



# **Communication Effects of Wounding Stress and Storage Temperature on the Accumulation of Chlorogenic Acid Isomers in Potatoes** (*Solanum tuberosum*)

Ana M. Torres-Contreras<sup>1</sup> and Daniel A. Jacobo-Velázquez<sup>2,\*</sup>

- <sup>1</sup> Tecnologico de Monterrey, Escuela de Ingeniería y Ciencias, Av. Eugenio Garza Sada 2501, Monterrey C.P. 64849, Mexico; marieltorres2811@gmail.com
- <sup>2</sup> Tecnologico de Monterrey, Escuela de Ingeniería y Ciencias, Av. General Ramón Corona 2514, Zapopan C.P. 45201, Mexico
- \* Correspondence: djacobov@tec.mx; Tel.: +52-312-119-1650

**Abstract:** Wounding stress is an effective strategy to increase the content of bioactive compounds in horticultural crops. Potato tubers subjected to wounding stress accumulate chlorogenic acid (CGA) and CGA isomers (neo-CGA and crypto-CGA), which are phenolics that prevent and treat different chronic and degenerative diseases. In this study, the effects of wounding stress and storage temperature (10 °C and 20 °C for 168 h) on the accumulation of CGA isomers in potatoes were evaluated. Results indicated that CGA accumulation was favored when wounded potatoes were stored at 20 °C for 120 h, obtaining a 1923.1% higher concentration when compared with samples before storage. Furthermore, wounded potatoes stored at 10 °C for 120 h showed the highest neo-CGA increase in concentration (712.2%). Likewise, the highest crypto-CGA concentration (84.9% higher than control samples) was quantified in wounded potatoes stored at 20 °C for 144 h. Based on the results from both the present study and previous reports, a strategy that summarizes effective postharvest stress conditions that induce the accumulation of specific CGA isomers in potatoes is presented. The tissue with an increased content of bioactive compounds could be used as raw material to produce functional foods or could be subjected to downstream processing to produce dietary supplements.

**Keywords:** postharvest abiotic stresses; wounding stress; wound-healing; chlorogenic acid isomers; crypto-chlorogenic acid; neo-chlorogenic acid; functional foods; wound-induced biosynthesis

## 1. Introduction

Phenolic compounds are plant secondary metabolites that have relevant applications on the prevention and treatment of chronic and degenerative diseases [1]. Therefore, it is highly relevant to develop simple and effective technologies that increase the phenolic content and improve the health benefits of horticultural crops. In this context, different strategies have been developed to increase the phenolic content of different crops, including genetic engineering [2] and the application of controlled abiotic stresses [3]. Among the different types of phenolic compounds, chlorogenic acid (CGA) and its metabolites produced by gut microbiota fermentation (i.e., dihydrocaffeic acid) demonstrated potential to prevent a wide range of diseases, including those related to metabolic syndrome [4] and different types of cancers [5–7].

The application of wounding stress in potatoes has been regarded as an effective postharvest treatment that could be easily applied and scaled-up to an industrial scale to induce the over-accumulation of CGA and its isomers (crypto-CGA, and neo-CGA) [8–10]. In this context, it has been suggested that by selecting the appropriate wounding intensity and storage time, the accumulation of specific CGA isomers could be triggered [8]. For instance, in a previous study, whole potatoes were wounded to produce slices, pie-cuts, and



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**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 (https:// creativecommons.org/licenses/by/ 4.0/). shreds, and the wounded tissue was stored at 10  $^{\circ}$ C for 144 h. The authors reported that the highest accumulation of CGA and neo-CGA was obtained at 96 h of storage, whereas the highest crypto-CGA content was observed at 144 h [8]. In a further study, the authors added amylolytic enzymes on wounded potatoes, as a strategy to increase the carbon source needed for CGA biosynthesis, resulting in a higher accumulation of the bioactive compounds [9].

