

Effect of wounding intensity on physiological and quality changes of strawberry fruit

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All relevant data are within the paper and its Supporting Information files.

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Abstract: Wounding makes fresh-cut product more perishable than whole fruit. The effect of wounding intensity on respiration rate and nutritional quality of fresh-cut 'Candonga' strawberries was investigated. Fruit were submitted to six levels of cutting intensity - whole fruit (WHO), 4, 16, 64, and 128 pieces and chopped (CHO) samples. Respiration rate, and the main nutritional parameters were evaluated at the processing day and after 2 days of storage at 5°C. Results showed that wounding intensity significantly influenced respiration rate, ascorbic and dehydroascorbic acids, total phenolic content, and antioxidant capacity. Respiration rate increased with wounding intensity up to the level of 64 pieces (10.01 $\mu\text{g kg}^{-1} \text{s}^{-1}$) compared to WHO (5.5 $\mu\text{g kg}^{-1} \text{s}^{-1}$) and then decreased in the CHO samples (2.81 $\mu\text{g kg}^{-1} \text{s}^{-1}$). At Day 2, the stress caused by the high intensity of cutting (64 pieces and CHO) induced a higher degradation of ascorbic acid, phenolic compounds, and antioxidant capacity. Stress-related changes decrease when the wounding damage was so high that it completely compromises the functionality of the cells (from 64 pieces up). These results should be considered for processing and packaging optimization of minimally processed strawberries-based products.

1. Introduction

The continuous physiological activity of living plant tissues induces severe compositional and structural variations, also associated to ripening and senescence of fresh produce during postharvest life. Tissue responses cannot be blocked but it is possible to delay them within certain limits in order to prolong fruit shelf life (El-Ramady *et al.*, 2015). The physiological stresses due to physical damage and wounding occurring during minimal processing, make fresh-cut products more perishable than whole fruit (Nicola and Fontana, 2014). Immediate response of plant cells start from a wound signal formed in adjacent and distant tissues, which gives rise to a wide range of different physiological and biochemical reactions. Common are respiration rate and ethylene production increase, variation in product quality, synthesis and/or loss of phytochemicals with consequent decrease of nutritional content, stimulation of enzymatic activity

and bacterial spoilage (Brecht, 1995; Surjadinata and Cisneros-Zevallos, 2003). The complex interrelationship among the different effects of wounding on physiological processes of fresh-cut products, are comprehensively described by Saltveit (1997). Wound responses could vary depending on different factors: species and cultivar of the product, maturity stage, temperature of processing and storage, cutting-type and sharpness of the blades, but also on process temperature (El-Ramady *et al.*, 2015), O₂ and CO₂ levels, and water vapor pressure (Brecht, 1995). The basis for the wound-induced changes include altered genes expression and changes in enzyme activities involved in an effort to heal the damaged tissues providing defense mechanisms of the plant aimed to prevent further and more serious damages (Chung *et al.*, 2006). During storage time the increase in respiration rate is usually responsible for the aging of the products due to consumption of reserve energy during redox process. As a consequence, the higher the respiration rate, the shorter is the storage life and therefore the faster is the quality deterioration. Respiration is a function of the climacteric or non-climacteric behavior of the product and of the physiological age of climacteric fruit (Gunes and Lee, 1997). As already reported wounding induces an increase in enzyme activity, in particular higher activities of phenylalanine ammonia-lyase (PAL), peroxidase (POD) and polyphenol oxidase (PPO) are observed. (Saltveit *et al.*, 2005 b). The main effect of the post-cutting interaction of substrates with enzymes, such as ascorbate oxidase, PPO, and POD, is the degradation of phytonutrients. Phenols oxidation and the resulting browning may induce a reduction in nutrient content resulting often in degradation of color, texture and flavor of fresh-cut products (Saltveit, 1997; Francis *et al.*, 2012). It is possible to reduce wound-induced browning with the application of antioxidant or calcium-based active compounds and treatments that interfere with the synthesis or oxidation mechanisms of the phenolic compound precursors (Brecht, 1995; Saltveit, 1997; Saltveit *et al.*, 2005 a). However, according to Francis *et al.* (2012), the induced synthesis of phenolic compounds after cutting caused an increase in nutritional value for lettuce, celery, carrot, parsnips, and sweet potato, while in the same study a decrease of phenols was observed in cut zucchini, radish, potato, and red cabbage, pointing out the influence of product on the wound-induced response type. Amodio *et al.* (2014) described the consecutive reaction mechanism that regulates the phenolic content in fresh-cut produce

