots (*Daucus carota* L.). *J. Food Sci.* **2003**, *68*, 2735–2740.

45. Degl'Innocenti, E.; Guidi, L.; Pardossi, A.; Tognoni, F. Biochemical study of leaf browning in minimally processed leaves of lettuce (*Lactuca sativa* L. var. *acephala*). *J. Agric. Food Chem.* **2005**, *53*, 9980–9984.
46. Reyes, L.F.; Villarreal, J.E.; Cisneros-Zevallos, L. The increase in antioxidant capacity after wounding depends on the type of fruit or vegetable tissue. *Food Chem.* **2007**, *101*, 1254–1262.
47. Aguayo, E.; Escalona, V.H.; Rtés, F.A. Metabolic Behavior and Quality Changes of Whole and Fresh Processed Melon. *J. Food Sci.* **2004**, *69*, SNQ148–SNQ155.
48. Rivera-López, J.; Vázquez-Ortiz, F.A.; Ayala-Zavala, J.F.; Sotelo-Mundo, R.R.; González-Aguilar, G.A. Cutting Shape and Storage Temperature Affect Overall Quality of Fresh-cut Papaya cv. “Maradol”. *J. Food Sci.* **2005**, *70*, s482–s489.
49. Robards, K.; Prenzler, P.D.; Tucker, G.; Swatsitang, P.; Glover, W. Phenolic compounds and their role in oxidative processes in fruits. *Food Chem.* **1999**, *66*, 401–436.
50. Chen, H.; Cheng, Z.; Wisniewski, M.; Liu, Y.; Liu, J. Ecofriendly hot water treatment reduces postharvest decay and elicits defense response in kiwifruit. *Env. Sci. Pollut. Res.* **2015**, *22*, 15037–15045.
51. Kim, Y.; Lounds-Singleton, A.J.; Talcott, S.T. Antioxidant phytochemical and quality changes associated with hot water immersion treatment of mangoes (*Mangifera indica* L.). *Food Chem.* **2009**, *115*, 989–993.
52. Chaurasiya, R.S.; Umesh Hebbar, H. Extraction of bromelain from pineapple core and purification by RME and precipitation methods. *Sep. Purif. Technol.* **2013**, *111*, 90–97.
53. Jutamongkon, R.; Charoenrein, S. Effect of temperature on the stability of fruit bromelain from smooth cayenne pineapple. *Kasetsart J. Nat. Sci.* **2010**, *44*, 943–948.
54. Vogt, T. Phenylpropanoid biosynthesis. *Mol. Plant* **2010**, *3*, 2–20.
55. Amodio, M.L.; Derossi, A.; Colelli, G. Modeling phenolic content during storage of cut fruit and vegetables: A consecutive reaction mechanism. *J. Food Eng.* **2014**, *140*, 1–8.
56. Ardila, H.; Baquero, B.; Martínez, S. Inducción de la actividad de la enzima fenilalanina amonio liasa en clavel (*Dianthus caryophyllus* L.) por elicitors del hongo *Fusarium oxysporum* f. sp. *Dianthi* raza 2. *Rev. Colomb. Quím.* **2007**, *36*, 151–167.
57. Gasull, E.; Becerra, D. Caracterización de polifenoloxidasas extraídas de pera (cv. Packam's Triumph) y manzana (cv. Red Delicious). *Inf. Tecnol.* **2006**, *17*, 69–74.
58. Das, J.R.; Bhat, S.G.; Gowda, L.R. Purification and Characterization of a Polyphenol Oxidase from the Kew Cultivar of Indian Pineapple Fruit. *J. Agric. Food Chem.* **1997**, *45*, 2031–2035.
59. Poh, S.S.; Abdul Majid, F.A. Thermal stability of free bromelain and bromelain-polyphenol complex in pineapple juice. *Int. Food Res. J.* **2011**, *18*, 1051–1060.
60. Pardo, M.F.; López, L.M.I.; Canals, F.; Avilés, F.X.; Natalucci, C.L.; Caffini, N.O. Purification of balansain I, an endopeptidase unripe fruits of *Bromelia balansae* Mez (Bromeliaceae). *J. Agric. Food Chem.* **2000**, *48*, 3795–3800.
61. Vallés, D.; Furtado, S.; Cantera, A.M.B. Characterization of new proteolytic enzymes from ripe fruits of *Bromelia antiacantha* Bertol. (Bromeliaceae). *Enzym. Microb. Technol.* **2007**, *40*, 409–413.
62. Kang, H.M.; Saltveit, M.E. Antioxidant capacity of lettuce leaf tissue increases after wounding. *J. Agric. Food Chem.* **2002**, *50*, 7536–7541.
63. Campos-Vargas, R.; Nonogaki, H.; Suslow, T.; Saltveit, M.E. Heat shock treatments delay the increase in wound-induced phenylalanine ammonia-lyase activity by altering its expression, not its induction in Romaine lettuce (*Lactuca sativa*) tissue. *Physiol. Plant.* **2005**, *123*, 82–91.
64. Liu, J.; Sui, Y.; Wisniewski, M.; Droby, S.; Tian, S.; Norelli, J.; Hershkovitz, V. Effect of heat treatment on inhibition of *Monilinia fructicola* and induction of disease resistance in peach fruit. *Postharvest Biol. Technol.* **2012**, *65*, 61–68.
65. Cantos, E.; Tudela, J.A.; Gil, M.I.; Espín, J.C. Phenolic compounds and related enzymes are not rate-limiting in browning development of fresh-cut potatoes. *J. Agric. Food Chem.* **2002**, *50*, 3015–3023.
66. Gong, Y.; Mattheis, J.P. Effect of ethylene and 1-methylcyclopropene on chlorophyll catabolism of broccoli florets. *Plant Growth Regul.* **2003**, *40*, 33–38.
67. Murata, M.; Tanaka, E.; Minoura, E.; Homma, S. Quality of cut lettuce treated by heat shock: Prevention of enzymatic browning, repression of phenylalanine ammonia-lyase activity, and improvement on sensory evaluation during storage. *Biosci. Biotechnol. Biochem.* **2004**, *68*, 501–507.
68. Trejo-Márquez, M.A.; Ramírez-Villatoro, G.; Camacho De La Rosa, N.A. Polyphenol oxidase and peroxidase activities in mangoes stored at chilling temperature. *Proc. Acta Hort.* **2010**, *864*, 395–402.