CGA is produced in cut tissue as part of the wound-healing process and serves as a precursor for the biosynthesis of suberin and lignin [11–17]. Temperature is one of the major factors affecting the biosynthesis rate of suberin and lignin [12,18], and thus it may also alter the accumulation of CGA and its isomers. Therefore, the objective of the present study was to determine the effects of wounding stress, storage time (168 h), and temperature (10 °C and 20 °C) on the accumulation of phenolic compounds in potato tubers, including CGA and its isomers. At the end, practical recommendations to use potatoes as biofactories of CGA isomers are presented, based on data herein obtained and from previous reports.

#### 2. Materials and Methods

#### 2.1. Chemicals, Plant Material, Processing, and Storage Studies

CGA, neo-CGA, crypto-CGA, ferulic acid (FA), *p*-coumaric acid (*p*-CA), 3,5-dicaffeoylquinic acid (3,5-diCQA), methanol (HPLC grade), water (HPLC grade), orthophosphoric acid, and formic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). White potatoes (*S. tuberosum* L.) were obtained from a local market (HEB, Monterrey, N.L. Mexico), sorted, washed, and disinfected with chlorinated water (200 ppm, pH 6.5). Pie-cuts were obtained by cutting the potatoes into slices (thickness ~3 mm), and each slice was then cut into four pieces, making two perpendicular cuts. Potatoes were cut using a commercial knife. Whole potatoes were used as the control. Pie-cuts were selected based on a previous study, where the accumulation of phenolics was evaluated on potatoes subjected to different cutting styles (whole potatoes, slices, pie-cuts, and shreds) and stored at 10 °C, where it was reported that pie-cuts stored for 96 h showed the highest accumulation of phenolics [8].

To study the effects of temperature and storage time on the accumulation of individual phenolics, whole and pie-cut potatoes (~200 g) were placed in plastic containers with a capacity of 5.7 L (Sterilite, Townsend, MA, USA) and stored in an incubator (VWR, Radnor, PA, USA) for 168 h at 10° and 20 °C. Samples were collected at 96, 120, 144, and 168 h. Sampling time started at 96 h of storage, based on a previous study that reported the highest accumulation of CGA isomers at 96 h of storage when the tissue was stored at 10 °C [8]. Containers were ventilated every 12 h to maintain CO<sub>2</sub> accumulation below 0.15% in the headspace and avoid anaerobic metabolism in the tissue. The CO<sub>2</sub> headspace accumulation was determined with an F-950 gas analyzer (Felix Instruments, Camas, WA, USA).

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

Extraction, identification, and quantification of individual phenolic compounds in potato extracts were determined in accordance with a method previously reported by Torres-Contreras et al. [8]. For the extraction of phenolic compounds, potato tissue (5 g) was homogenized with methanol (20 mL) using a tissuemizer (advanced homogenizing system, VWR, Radnor, PA, USA), and then centrifuged ( $10,000 \times g$ , 15 min, 4 °C). The supernatant was microfiltered using nylon membranes ( $0.45 \mu m$ , VWR, Radnor, PA, USA), and used as a phenolic extract.

Thereafter, 10  $\mu$ L of the phenolic extract was injected to the HPLC-diode array detection (DAD) system, which was composed of a quaternary pump, an autosampler, and a DAD detector (1260 Infinity, Agilent Technologies, USA). Compounds were separated on a 4.6 mm × 250 mm, 5  $\mu$ m C18 reverse phase column (Luna, Phenomenex, Torrance, CA, USA). The mobile phases consisted of 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 Millenium software V3.1 (Waters Corp, Mildford, MA, USA). The identification of individual phenolics was based on their DAD spectra and elution time when compared with authentic standards and a previous report [8].

To identify and quantify individual phenolics, calibration curves of authentic standards of CGA, neo-CGA, crypto-CGA, FA, *p*-CA, and 3,5-diCQA at a range of 5–250 ppm were obtained. The concentration of phenolics was expressed as the mg of each individual compound per kg of potatoes on a dry weight (DW) basis. To express the results on a DW basis, the moisture of content samples was determined by the air-oven method.