during storage and how their variation is related to cut intensity. They observed, for example, that there was an increase in k_1 values (the rate constant for the *de novo* synthesis of the phenols), when fresh-cut lemons were cut as half-slices rather than slices. This result was also in agreement with the increase in PAL activity resulting from the higher level of wounding on vegetable tissues. At the same time, the cut intensity did not affect the rate of phenolic oxidation. The authors stated that one of the most important factor affecting the phenolic content and synthesis is the biological variability; in fact, each product shows a particular combination of factors that can contribute to the amount and composition of wound-induced phenolics (Francis *et al.*, 2012). Fernando Reyes *et al.* (2007) stated that the final concentration of phenolic compound in cut products is strongly affected also by the type of tissue and the initial level of reduced ascorbic acid.

Strawberry-based product attracted in the recent years the food industry due to the high amount of bioactive compounds (vitamin C, anthocyanins and flavonols). For this reason strawberries are one of the richest fruits in term of antioxidant capacity (Cordenunsi *et al.*, 2002; Pertuzatti and Barcia, 2015). Understanding the stress-induced changes is important in order to develop reliable approaches to control the stress responses, and improve the quality of minimally processed fresh products, particularly when the fruit is subjected to a pronounced mechanical damage. This makes very interesting the investigation on the effect of wounding on soft and fresh fruit like strawberries. Therefore, the main objective of the present study was to determine the effect of wounding intensity, on physiological and quality changes of fresh strawberry fruit, with a particular focus on the respiration rate and nutritional compounds.

2. Materials and Methods

Sample preparation

'Candonga' strawberries (*Fragaria x ananassa* Duch.) were purchased from local stores in Foggia (South Italy) and stored at 5°C overnight. In the next morning, fruit with uniform color and size, free of physical defects and decay, were divided into six groups (treatments), each one corresponding to one different level of wounding intensity: whole fruit (no cutting), cutting into 4, 16, 64, 128, pieces and chopped defined as WHO, P4, P16, P64, P128 and

CHO, respectively. About 12-15 fruit were cut (300 g) for each replicate and 150 g of product were used for initial determinations, whereas the remaining samples were stored at 5°C under a continuous flow of humidified air for 2 days. Three replicates were used for each cutting intensity treatment and for each quality parameter determination. Vitamin C, total phenolic and anthocyanin content, antioxidant capacity, soluble solid content, pH value, titratable acidity and sugar/acid ratio were evaluated at the processing day (Day 0) and after storage (Day 2). Separate samples were used for respiration rate measurement.

Respiration rate

Respiration rate of fresh-cut strawberries was measured in static conditions as described in Kader (2002). Respiration rate (expressed as $\mu\text{g kg}^{-1} \text{ s}^{-1}$ of CO_2) was determined at 120 min after cutting. Separate samples (about 300 g each) of strawberries were placed in 5 L sealed glass jars with a plastic septum for sampling gas; jars were closed after an equilibration time of about 1 hours. From each jar, a gas sample (0.5 mL) was collected after the required time to accumulate CO_2 in the headspace up to a concentration of 0.1-0.2%, and injected into a gas chromatograph (Shimadzu, model 17 A, Kyoto, Japan), equipped with a thermal conductivity detector (200°C). Separation of CO_2 was achieved on a Carboxen 1006 plot (30 m \times 0.53 mm, Supelco, Bellefonte, PA, USA), with a column flow of 7 mL min^{-1} , and an oven temperature of 180°C.

