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Valorization of Carrot Pomace: UVC Induced Accumulation of Antioxidant Phenolic Compounds

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Abstract: Carrot pomace is the main waste residue obtained during carrot juice extraction. Plant tissues respond to abiotic stresses (i.e., wounding stress and ultraviolet C (UVC) radiation) by accumulating bioactive compounds. Due to the mechanical damage occurring during juice extraction, carrot pomace undergoes extreme wounding stress. In this study, the effects of UVC light (11.8 W m^{-2}, 0–120 min) and storage time (48 h, 25 °C) on the accumulation of phenolics compounds and the antioxidant activity (AOX) of carrot pomace were evaluated. Carrot pomace that was non-treated with UVC (control) showed a 709.5% increase in total phenolics at 48 h. A high correlation of AOX values against total phenolics (R^2 = 0.87) was observed, indicating that phenolics were the main contributors to the AOX of the tissue. After UVC treatment, the pomace that was radiated for 120 min with UVC showed an increase (40.4%) in chlorogenic acid (CHA) content. At 24 h, protocatechuic acid and 3,5-dicaffeoylquinic acid, which were not detected before storage, showed accumulation by 166.5 mg/kg and 169.4 mg/kg, respectively, in UVC treated pomace. Chlorogenic acid showed the highest increase (143.6%) at 48 h in the control. Valorization of carrot pomace was achieved by increasing its concentration of antioxidant phenolics through UVC radiation.

Keywords: carrot waste; valorization; by-products; health-promoting compounds; chlorogenic acid; ultraviolet C radiation; antioxidant capacity; circular economy; sustainable food systems

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1. Introduction

The global population is expected to grow from 7.7 billion in 2019 to 9.7 billion in 2050 [1]. Furthermore, the annual waste production is expected to increase by 70% in the next 40 years [2]. In this context, the valorization of food waste is gaining attention due to the considerable impact that it could have on overcoming the contradiction of 820 million people with hunger and malnutrition while others are over-consuming foods, together with an increase in food waste production [3,4].

The pomace produced from the agri-food industry could be valorized due to the presence of bioactive molecules that could be extracted, purified, and commercialized by the food, cosmetic, and pharmaceutical industries [5]. However, in the case of pomace that is obtained after juice extraction from plant material during the juice manufacturing process, a significant amount of the bioactive compounds is extracted with the juice, resulting in pomace with a low content of nutraceuticals. Therefore, it would be interesting to find technologies that increase the bioactive compound content in plant food waste before further commercial use.

In this context, the application of controlled abiotic stresses (i.e., wounding stress, ultraviolet (UV) light, and modified atmospheres) in plant tissue induces the biosynthesis of secondary metabolites with health-promoting properties [6,7]. Due to the mechanical

damage occurring during juice extraction, carrot pomace is under extreme wounding stress. Therefore, it is likely that storing the tissue under the appropriate conditions would result in the accumulation of secondary metabolites. Previous reports have demonstrated that wounding stress application in carrots, either applied alone or in combination with UV radiation, results in the biosynthesis of antioxidant phenolic compounds [8,9]. The main phenolic compound that is accumulated is the chlorogenic acid (CHA), which has been related to the prevention and treatment of the metabolic syndrome [10], colon cancer [11,12], and the improvement in cognitive abilities and neural development [13].

Carrot processing waste is around 175,000 tons annually in the United States [14]. Previous reports on the valorization of carrot waste have been mainly focused on extracting nutraceuticals such as carotenoids [14] and dietary fiber [15]. In the present study, the effects of UVC light (11.8 W m^{-2}, 0, 30, 60, and 120 min) and storage time (48 h, 25 °C) on the accumulation of phenolics compounds and antioxidant activity (AOX) of carrot pomace were evaluated as tools to valorize the waste residue prior to its further use as a raw material for the extraction of nutraceuticals or for its conversion to a food ingredient.

2. Materials and Methods

2.1. Chemicals and Plant Material

Methanol, Na_2CO_3, 0.1N NaOH, and orthophosphoric acid were purchased from Desarrollo de Especialidades Quimicas S.A. de C.V. (San Nicolas de los Garza, N.L. Mexico). Chlorine (Cloralex®, 6% sodium hypochlorite) and carrots (*Daucus carota*) of commercial maturity were purchased in a local supermarket (HEB, Monterrey, N.L. Mexico). All other chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Pomace Obtention, UVC Radiation, and Storage Studies

To obtain carrot pomace, juice from whole carrots was extracted as previously described [16]. Before juice extraction, the tissue was sanitized by submerging the carrots for 5 min in a chlorinated aqueous solution (200 ppm, pH 6.5). The juice was extracted with a juice extractor (Black & Decker, JE1500, Towson, MD, USA) and filtered through eight layers of cheesecloth to recover the carrot pomace.