### 2.3. Statistical Analysis

Replication was achieved by repeating treatment under the same conditions. The control (whole potatoes) and wounding stress conditions were run concurrently. All reported data were pooled from repeated independent treatments. There were five replicates per treatment (n = 5). Statistical analyses were performed using the values obtained from the 5 experimental repetitions. Data represent the mean value of samples  $\pm$  standard error of the mean. Analyses of variance (ANOVA) was conducted to determine the main effects and interactions using JMP software version 11.0 (SAS Institute Inc. Cary, NC, USA), and mean separation was performed using the LSD test (*p* < 0.05).

#### 3. Results

### 3.1. Identification of Individual Phenolic Compounds in Whole and Wounded Potatoes

The tentative identification of phenolic compounds in potato tubers was performed by HPLC–DAD (Figure 1). Phenolic compounds identified included CGA, neo-CGA, crypto-CGA, 3,5-diCQA, *p*-CA, and FA.

The results agreed with previous studies reporting the phenolic profiles of potato tubers [8–10]. Chromatograms of wounded potatoes were qualitatively similar after 96 h of storage at 10 °C (Figure 1B) and 20 °C (Figure 1C), whereas a significant increase in most phenolics was detected in wounded potatoes when compared with whole potatoes (Figure 1A). Whole potatoes showed the presence of neo-CGA, crypto-CGA, and 3,5-diCQA at low concentrations. Moreover, wounded potatoes stored for 96 h showed an additional compound (peak 6) which corresponded to the FA. Likewise, pie-cuts stored at 20 °C also presented the accumulation of p-CA.



**Figure 1.** Typical HPLC–PDA chromatograms (shown at 320 nm) from methanol extracts of pie-cut potatoes before (**A**) and after 96 h of storage at 10 °C (**B**), and 20 °C (**C**). Identification of individual phenolic compounds in the potatoes was based on their retention time and spectra characteristics ( $\lambda$  max) when compared with authentic standards (**D**).

# 3.2. Effects of Wounding Stress and Storage Temperature on the Accumulation of Individual *Phenolic Compounds in Potatoes*

The quantification of individual phenolics before and during the storage of the control (whole potatoes) and wounded potatoes (pie-cuts) is shown in Table 1. All the factors evaluated significantly affected CGA content in the potatoes. The highest CGA accumulation was observed in pie-cuts stored at 20 °C for 120 h, which was 1923.1% higher when compared with the control before storage (CBS). After 120 h of storage, the concentration of CGA started to decrease in samples stored at 20 °C. The results also indicated that a higher temperature accelerates the accumulation of CGA, since the concentration of CGA in pie-cuts stored at 20 °C for 96 h showed a 144.6% higher CGA when compared with pie-cuts stored at 10 °C for the same storage time.