Compositional attributes

Vitamin C content was measured in 5 grams of fresh homogenized strawberry tissue as L-ascorbic acid (AA) and L-dehydroascorbic acid (DHA) contents expressed as g of AA, DHA or total vitamin C (AA + DHA) per 1 kg of fresh weight (g kg^{-1}) following the procedure by Zapata and Dufour (1992) with slight modifications.

Total phenolic content (TPC) was analyzed using the Folin-Ciocalteu method of Singleton and Rossi (1965), with some modifications where five grams of fresh tissue were homogenized in an Ultraturrax (IKA T18 basic, Wilmington, NC, USA) with 10 mL of extraction buffer containing 200 mL of distilled water, 800 mL of methanol and 2 mM (84 mg L^{-1}) of sodium fluoride (NaF). The absorbance was read at 725 nm compared with a blank (prepared in the same way, replacing the sample with 100 μL of distilled water) using a spectrophotometer (UV-1700

Shimadzu, Jiangsu, China). TPC was calculated based on the calibration curve of gallic acid and results were expressed as g gallic acid equivalents per 1 kg fresh weight (g kg^{-1}).

Total anthocyanin content (TAC) was determined following the protocol described by Cordenunsi *et al.* (2002), with small modifications using hydrochloric acid/methanol mixture as extraction medium. 700 μL of extract plus 300 μL of 1% HCl-MeOH solution were put in cuvettes and absorbance was read immediately in a spectrophotometer at 510 nm. Results were expressed g of pelargonidin-3-glucoside (PG-3-glu) equivalents per 1 kg of fresh weight (g kg^{-1}).

The antioxidant capacity (AC) assay was conducted on the same extract made for TPC, following the method of Brand-Williams *et al.* (1995), with slight modifications. Fifty μL of extract were mixed with 950 μL of DPPH (2, 2-Diphenylpicrylhydrazyl) solution and absorbance was read at 515 nm after 24 h. Trolox (6-Hydroxy-2, 5, 7, 8-tetramethylchromane-2-carboxylic acid) was used as a standard and results were expressed in g of Trolox equivalents per 1 kg of fresh weight (g kg^{-1}).

Total soluble solids (TSS) were obtained by measuring the refractive index of fresh strawberry juice using a digital refractometer (Atago RX-7000cx; Atago Co. Ltd., Japan) at 25°C and expressed as percentage. One gram of sample was used to determine the pH and titratable acidity (TA), with an automatic titrator (T50 M Terminal, METTLER TOLEDO, Switzerland) against a volume of 0.1 N NaOH until reaches the final pH of 8.2. TA was expressed as g of citric acid equivalent per 1 kg of product (g kg^{-1}). TSS/TA ratio was also calculated.

Statistical analysis

Data were subjected to a 2-way ANOVA (for treatment and sampling time); treatment means were separated by Tukey's test at $P < 0.05$ using Stat Graphics Centurion XVI.I (Stat Point Technologies, Inc., Warrenton, VA USA) software.

3. Results

Wounding intensity had a significant effect on all parameters except pH; the same for storage time with exception of total phenolic content, while the interaction between the two factors showed a significant effect on respiration rate, ascorbic and dehydroascorbic acids, total vitamin C, total phenolic content and antioxidant capacity but no significant effect

on TAC, TSS, pH, TA and TSS/TA ratio. Following, these effects are described in detail. The effect of wounding intensity on the respiration rate of 'Candonga' strawberry fruits after 120 min post-cutting at 5°C is shown in figure 1. Wounding induced a significant raise in respiration of strawberries tissues that resulted to be increasing with cutting intensity up to a certain point from 5.5 (WHO) to 10.01 $\mu\text{g kg}^{-1} \text{s}^{-1}$ of CO_2 (P64). Further increase of wounding beyond P128 did not stimulate respiration rate: a significant decrease up to a minimal level of 2.81 $\mu\text{g kg}^{-1} \text{s}^{-1}$ was observed for chopped samples.