The pomace was weighed (500 g) and placed in open plastic containers (33.7 × 21.9 × 12.4 cm). The samples were positioned at 50 cm under the UVC light (30W germicidal UV light, G30T8, General Electric, Fairfield, CT, USA) and radiated for 0 (control), 30, 60, and 120 min at an intensity of 11.8 W/m^2. The UVC treated carrot pomace and the control were stored in an incubator (VWR, Randor, PA, USA) for 48 h at 25 °C. Samples were collected at 0, 24, and 48 h of storage. The tissue was immediately flash-frozen in liquid nitrogen, placed at −80 °C until freeze-dried (Labconco, Kansas City, MO, USA), and then ground to a fine powder until assayed for phytochemical analyses.

2.3. Total Phenolics and Antioxidant Activity

Freeze-dried carrot powder (0.5 g) was added with methanol (10 mL), mixed with a vortex for 2 min, and incubated overnight at 4 °C. After that, the mixture was vortexed again for 2 min and centrifuged (10,000× *g*, 15 min, 4 °C), and the supernatant was collected as methanolic extract to analyze total phenolics, antioxidant activities, and the phenolic profiles. The total phenolics were demined following the Folin–Ciocalteu method as reported by Swain and Hillis [17] but adapted for a 96-well microplate format [18]. The concentration of total phenolics was expressed as milligrams of CHA equivalents per km^{-1} of carrot pomace on a dry weight (DW) basis.

The antioxidant capacity of the methanol extract was determined with the oxygen radical absorbance capacity (ORAC) assay. The ORAC value was obtained with the procedure described by Wu et al. [19] for hydrophilic ORAC. Results were expressed as milligrams of Trolox equivalents (TE) per km^{-1} of carrot pomace DW.

2.4. Identification and Quantification of Individual Phenolic Compounds by High-Performance Liquid Chromatography–Diode Array Detection (HPLC–DAD)

The phenolic profiles of the carrot pomace that was treated and non-treated with UVC light were determined following a method previously described [20,21]. Briefly, the methanolic extract (10 µL) was injected in an HPLC-diode array detection (DAD) system equipped with a quaternary pump, an autosampler, and a DAD detector (1260 Infinity, Agilent Technologies, Santa Clara, CA, USA). The individual phenolic compounds were separated on a 4.6 mm × 250 mm, 5 µm C18 reverse phase column (Luna, Phenomenex, Torrance, CA, USA). The mobile phases were water (phase A) and methanol:water (60:40, *v*:*v*, phase B) adjusted to pH 2.4 with orthophosphoric acid. The gradient solvent system was 0/100, 3/70, 8/50, 35/30, 40/20, 45/0, 50/0, and 60/100 (min/% phase A) at a constant flow rate of 0.8 mL/min. Chromatographic data were processed with Millennium software V3.1 (Waters Corp, Milford, MA, USA). The identification of individual phenolics was based on their DAD spectra and elution time when compared with authentic standards and previous reports [20,21].

2.5. Statistical Analysis

Replication was achieved by repeating treatment under the same conditions. The control (carrot pomace non-treated with UVC light) and the carrot pomace treated with UV light were run concurrently. All reported data were pooled from repeated independent treatments. There were three replicates per treatment (n = 3). Statistical analyses were performed using the values obtained from the 3 experimental repetitions. Data represent the mean value of samples ± the standard error of the mean. Significant differences between mean values were determined by one-way analyses of variance, followed by an LSD test ($p < 0.05$) using JMP software version 11.0 (SAS Institute Inc., Cary, NC, USA).