Temperature (°C)		Concentration (mg/kg) <sup>1,2</sup>				
	Sample	Storage Time (h)	CGA	neo-CGA	crypto-CGA	3,5-diCQA
	Control <sup>3</sup>	0	$8.14\pm0.6~{\rm f}$	$7.99\pm0.3~\mathrm{e}$	$12.19\pm0.9$ f,g	$8.11\pm0.4~\mathrm{d,e}$
10	Wholes	96 120	$9.24 \pm 0.4$ e,f $12.15 \pm 0.4$ e,f $11.26 \pm 0.4$ e,f	$7.75 \pm 0.2 \text{ e}$ $22.51 \pm 0.9 \text{ c,d}$ $18.27 \pm 0.4 \text{ d}$	$15.59 \pm 0.5$ c,d,e,f $11.43 \pm 0.6$ g $11.00 \pm 0.2$ r	$7.55 \pm 0.1 \text{ e,f}$ $7.58 \pm 0.1 \text{ e,f}$
		144 168	$11.26 \pm 0.4$ e,f $10.69 \pm 0.3$ e,f	$18.37 \pm 0.4$ d 22.53 ± 1.2 c,d	$11.00 \pm 0.3$ g $11.66 \pm 0.7$ g	$9.29 \pm 0.3$ B,C $9.04 \pm 0.6$ c
	Pie-cuts	96 120 144 168	$\begin{array}{c} 49.81 \pm 3.4 \text{ d} \\ 134.0 \pm 9.7 \text{ b,c} \\ 133.14 \pm 10.8 \text{ b,c} \\ 139.12 \pm 6.0 \text{ b} \end{array}$	$\begin{array}{c} 21.12 \pm 0.8 \text{ d} \\ 64.90 \pm 5.7 \text{ a} \\ 47.95 \pm 4.3 \text{ b} \\ 61.63 \pm 6.6 \text{ a} \end{array}$	$18.12 \pm 1.4 \text{ b,c,d} \\ 15.64 \pm 0.4 \text{ c,d,e,f} \\ 18.18 \pm 4.0 \text{ b,c,d} \\ 16.62 \pm 1.4 \text{ c,d,e} \\ \end{cases}$	$\begin{array}{c} 6.92 \pm 0.1 \text{ f} \\ 7.45 \pm 0.2 \text{ e,f} \\ 7.40 \pm 0.2 \text{ e,f} \\ 7.20 \pm 0.0 \text{ f} \end{array}$
20	Wholes	96 120 144 168	$\begin{array}{c} 10.42\pm 0.4 \text{ e,f} \\ 21.44\pm 1.3 \text{ e} \\ 13.12\pm 1.0 \text{ e,f} \\ 12.19\pm 0.4 \text{ e,f} \end{array}$	$\begin{array}{c} 8.92 \pm 0.2 \text{ e} \\ 24.39 \pm 0.5 \text{ c,d} \\ 23.56 \pm 4.0 \text{ c,d} \\ 29.22 \pm 1.5 \text{ c} \end{array}$	$\begin{array}{c} 14.53 \pm 1.1 \text{ d,e,f,g} \\ 13.59 \pm 0.3 \text{ e,f,g} \\ 22.55 \pm 1.1 \text{ a} \\ 13.34 \pm 0.9 \text{ e,f,g} \end{array}$	$\begin{array}{c} 9.00 \pm 0.1 \text{ c} \\ 10.08 \pm 0.4 \text{ a,b} \\ 10.77 \pm 0.6 \text{ a} \\ 9.89 \pm 0.3 \text{ b} \end{array}$
	Pie-cuts	96 120 144 168	$\begin{array}{c} 121.83 \pm 4.1 \text{ c} \\ 164.68 \pm 8.2 \text{ a} \\ 126.87 \pm 9.3 \text{ b,c} \\ 121.9 \pm 1.7 \text{ c} \end{array}$	$\begin{array}{c} 17.68 \pm 3.2 \text{ d} \\ 18.83 \pm 0.6 \text{ d} \\ 22.50 \pm 2.3 \text{ c,d} \\ 23.71 \pm 2.0 \text{ c,d} \end{array}$	$\begin{array}{c} 25.41 \pm 2.5 \text{ a} \\ 21.27 \pm 0.9 \text{ b} \\ 18.47 \pm 1.4 \text{ b,c} \\ 20.89 \pm 1.9 \text{ b} \end{array}$	$\begin{array}{c} 8.07 \pm 0.3 \text{ d,e} \\ 8.52 \pm 0.4 \text{ c,d} \\ 7.20 \pm 0.1 \text{ e,f} \\ 9.04 \pm 0.4 \text{ c} \end{array}$
Significance <sup>4</sup> Wounding stress		***	***	***	***	
Temperature			***	***	***	***
Storage time			***	***	***	***
Wounding stress × temperature			**	***	N.S.	N.S.
Wounding stress $ imes$ storage time			***	***	***	***
Temperature $\times$ storage time			***	***	**	**
Wounding stress $\times$ temperature $\times$ storage time			***	***	***	*

Table 1. Concentration of chlorogenic acid isomers in whole and pie-cut potatoes during storage at 10 °C or 20 °C for 168 h.