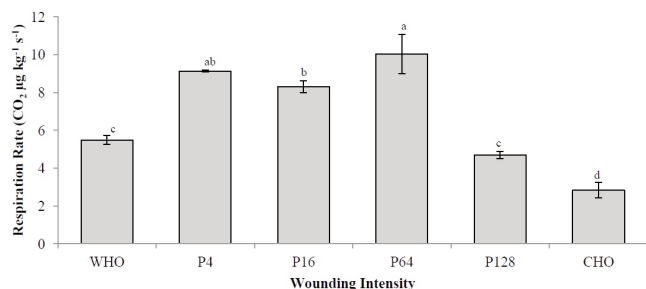


Fig. 1 - Effect of wounding intensity on respiration rate at 5°C of 'Candonga' strawberry fruit. WHO, P4, P16, P64, P128 and CHO stay for whole fruit, cut into 4, 16, 64, 128 pieces and chopped. Error bar represent st. dev of mean values (n=3). Different lowercase letters indicate significant difference among treatment according to Tukey's test ($P \leq 0.05$).

The effect of wounding intensity on AA, DHA and total vitamin C content (AA+DHA) of fresh-cut 'Candonga' strawberries is shown in figure 2. At Day 0, AA and total vitamin C were not significantly affected by wounding intensity, with almost similar values referred to ascorbic acid among treatments ranging from 0.37 (P64) to 0.44 g kg^{-1} (P4). On the other hand, an increase in DHA content could be observed already at Day 0 with a significant difference among treatments ranging between 0.067 g kg^{-1} in the WHO and 0.22 g kg^{-1} in the treatment P128. The mean values of total vitamin C content at Day 0 ranged from 0.51 (WHO) to 0.61 (P128) g kg^{-1} . P128 and CHO samples showed in fact significantly lower values of ascorbic acid (0.27 and 0.17 g kg^{-1} , respectively) than other treatments, with 64-piece sample showing an intermediate behavior (0.41 g kg^{-1}). Moreover a significant increase of DHA with the increase of wounding intensity was observed for all samples starting from P16. The lowest and highest DHA level at Day 2 were found with the WHO and CHO samples (0.12 and 0.55 g kg^{-1} , respectively). The highest level of wounding intensity (CHO) induced about a 5-fold increase in DHA content.

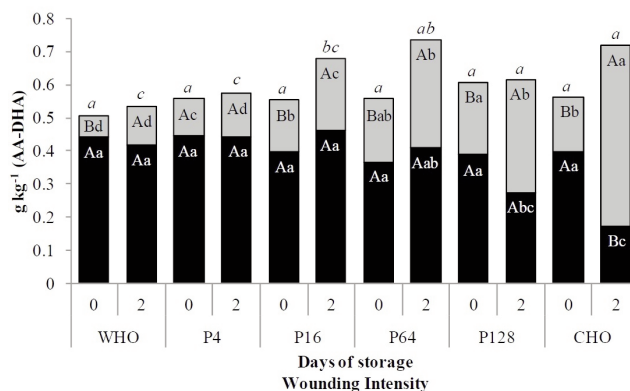


Fig. 2 - Effect of wounding intensity on ascorbic acid (AA, black bars, g kg^{-1}), dehydroascorbic acid (DHA, grey bars, g kg^{-1}) and total vitamin C content (sum of AA and DHA, g kg^{-1}) of fresh-cut 'Candonga' strawberries at day 0 and day 2 of storage at 5°C. WHO, P4, P16, P64, P128 and CHO stay for whole fruit, cut into 4, 16, 64, 128 pieces and chopped. Mean value (n=3) is reported. Different lowercase and uppercase letters, indicate significant differences among treatments and storage times, respectively, according to Tukey's test ($P \leq 0.05$). Different italic letters indicate significant differences among treatments for total vitamin C content for each storage time.