3. Results and Discussion

3.1. Total Phenolics and Antioxidant Activity

The effects of UVC radiation and storage time on the accumulation of total phenolics and the antioxidant activity of carrot pomace are shown in Figure 1. The total phenolic content of the carrot pomace obtained immediately after juice extraction was 1530 mg CHA equivalents kg^{-1} (Figure 1A). Compared with most plant materials, carrot pomace contains a low content of total phenolics. For instance, blueberry, purple potato, and sweet cherry contain 41,800, 7810, and 20,980 mg/kg of total phenolics, respectively [22]. Similarly, the AOX of carrot pomace that is obtained after juice extraction was 5626 mg TE kg^{-1} (Figure 1B), which is also low compared with other edible plant tissue such as green pepper (40,046 mg TE kg^{-1}), broccoli (33,038 mg TE kg^{-1}), and tomato (16,529 mg TE kg^{-1}) [23]. The carrot pomace retained 51% of the total phenolics and 28.1% of the antioxidant activity from whole carrots, which have been previously reported to be around 3000 mg/kg and 20,000 mg/kg, respectively [24].

During storage, the carrot pomace that was non-treated with UVC (control) showed increases in the total phenolics by 241.6% and 709.5% at 24 h and 48 h, respectively, compared with the control before storage (CBS, Figure 1A). Regarding the AOX value, non-significant increases were detected at 24 h of storage, whereas at 48 h, the AOX increased by 80.1% in the control compared with the CBS (Figure 1B). The total phenolics observed after the storage of carrot pomace (12,390 mg/kg) reached higher values than those previously reported for whole carrots (3000 mg/kg) [24]. However, the AOX value observed in the stored carrot pomace (10,133 mg TE kg^{-1}) was 51% of the value reported for whole carrots (20,000 mg/kg) [24].