<sup>1</sup> Data represent the mean of 5 repetitions  $\pm$  standard error of the mean. <sup>2</sup> Different letters in the same column indicate statistical differences by the LSD test (p < 0.05). <sup>3</sup> The control was whole potatoes before storage, and is referred in the text as the control before storage (CBS). <sup>4</sup> Asterisks indicate that main effects and interactions were significantly different by analyses of variance (ANOVA). N.S.—non significant, \* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001. Abbreviations: CGA, chlorogenic acid; 3,5-dicQA, 3,5-dicaffeoylquinic acid.

All the interactions between the factors evaluated (wounding stress, temperature, and storage time) significantly affected the concentration of neo-CGA in the potatoes. The highest accumulation of neo-CGA was observed in pie-cuts after 120 h of storage at 10 °C, showing 712.2% higher levels when compared with the CBS.

Regarding crypto-CGA, its concentration was significantly affected (p < 0.001) by wounding stress, temperature, and storage time (p = 0.0058). Likewise, except for the interaction between wounding stress × temperature, all other interactions between the factors evaluated (wounding stress, temperature, and storage time) significantly (p < 0.01) affected crypto-CGA content in potato samples. Whole potatoes stored at 10 °C did not show changes in the concentration of crypto-CGA during storage. However, when the whole tissue was stored at 20 °C for 144 h, a higher crypto-CGA (84.9%) was observed when compared with the CBS. Moreover, the accumulation of crypto-CGA was acerated by the application of wounding stress in the potatoes, where samples stored for 96 h at 20 °C showed a 108.4% higher content when compared with the CBS.

Finally, the 3,5-diCQA content was significantly affected (p < 0.001) by wounding stress, temperature, and storage time. As observed for crypto-CGA, except for the interaction between wounding stress and temperature, all other interactions between the evaluated factors significantly affected 3,5-diCQA content in potato samples. Only a mild increase (32.8%) in the concentration of 3,5-diCQA was observed in the whole tissue stored at 20 °C for 144 h.

When evaluating the total concentration of CGAs (calculated as the sum of CGA, neo-CGA, and crypto-CGA content), the highest accumulation of total CGAs was detected on pie-cuts stored for 120 h at both temperatures (10 °C and 20 °C), showing increases of around 511% when compared with the CBS. Interestingly, although the total amount of CGAs in pie-cuts stored for 10 °C and 20 °C was similar, differences in the CGAs' profile was observed. For instance, pie-cuts stored at 10 °C for 120 h showed a higher neo-CGA accumulation when compared with samples stored at 20 °C, whereas pie-cuts stored at 20 °C showed a higher accumulation of CGA. Thereafter, samples stored at 10°C maintained the increase in CGA content, whereas for samples stored at 20°C, a decrement began after 120 h of storage.

#### 4. Discussion

Chlorogenic acid and its isomers represent ~90% of total phenylpropanoids in potatoes [19,20]. The increase in phenolic compounds in potatoes induced by wounding stress has previously been reported [8–10,19,20]. For instance, Ramamurthy et al. [19] reported a 3–4-fold increase in the total phenolic compound content of potato slices after 8 days of storage at 25 °C. Similarly, Reyes and Cisneros-Zevallos [21] found an increase of 60% in total phenolics in purple-flesh potato slices stored at 15 °C for 48 h. Likewise, Torres-Contreras et al. [8] reported an increment in total phenolics by 100% and 60% in slices and pie-cuts, respectively, after 96 h of storage at 10 °C. However, to the best of our knowledge, this is the first report in the literature comparing the effect of storage temperature (10 °C and 20 °C) on the accumulation of CGA isomers in potato tubers. This is particularly relevant when potatoes are intended to be used as biofactories for antioxidant phenolic compounds.