The effect of wounding intensity on TPC, TAC, and AC of fresh-cut 'Candonga' strawberries is shown in Table 1. The different cutting stress significantly affected the TPC of fresh-cut strawberries. At Day 0, P128 had significantly higher ($P < 0.05$) total phenolic content compared to other treatments with the exception of P64, with P16 showing the lowest amount (2.27 g kg^{-1} of gallic acid equivalent) and, together with P4 (2.37 g kg^{-1}), resulting in significantly lower content of phenolic compounds than whole fruit. There was no significant difference in TAC both at Day 0 and 2 although the mean TAC value of sample P4 showed slight increase during storage time. The mean value of TAC ranged from 0.19 (P4) to 0.22 (CHO) g kg^{-1} of PG-3-glu (Table 1). Wounding intensity also significantly affected antioxidant capacity of fresh 'Candonga' strawberries. At Day 0, samples cut into 16 (6.35 g kg^{-1} of Trolox equivalent) and 64 (6.59 g kg^{-1}) pieces had significantly higher AC than the CHO (5.41 g kg^{-1}) and WHO (5.20 g kg^{-1}) samples. A similar trend was also observed after 2 days from cutting when P16 sample showed significantly higher AC value when compared to the CHO samples, although this difference was not significant if compared to the rest of the treatments. A particular behavior was observed for CHO strawberries (Table 1): no significant effect of storage time was observed on the TPC, TAC, and AC values of this sample indi-

cating that these compounds were quite preserved during cold storage, and did not show after two days of storage great variations if compared with other samples. In addition, this sample showed also the lowest respiration and no significant difference in total vitamin C if compared to the WHO samples. These characteristics may be therefore exploited to maintain the quality of fresh-blended products.

TSS, pH value, TA and TSS/TA ratio of fresh-cut strawberry subjected to different wounding intensity and stored for two days at 5°C were significantly affected by wounding intensity (with exception of pH) and storage time, but not by their interaction (data not shown). The mean values of TSS ranged from 7.6 (WHO at Day 0) to 8.6% for WHO, P16 and CHO samples at Day 2. Almost similar pH values (3.9 to 4.0) were determined for all treatments and sampling times. TA ranging from 0.07 to 0.08 g kg⁻¹ of citric acid showed almost no differences among treatments and sampling time except for P64 which had a slightly lowest value after two days. Similarly, almost no differences in the TSS/TA ratio were recorded. The highest and lowest values were 9.4 (P4 at day 0) and 12.1 (P16 at Day 2).

4. Discussion and Conclusions

The effect of wounding intensity on respiration rate and compositional values of fresh 'Candonga' strawberry fruit was clearly determined. In general, high degree of wounding intensity (P128 and CHO) caused a significant decrease in respiration rate. In addition, the chopped sample did not show any significant difference in TPC, TAC and AC during storage time.

Respiration rate gives an immediate overview of the metabolism of a commodity (Fig. 1), where higher respiration is an indicator of accelerated metabolism which is usually inversely related to shelf-life. Strawberry is among the commodities with highest respiration rate (Saltveit, 2002), that can be further increased as a cut consequence. According to Surjadinata and Cisneros-Zevallos (2003), an increase in respiration may occur due to simultaneous enzyme synthesis and decrease. The newly synthesized enzymes could in fact be degraded by an inactivation system. It is possible that, after a certain cutting degree, the very high tissue damage compromised the cell functionality. The transition from respiring to non-respiring tissues after wounding is probably related to the damage of the membrane system or mitochondria and consequent disruption of oxidative phosphorylation. Changes in ammonium dihydrogen phosphate (ADP) and ammonium transferase phosphate (ATP) concentrations in wounded tissue indicate that oxidative phosphorylation failed to keep place with ATP utilization in injured tissues (Lafta and Fugate, 2011). According to these authors, a 41% reduction in ATP concentration and a simultaneous increase in ADP (31%) were observed between day 1 and day 4 after incremental injury of sugar beet root. Costa *et al.* (2011), reported that respiration rates of fresh-cut strawberries was higher than whole fruit and that low storage temperature significantly influenced this parameter. This is possibly due to the fact that cutting increases the surface exposed to the air, and as a consequence, oxygen is able to diffuse into the internal cells more rapidly. Moreover, injured cells show an increased metabolic activity (Nilsson and Hedenqvist, 2011; Saltveit, 1997). Thus, respiration is stimulated by physical damages given to the