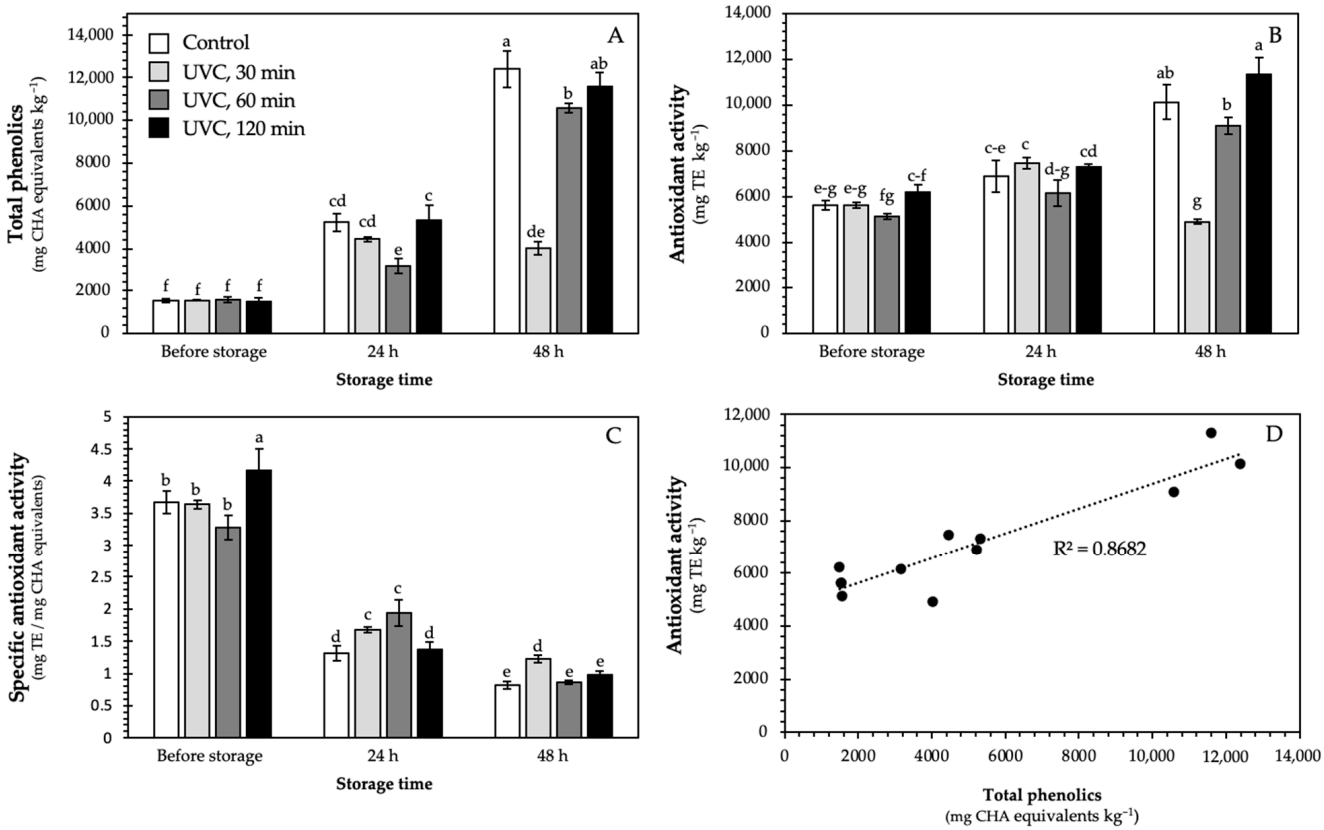

Figure 1. Total phenolic content (**A**), antioxidant activity (**B**), specific antioxidant activity (**C**), and correlation of antioxidant activity against phenolic content (**D**) of carrot pomace treated with UVC for 0 (control), 30, 60, or 120 min, and stored at 25 °C for 48 h. Data represent the mean of 3 repetitions ± the standard error of the mean. Different letters among bars (a, b, c, d, e, f, and g) indicate a statistical difference between treatments using the LSD test ($p < 0.05$). Abbreviations: CHA: chlorogenic acid; TE: Trolox equivalents.

To better understand the capacity of phenolics in carrot pomace to neutralize free radicals, the specific antioxidant activity was calculated (AOXs, Figure 1C). The AOXs is defined as the ratio of total antioxidant activity per total soluble phenolics and expressed as milligram of TE per milligram of CHA equivalents [25]. In this context, the AOXs of carrot pomace (3.67 mg TE/mg CHA equivalents) is 44.8% lower as compared with the AOXs of whole carrots [24], indicating that the phenolic compounds retained in carrot pomace after juice extraction have a lower capacity to neutralize free radicals. Furthermore, the AOXs decreased from 3.67 to 0.81 mg TE/mg CHA equivalents in the control (Figure 1C). This result indicates that the mixture of phenolics present in the carrot pomace before storage possesses a higher capacity to neutralize free radicals compared to the non-treated UVC stored tissue. The antioxidant activity of phenolic compounds depends on the number of hydroxyl groups present in their chemical structure [26,27]. Therefore, their relative concentrations would determine how phenolics would interact to neutralize free radicals. These interactions could be synergistic, additive, or antagonistic [28–30].

The increase in phenolic content observed during storage of the carrot pomace that was non-treated with UVC radiation (control) could be attributed to the wound response of the plant tissue [31]. During carrot juice production, whole carrots are subjected to extreme wounding stress. The typical stress response of plants to wounding is the production of phenolic compounds, which serve as precursors for the lignin biosynthesis needed by the wounded tissue to decrease water loss [20,32]. The accumulation of soluble phenolics in the tissue indicates that carrot pomace retains the metabolic machinery needed for phenolic biosynthesis, responding to extreme mechanical damage by producing phenolics as a stress defense mechanism.

The application of UVC radiation did not induce an immediate change in the total phenolics (Figure 1A) and antioxidant activity (Figure 1B) of carrot pomace. These results agree with a previous report, where the application of UVC radiation did not modify the total phenolic content in carrot slices, pie-cuts, and shreds [8]. Interestingly, the carrot pomace treated with 120 min of UVC radiation showed a slightly higher amount of AOXs than the CBS (Figure 1C), indicating that UVC light modified the phenolic profile, generating a combination of compounds with a higher capacity to neutralize free radicals.

At 24 h of storage, the concentration of soluble phenolics increased in all samples, while carrot pomace subjected to 60 min of UVC light showed the lowest increase (Figure 1A). At 48 h, the tissue treated with 30 min of UVC light did not show a further increase in total phenolics, while the carrot pomace treated with 60 and 120 min of UVC continued to increase. No significant difference was detected in the phenolic content between the 120 min UVC treatment and the control, whereas the 60 min UVC treatment showed a slightly lower (−14.6%) accumulation of total phenolics (Figure 1A). The antioxidant activity showed a similar behavior (Figure 1B), indicating that the phenolic compounds are the main contributors to the AOX value of carrots (Figure 1D). This is demonstrated with the R^2 value obtained for the correlation of AOX against the total phenolics (R^2 = 0.8782, Figure 1D), which was higher than 0.7 [25].