In potatoes, phenolic compounds biosynthesized during the wound-healing process are utilized for the biosynthesis of suberin and lignin [11,13,22,23]. These polymers are produced as a defense mechanism to prevent water loss in wounded plant tissues [13,15,19,22–24]. The biosynthesis of both polymers starts at the phenylpropanoid metabolism, and it is well known that this is induced by wounding stress in plants [16,25]. This pathway involves enzymes such as the phenylalanine ammonia lyase (PAL), cinnamyl alcohol dehydrogenase (CAD), and peroxidase (POD), which are involved in lignin and suberin biosynthesis [25]. The activation of the phenylpropanoid metabolism by wounding in potatoes has been previously documented by an increase in PAL activity [21,26,27]. Likewise, Cai et al. [28] studied the change in the activities of enzymes related to lignification enzymes in loquat fruit flesh stored at different temperatures after harvest. The authors reported higher enzymatic activities at 20 °C when compared with 12 °C after 8 days of storage, suggesting that the difference in the CGAs profiles detected between pie-cuts stored at 10 °C and 20 °C can be attributed to the effect of temperature on enzymes involved in CGA production, and their utilization for suberin and lignin biosynthesis.

In agreement with previous reports, the major phenolic compound accumulated in wounded potatoes was CGA [8–10]. Although the specific route of the biosynthesis of CGA in potatoes has not been elucidated, the supposed biosynthetic routes involve hydroxycinnamoyl-CoA quinate hydroxycinnamoyl transferase (HQT) and/or hydroxycinnamoyl-CoA shikimate/quinate hydroxycinnamoyl transferase (HCT) [17]. HQT catalyzes CGA synthesis in potatoes [29], whereas HCT is involved in CGA mobilization toward suberin [17]. Our results suggested that during the first 96 h of storage of the wounded potatoes, the balance between the individual CGA biosynthesis rate and the utilization rate for suberin and lignin production differ, affecting the CGA profile of the tissue. However, to better understand this phenomenon, it would be needed to determine the enzymatic activity of HQT and HCT to elucidate how the accumulation of specific CGAs is favored depending on the storage temperature selected for the wounded tissue. Likewise, it is important to point out that the specific role of CGA isomers (neo-CGA and crypto-CGA) as precursors for the biosynthesis of lignin and suberin remains unknown [30], and their involvement as precursors for the biosynthesis of lignin has not been elucidated. Indeed, the biosynthesis pathway of chlorogenic acids isomers (neo-CGA and crypto-CGA) also

remains unknown [30]. However, in transgenic tomato fruits [31] and tobacco plants [32], where the biosynthesis of lignin was down-regulated, higher levels of neo-CGA, crypto-CGA, and CGA were detected, indicating that CGA and its isomers are involved in lignin biosynthesis.

Regarding the other phenolic compounds identified (FA, *p*-CA, and 3,5-diCQA), only mild changes on their concentration were detected during storage of the whole and wounded tissue. For instance, FA and *p*-CA were not detected in the CBS sample, whereas their highest content of 6.97 mg/kg and 9.20 mg/kg were detected in pie-cut and whole potatoes stored at 20 °C for 168 h and 144 h, respectively (data not shown). Likewise, only a mild increase in 3,5-diCQA content was observed in whole carrots stored for 144 h at 20 °C. The low accumulation of FA and *p*-CA in the wounded tissue supports previous findings indicating that their biosynthesis and utilization rates (for lignin and suberin production) are similar, giving only a slight positive balance on the accumulation of both lignin and suberin precursors [8,33]. Finally, in accordance with previous reports, the low accumulation of 3,5-diCQA detected in the wounded tissue and the slight, but significant, increase in the whole tissue, support the previous hypothesis that this compound is not involved as a precursor of lignin and suberin [8].

# *Practical Approaches for the Use of Potatoes as Biofactories of CGA Isomers and Its Potential Use as Raw Material or in the Production of Functional Foods and Ingredients*

Recently, it was proposed that fruits and vegetables with an increased content of bioactive compounds, induced by the application of postharvest treatments, could be used as raw material to produce food and beverages with enhanced nutraceutical value [34–40]. Following this approach, products such as carrot juice [37] and purée [38] with an enhanced content of CGA have been produced. In the specific case of the carrot purée, it was tested on animal subjects to validate its pharmacological effect to enhance the cognitive abilities and neural development of rats. Moreover, food ingredients using the raw material of stressed carrot tissue with enhanced levels of CGA have been developed and added to highly consumed foods such as tortillas [35] and sausages [36]. In this context, potatoes containing higher levels of CGA due to wounding stress could also be used as ingredients to produce functional foods. Based on previous reports and the results obtained in the present study, Figure 2 presents different postharvest wounding stress and storage conditions that could be applied in potatoes to enhance the accumulation of specific CGA isomers.