Table 1 - Effect of wounding intensity on total phenolic content (TPC, in g kg⁻¹ of gallic acid equivalent), total anthocyanin content (TAC, in g kg⁻¹ of pelargonidin-3-glucoside), and Antioxidant Capacity (AC, in g kg⁻¹ of Trolox equivalent) of fresh-cut 'Candonga' strawberries at day 0 and day 2 of storage at 5°C

	Days at 5 °C	Wounding intensity					
		WHO	P4	P16	P64	P128	CHO
Total phenolic content	0	2.88±0.07 Ab	2.37±0.16 Bc	2.27±0.04 Bc	2.95±0.02 Aab	3.23±0.08 Aa	2.94±0.05 Ab
	2	2.62±0.12 Bb	3.19±0.18 Aa	3.25±0.17 Aa	2.52±0.15 Bb	2.87±0.10 Bab	2.58±0.07 Ab
Total anthocyanin content	0	0.19±0.004 ns	0.19±0.007 Ans	0.20±0.004 ns	0.21±0.018 ns	0.20±0.019 ns	0.20±0.010 ns
	2	0.21±0.015 ns	0.21±0.002 Bns	0.21±0.014 ns	0.22±0.002 ns	0.22±0.021 ns	0.22±0.012 ns
Antioxidant capacity	0	5.89±0.03 Abc	6.02±0.05 Aabc	6.35±0.12 Aa	6.59±0.09 Aa	6.27±0.12 Aab	5.41±0.06 Ac
	2	5.20±0.22 Bab	5.98±0.44 Bab	6.16±0.02 Ba	5.44±0.03 Bab	5.67±0.09 Aab	4.62±0.10 Ab

IWHO, P4, P16, P64, P128 and CHO stay for whole fruit, cut into 4, 16, 64, 128 pieces and chopped; mean values (n=3) ± standard deviations are reported.

Different lowercase and uppercase letters, indicate significant differences among treatments and storage times, respectively, according to Tukey's test ($P \leq 0.05$).

fruits: the more the severity of damage, the more the degree of respiration rate increase (Kader, 1987; Zhu *et al.*, 2001). The main consequence of an increase in CO₂ and ethylene production as a response to cutting process (Saltveit, 1997) could be a reduction of the fresh-cut product shelf life. Moreover, moisture in the cut surface may impede gas diffusion, and this, together with increased respiration, could possibly lead to anaerobiosis, causing further deterioration of the tissues (Saltveit, 1997; Surjadinata and Cisneros-Zevallos, 2003). However, wound-induced respiration depends on the type of tissue, temperature, controlled atmospheres and degree of cutting (Zhu *et al.* 2001). As described by Surjadinata and Cisneros-Zevallos (2003) respiration rate of carrot tissues after wounding showed a typical increase (resulting in a maximum peak) and then a decrease reaching steady-state respiration values similar to that of whole carrot. In some plant tissues, such as potato, this behavior may be related to the oxidation of fatty acids and carbon dioxide, being these reactions responsible for increased respiration after wounding (Gunes and Lee, 1997). According to Surjadinata and Cisneros-Zevallos (2003), wounding stimulates enzymatic activity of phosphofructokinase and cytochrome oxidase from the respiration pathway, which catalyse the phosphorylation of fructose-6-phosphate to fructose-1,6-bisphosphate (a key regulatory step in the glycolytic pathway) and the electrons transfer to oxygen, respectively. A higher enzymatic activity could be due to activation of already present enzyme or to *de novo* synthesis, as suggested by Surjadinata and Cisneros-Zevallos (2003). Their higher activity results in an increase of respiration rate in wounded tissues (Lafta and Fugate, 2011).