The combined effect of wounding and UVC radiation to induce the biosynthesis of phenolic compounds in carrots has been previously reported [8,9]. The authors determined the mechanism by which the tissue responds to the combination of both stresses, leading to the accumulation of phenolics. In wounded carrot tissue, UVC radiation induces the production of reactive oxygen species (ROS), and together with ethylene and jasmonic acid, which are produced as a response to wounding stress, activates the expression of genes and enzymes related to the biosynthesis of phenolic compounds [9]. A similar phenomenon could occur in carrot pomace that is treated with UVC radiation, where both stresses are combined. Interestingly, UVC radiation did not induce a higher accumulation of total phenolics compared to the control. The soluble phenolics quantified in the tissue result from a balance between their biosynthesis rate and their utilization rate for the production of other metabolites such as lignin [20,21]. Likewise, it also depends on the availability of the carbon source needed for their biosynthesis [33,34]. Therefore, it is likely that depending on the dose of UVC radiation applied, the concentration of signaling molecules that modulate different mechanisms in the tissue are activated. For instance, results suggest that samples treated with 30 min of UVC radiation present a higher lignification rate than the control, while higher UVC doses also increase the phenolic biosynthesis rate, displaying a similar behavior to the one observed in the control. Furthermore, results also suggest that the carbon source is the limiting factor in obtaining a higher accumulation of phenolics in the tissue since the carrot pomace treated with UVC radiation did not show a higher phenolic content compared to the control at 48 h of storage.

3.2. Phenolic Profile and Individual Phenolic Compounds

A typical chromatogram showing the phenolic compound identified in carrot pomace that is treated with UVC radiation for 30 min and stored at 25 °C is shown in Figure 2. The compounds identified include protocatechuic acid, gallic acid derivative, 3,5-dicaffeoylquinic acid, chlorogenic acid, and ferulic acid. The phenolic profile obtained herein is similar to those previously reported for whole carrots [20,24]. For the carrot pomace obtained immediately after juice extraction, CHA was the only phenolic compound identified (Figure 3). This result indicates that the other phenolic compounds usually detected in whole carrots (protocatechuic acid, gallic acid derivative, 3,5-dicaffeoylquinic acid, 3-hydroxy dihydro chlorogenic acid, 4,5-dicaffeoylquinic acid, *p*-coumaric acid, and ferulic acid) are extracted with the juice [16,20].

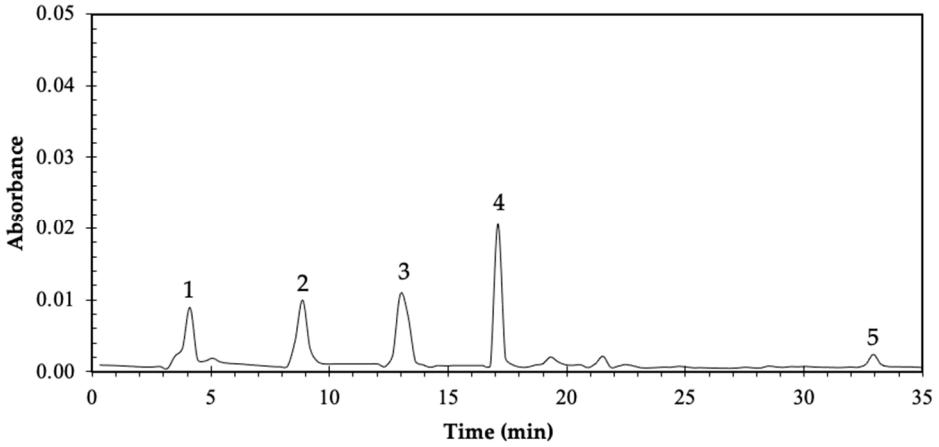

Figure 2. Typical HPLD-DAD chromatogram (shown at 320 nm) of methanolic extracts from carrot pomace obtained as a by-product after juice extraction, treated with UVC radiation for 30 min, and stored at 25 °C for 48 h. Peak identification: 1. protocatechuic acid; 2. gallic acid derivative; 3. 3,5-dicaffeoylquinic acid; 4. chlorogenic acid; 5. ferulic acid. Phenolic compounds were identified by comparing spectra characteristics of the chromatographic peaks with authentic chemical standards and previous reports [20,21].