**Figure 2.** Postharvest wounding stress and storage temperature conditions to trigger the accumulation of specific chlorogenic acid (CGA) isomers in potatoes. The tissue with an increased content of CGAs could be used as raw material to produce functional foods or dietary supplements.

Potatoes with increased levels of CGAs could be used to produce value-added products such as functional foods or dietary supplements. In accordance with the results obtained herein, if potatoes with a high content of CGA are desirable, pie-cut potatoes should be stored for 120 h at 20 °C, whereas if an enhanced concentration of crypto-CGA is expected, the tissue should be stored for 144 h at 20 °C, and to trigger the accumulation of neo-CGA, potato pie-cuts should be stored for 120 h at 10 °C. These raw materials with an increased content of CGA isomers could be used for the manufacturing of potato products fortified with CGA isomers. Examples of possible processed products include potato purée, or fried potatoes. Interestingly, regarding potato fries, it is likely that if stressed potatoes are used for their production, a lower content of acrylamide would be produced during frying, since asparagine content (a precursor for acrylamide formation) is highly decreased at the storage conditions of wounded potatoes [24]. In this context, potato fries with enhanced antioxidants and a lower acrylamide content would be obtained. However, this hypothesis should be further validated.

Figure 2 shows an additional alternative to increasing the content of neo-CGA in potatoes, which consists of adding amylolytic enzymes to pie-cut potatoes stored for 96 h at 20 °C, to increase the carbon source needed for CGA. In accordance with a previous report, it would improve the wound-induced accumulation of neo-CGA in potatoes [9]. For the potato tissue obtained by this procedure, the suggested use would be as raw material for the extraction and purification of the compound, since the followed bioprocess would affect the quality characteristics of the tissue (i.e., color, flavor, texture, etc.), impeding its further transformation into a processed food.

### 5. Conclusions

In the present study, the combined effect of temperature and wounding stress on the accumulation of antioxidant phenolic compounds in potato tubers was reported. The study was focused on evaluating the effect of both abiotic stresses (wounding and temperature) on CGA and CGA isomers (neo-CGA and crypto-CGA) content, since 90% of phenolic composition in potatoes corresponds to these three hydroxycinnamic acids. To the best of our knowledge, this is the first report in the literature that studied the effect of temperature on the accumulation of CGAs in wounded potatoes. Previous reports were focused on evaluating the effect of temperature on suberin and lignin accumulation, where a detailed study on the phenolic profile was overlooked. Herein, it was demonstrated that by modifying the temperature, the profile of CGAs could be altered, and thus appropriate storage conditions could be selected to trigger the accumulation of specific CGA isomers has not been reported; thus, further studies should be focused on understanding the biosynthesis pathway of CGA isomers in potatoes under wounding stress.

Interestingly, as observed in the chromatogram showing the phenolic profile of potato tubers (Figure 1), CGA and its isomers were well separated from each other, as their retention times in the column were 11.3, 16.3, and 20.2 min for CGA, neo-CGA, and crypto-CGA, respectively. Therefore, this chromatographic method could be used as a starting point to scale up the purification of CGA and its isomers for further uses in the pharmaceutical and dietary supplement industries. Likewise, to use stressed potatoes for human consumption, it would be desirable to have more than one isomer accumulated in the tissue. As such, pie-cut potatoes should be stored at 10 °C for 120 h, which would result in a high accumulation of CGA and neo-CGA in the tissue, when transforming into processed food. However, further research should evaluate the effect of wounding and storage time on the quality attributes of potatoes, as well as on the stability of CGA and its isomers when the tissue is subjected to different food processing conditions.

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