On the processing day total vitamin C content did not vary among the treatments, most probably because ascorbic acid was oxidized to dehydroascorbic. The same vitamin C content trend showed in figure 2 was observed by Costa *et al.* (2011), and may be ascribed to the fact that tissue had only slightly responded to the stress. After 2 days from cutting a slight increase in AA was observed for samples P16 and P64 while a noticeable reduction was found with the increase of cutting intensity. As a results of enzymatic and non enzymatic oxidation of ascorbic acid, prolonged storage period, mechanical damages or thermal treatment, DHA amount is known to increase. The oxidized form thus represents the majority of vitamin C (Davey *et al.*, 2000; Lee and Kader, 2000). In the present study different behaviors were observed: as for P128 and CHO sam-

ples the decrease in AA amount due to oxidation was accompanied by an increase in DHA amount ending in a rise of total vitamin C content of the products. Since this increase was proportional with the decrease in AA concentration, it can be supposed that no further oxidation of DHA into 2,3-diketogulonic acid occurred. Regarding P16 and P64 samples at day 2, higher AA amounts were detected, suggesting the occurrence of new synthesis or the presence of different sources of ascorbic acid. Cordenunsi *et al.* (2005), reported that ascorbic acid synthesis in strawberries occurs during the storage period, and that temperature may affect it. As for sweet pepper fruit, it was observed that wounding stress activated both biosynthesis and metabolism reduction of ascorbic acid, leaving unaffected the level of AA in the product (Imahori *et al.*, 1997). Moreover, Wolucka and Van Montagu (2003) proposed a new vitamin C biosynthesis pathway in which L-gulose and L-gulono-1,4-lactone act as direct precursors of ascorbic acid in plant tissue during storage. As for the present work it was possible to suppose that new synthesized ascorbic acid replaced the amount which was oxidized. As a result, ascorbic acid content did not decrease over time while a huge increment in DHA occurred for P64 sample. Some authors observed in different strawberry cultivars that DHA evolution with storage time, when associated with ascorbic acid retention, can be considered an evidence of a redox system (AA/DHA) triggered during cold storage, reported to be a cultivar-specific more than fruit-specific process (Cordenunsi *et al.*, 2005). Moreover, to date, many of the products deriving from DHA degradation are still unclear, although 2,3-diketogulonic acid, threose and oxalic acid, glyoxal, methyl glyoxal and diacetyl have been identified or hypothesized as byproduct of dehydroascorbic acid decomposition (Fayle *et al.*, 2000). Ascorbate, its product of oxidation (DHA) and consequently DHA metabolism play significant roles in the apoplast (Lin and Varner, 1991). For this reason DHA results to be a key-factor in ascorbate catabolism, and it could be oxidized to oxalate or hydrolyzed to 2,3-diketogulonate and downstream carboxypentonates. The prevalence of one of the two reactions (oxidation or hydrolysis) is dependent on the status of the reactive oxygen species (Parsons *et al.*, 2011). In general, DHA/AA ratio tends to rise during storage time although the oxidized form is unstable and is easily decomposed, this leading to a decrease in its biological activity. The changes in the form of ascorbic acid result to be important from a technological and a

nutritional point of view (Lee and Kader, 2000; Cordenunsi *et al.*, 2005).

A possible explanation for what is showed in Table 1 may be found in the prevalence of phenolics *de novo* synthesis with respect to their oxidation as response to cutting stress. As described by Kang and Saltveit (2002), the cutting related to processing of fresh-cut fruit and vegetables may induce an increase in their antioxidant capacity, enhancing synthesis and accumulation of phenols. In fact, wounding stimulates the activity of phenylalanine ammonia-lyase, which is responsible for the catalyzation of the first step of phenylpropanoid metabolism due to which tartaric acid is converted into chlorogenic acid. An increase in its activity leads to accumulation of phenols, enzymatic oxidation and tissue discoloration (Saltveit, 2000; Adams and Brown, 2007). In contrast, however, wounding also induces antioxidant degradation, resulting in oxidation of active compounds such as ascorbic acid and phenolic compounds. Their final content is the balance result between production and oxidation rates, being these rates affected by storage temperature or cutting intensity as modeled by Amodio *et al.* (2014). However, in the case of a more serious damage, as for CHO samples, TPC degradation was supposed to be faster than their production, ending in the lowest value of TPC. At Day 2 however, TPC increased more clearly in P4 and P16 samples, which showed significantly higher values than other treatments, including the WHO. This is supported by literature, Torres-Contreras *et al.* (2014) report that phenolic content in white potato tubers that were subjected to different wounding stresses showed an accumulation of 100% and 65% TPC for slices and pieces, respectively, whereas shredded potatoes stored at 10°C for 96 hours showed 40% lower phenolic content if compared to the starting product. Similarly, an accumulation of wounding-induced TPC (approximately 60%) and an increase in the activity of PAL enzyme were found in purple-flesh sliced potato tissues stored at 15°C for 48 hours (Reyes and Cisneros-Zevallos, 2003). According to Surjadinata and Cisneros-Zevallos (2003), phenolic antioxidant accumulation is dependent on the level of wounding intensity. The phenolic content increased with wounding intensity by 97, 76, and 252% when cut as slices, pieces and shreds, respectively compared to non-wounded carrots (0.45-0.52 g kg⁻¹). Moreover, the same behavior was observed by Hu *et al.* (2014) on fresh-cut lotus root. After 7 days of storage PAL activity of fresh-cut lotus root slices was 68% higher compared to control. At