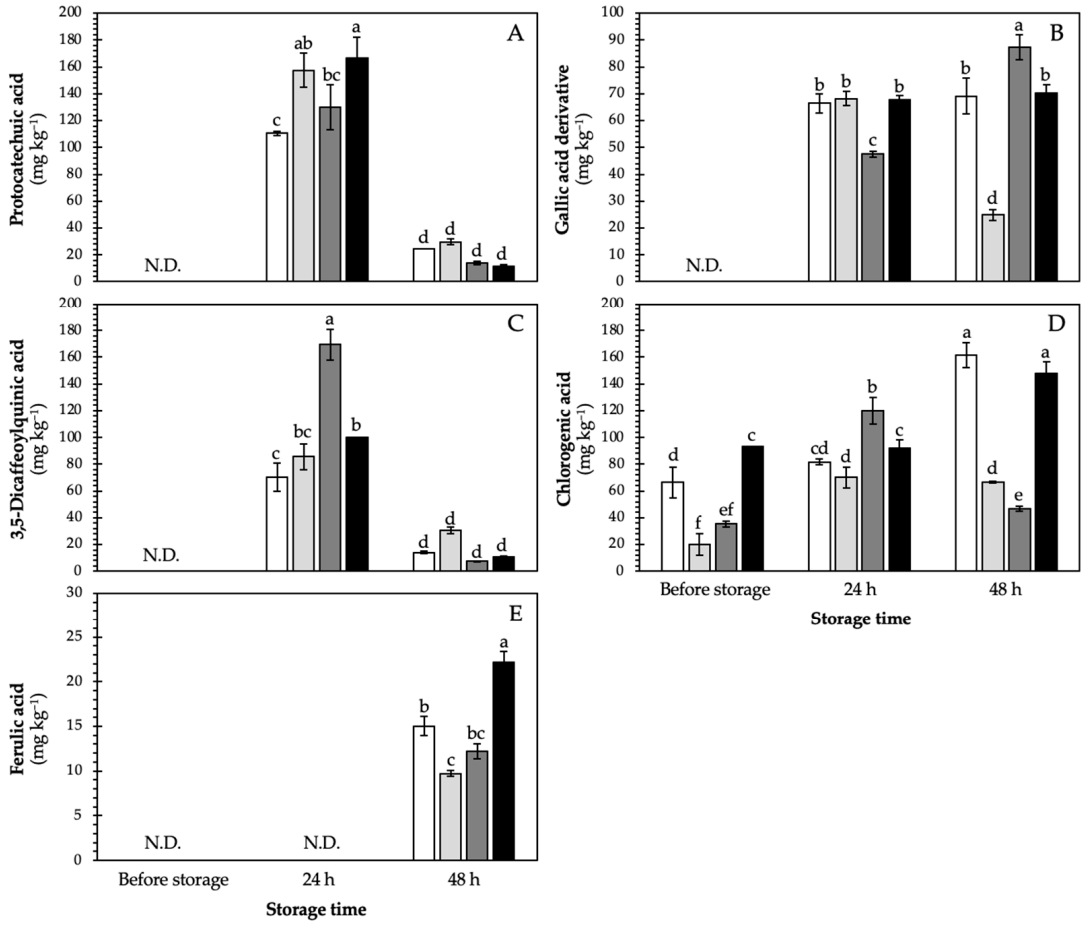

Figure 3. Concentration of individual phenolic compounds in carrot pomace treated with UVC for 0 (control), 30, 60, or 120 min, and stored at 25 °C for 48 h. Data represent the mean of 3 repetitions ± the standard error of the mean. Different letters among bars (a, b, c, d, e, and f) indicate a statistical difference between treatments using the LSD test ($p < 0.05$). Individual phenolic compounds identified and quantified were: protocatechuic acid (**A**); gallic acid derivative (**B**); 3,5-dicaffeoylquinic acid (**C**); chlorogenic acid (**D**); ferulic acid (**E**). N.D. = not detected.

Compared with the concentration of CHA previously reported for whole carrots (~500 mg/kg), the pomace retains around 12% of the content in whole carrots [24]. UVC did not induce the de novo biosynthesis of individual phenolics as an immediate stress response. However, the concentration of CHA decreased in carrot pomace that was irradiated for 30 and 60 min. These decreases could be attributed to the immediate increase in the concentration of ROS levels induced by UVC radiation, which could oxidize phenolic compounds [35]. Interestingly, the carrot pomace that was treated for 120 min with UVC light showed a slight increase (40.4%) in CHA content, suggesting that UVC radiation induces an immediate biosynthesis of the compound or that UVC radiation releases bound phenolics present in the carrot pomace [36]. The immediate increase in CHA content detected in the carrot pomace that was radiated for 120 min with UVC (Figure 3D) correlates with the higher AOXs identified in the tissue (Figure 1C).