the same time 130% increase in phenolic content was detected. Antioxidant activity also increased due to total phenol accumulation; however, wounding resulted in a significantly higher browning by increasing the PPO activity of the slices. Phenols represent the main structural and defense-related functions in plant cells via the phenylpropanoid pathway. Wounding of fruit and vegetables tissues obviously causes rupture of the cell membrane and this induce several physiological responses causing the combination of phenolics with the oxidative enzymes and/or the synthesis of different classes of phenolics to repair the wounding damage. In strawberries, TAC was reported to be in the range between 0.15 to 0.80 g kg⁻¹ (Padmanabhan *et al.*, 2016) and cold storage may induce their biosynthesis and accumulation (Holcroft and Kader, 1999). Accordingly, about 60% accumulation of TAC in sliced potatoes was described by Reyes and Cisneros-Zevallos (2003), and a similar increase was reported for anthocyanin content in pomegranates stored at low temperatures (Arendse *et al.*, 2014). The high amount of vitamin C, phenolics including anthocyanins and therefore antioxidant activity in strawberries, even after severe cutting stress, is very important for beneficial effects on consumer health (Giampieri *et al.*, 2012). The relatively higher values of AC determined for CHO sample could probably be due to the higher values of total vitamin C and phenolics. Antioxidant capacity of fruit is, in fact, strictly related to the presence of vitamin C and phenolic compounds, being these effective systems to scavenge oxygen radical, AC level is influenced by the occurrence of different active phytochemical compounds (Giampieri *et al.*, 2012). As reported by Tulipani *et al.* (2008), vitamin C in strawberries is the greatest contributor (>30%) to the total antioxidant capacity followed by anthocyanins (contributing for 25-40%). The remaining part was composed mainly of ellagic acid derivatives and flavonols (Padmanabhan *et al.*, 2016). Antioxidant activity is therefore an expression of total vitamin C and total phenolic content of the product, including anthocyanin and ellagitannins groups (Giampieri *et al.*, 2012).

As for pH, TSS and TA, no clear trend and very slight differences were observed, as also reported by Li *et al.* (2017) studying the effect of different cutting styles on postharvest quality of pitaya fruit. In this case, fresh-cut processing also showed little effect on the contents of vitamin C, TSS and TA. This behavior could be interesting since the nutritional quality of the products do not greatly change after treatments,

allowing to maintain their fresh-like state.

The results of the present study demonstrated that the application of wounding intensities could be used as simple emerging technology to induce the accumulation of TPC in plants (Torres-Contreras *et al.*, 2014) and selection of appropriate wounding intensity and/or abiotic stress can enhance the nutritional and functional and health-related values of fresh produce (Reyes and Cisneros-Zevallos, 2003; Hu *et al.*, 2014). These results should be taken into consideration for processing and packaging optimization of minimally processed products from fresh strawberries, although further investigations extending shelf life period would be necessary in order to make these results useful for industrial application.

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