At 24 h of storage, additional phenolic compounds were detected in the carrot pomace (protocatechuic acid, gallic acid derivative, and 3,5-dicaffeoylquinic acid), confirming the wound-induced biosynthesis of individual phenolic compounds. Protocatechuic acid and 3,5-dicaffeoylquinic acid showed their highest accumulation at 24 h of storage in the irradiated tissue. For protocatechuic acid, the treatment that showed the highest accumulation (166.5 mg/kg, 50.6% higher than the control) was the carrot pomace that was irradiated for 120 min, where no significant difference was detected when compared with the 30 min UVC treatment (Figure 3A). On the other hand, the 3,5-dicaffeoylquinic acid showed its highest accumulation (169.4 mg/kg, 140.2% higher than the control) in the carrot pomace that was treated with 60 min of UVC radiation (Figure 3C). A similar trend was observed for CHA, where the 60 min UVC treatment showed the highest accumulation at 24 h of storage, which was 46.8% higher than the control and 80.9% higher than the CBS. Regarding gallic acid derivative, the compound accumulated in the tissue at concentrations between 47.7 and 68.2 mg/kg, where the lowest content was observed in the tissue treated with UVC for 60 min, and no significant difference was observed between the other UVC treatments (30 and 120 min) and the control (Figure 3B). Interestingly, the treatment that showed the highest accumulation of hydroxycinnamic acids (3,5-dicaffeoylquinic acid and CHA) showed the lowest accumulation of gallic acid derivative, suggesting that the treatments redirected the use of shikimic acid as a precursor for the biosynthesis of aromatic amino acids rather than their conversion into 3-dehydroshikimic acid and for their further transformation into gallic acid [37].

At 48 h of storage, the concentration of protocatechuic acid and 3,5-dicaffeoylquinic acid decreased to low levels (Figure 3A,C). For the gallic acid derivative, the highest accumulation (26.5% higher than the control) was detected in the 60 min UVC treatment. Regarding CHA, the content remained constant in the 30 min UVC treated samples but decreased by 60.9% from 24 h to 48 h storage time. Interestingly, the control and 120 min UVC treated samples showed the largest CHA content increase, which was 143.6% and 129.9% higher than the CBS, respectively. In addition to the phenolic compounds mentioned, ferulic acid was also detected at 48 h of storage of the carrot pomace (Figure 3E). The highest accumulation of ferulic acid was observed in the tissue treated with UVC for 120 min, whose content was 46.6% higher than the control. The ferulic acid accumulation also supports that the wound-induced activation of lignin was activated in carrot pomace since it is an essential precursor for its biosynthesis [32]. As described for total phenolics, the accumulation of individual phenolics results from the balance between their biosynthesis rate and utilization rate for the production of other molecules or even their oxidation/degradation rate [38]. For instance, the high accumulation of CHA observed at 48 h of storage in the control and in the 120 min UVC treated samples indicates that its accumulation rate was higher than its utilization rate for lignin biosynthesis.

4. Conclusions

The present study demonstrated that carrot pomace obtained after juice extraction produces phenolic compounds during storage due to the extreme wounding stresses to

which the whole carrot tissue is subjected during juice extraction. UVC radiation allowed a higher accumulation of individual phenolic compounds at 24 h of storage of the tissue. Thus, UVC radiation could be used as an effective strategy for the valorization of carrot pomace, inducing a higher accumulation of antioxidant phenolic compounds. Carrot pomace could be transformed into an ingredient that can be used in the food industry or used as raw material to extract and purify antioxidant phenolic compounds, which can be further commercialized in the dietary supplement industry. Given the recent developments in extraction and isolation technologies, it would be feasible to implement efficient, low-investment processes at a large scale that address the recovery and purification of high-value bioactives from carrot pomace. Moreover, it is relevant to further research the effect of different abiotic stresses to establish alternative processing strategies and conditions that promote the biosynthesis and accumulation of nutraceutical compounds on carrot pomace and other food industry waste materials. In this regard, some non-thermal technologies, such as high hydrostatic pressure and pulsed electric fields, have been studied as strategies for inducing the biosynthesis of nutraceutical compounds in whole fruits and vegetables at a low processing cost. Therefore, exploring such technologies to elicit abiotic stress on pomaces and other vegetal waste by-products in the food industry would be possible.

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