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# Effect of slicing and storage temperatures on biochemical aspects of membrane integrity in two different genotypes of tomato

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**Abstract**

*Background:* Fresh-cut vegetables are subjected to multiple stressing agents: i) slicing that induce cellular decompartmentalization; ii) low refrigeration temperature responsible of chilling injury in the most sensitive products (e.g. tomatoes); iii) storage time which leads the tissue until senescence. In tomato slices, one of the most important issue is membrane that is responsible of several disorders related to the alteration of physiological processes, including ethylene biosynthesis.

*Results:* Electrolyte leakage and thiobarbituric acid reactive substances content of sliced tomatoes increased over time at both storage temperatures (4 and 15°C) compared to intact fruit for the commercial variety (cultivar) Jama used as reference. However, in the tomato Italian landrace Canestrino, electrolyte leakage in sliced fruits increased after 120 h of storage compared to intact tomatoes, whilst thiobarbituric acid reactive substances content rapidly increased over time at both storage temperatures. In the packages, higher values of ethylene content and carbon dioxide concentrations were detected in sliced tomatoes compared to intact fruits for both genotypes. In the most sensitive genotype for slicing (Jama), phospholipase C activities increased in tomato slices after 24 h of storage, while phospholipase D reached higher value only at 168 h after processing at 4 °C of storage.

*Conclusions:* The results evidence that the main damage in slices of full ripe tomatoes is more related to cutting, instead of chilling injury due storage temperatures with differences related to the genotype. Slicing enhanced membrane catabolism, ethylene production and enzyme activities of phospholipases with a significant effect of the genotype.

**Keywords:** electrolyte leakage, membrane lipid peroxidation, ethylene, carbon dioxide, phospholipases, fresh-cut tomato

**Abbreviations:** EL, electrolyte leakage; TBARS, thiobarbituric acid reactive substances; PLC, phospholipase C; PLD, phospholipase D; EC, electrical conductivity; FW, Fresh weight; WT, wild type.

## 1. Introduction

Fresh-cut fruits and vegetables or fresh-cut produce are defined as fruit and vegetable products subjected to peeling, slicing, and packaging in order to satisfy the consumer request for fresh products having high nutritional value.[1]

Although fresh-cut produce has the quality and sensory attributes of the intact commodity, their shelf life is dramatically reduced compared to intact fruit or vegetable. The metabolism of these products is mainly affected by cellular disruption due to slicing, chilling injury, and increase in ethylene production that triggers several catabolic processes leading to a more perishable product compared to the intact fruit or vegetable.[2] Indeed, the physiology of fresh-cut produce is essentially that of wounded tissues, thus ethylene plays a pivot role in triggering tissue senescence.[3]

Slicing hastens tissue deterioration and induces softening which are considered the major limiting factors affecting fresh-cut quality.[4,5] Loss of texture is related to both processes of cell wall disassembly (pectic matrix degradation) and changes in turgor pressure. [6,7,4] The first phenomenon is mainly related to enhancements in the activity of enzymes such as pectinmethylesterase (PME, EC 3.1.1.11) and polygalacturonases (eso-PG, EC 3.1.1.15 and endo-PG, EC 3.2.1.15).[8] Instead, the loss of turgor pressure is related to changes in plasmalemma integrity by leading to an increase in EL.

Membrane breakdown is a consequence of physical alteration in the double layer structure as a consequence of chilling injury damage[9], senescence, and the activity of reactive oxygen species (ROS) or membrane hydrolytic enzymes like phospholypases (PLC, EC 3.1.4.3; PLD, EC 3.1.4.4).[10-13] Indeed, Pinhero *et al*. [14] demonstrated that PLD enzyme suppression can preserve tissue integrity and reduce the loss in colour of fresh-cut tomato.

Chilling injury induces a gradually increase in the permeability of cellular membranes due to a lipid phase separation over a few days of storage at temperature below 10°C in tomato fruits. [9]

Wounding considered as abiotic stress, may trigger lipid metabolism signaling cascade that involves phospholipids degradation which are the main lipid component in tomato membranes.[15] According to the working model of phospholipids degradation for *Arabidopsis thaliana* suggested by Wang *et al.*[12], the wounding signal involves the translocation of PLD to membrane via an influx of $Ca^{2+}$. PLDs associated with membranes change their conformation and become active by initiating the lipolytic process. PLD releases second messengers as phosphatidic acid (PA) that directly stimulates the activities of acyl-hydrolyzing enzymes as PLC. Downstream this lipolytic pathway, free polyunsaturated fatty acids from membrane phospholipids are released and hydroperoxidated by lipoxygenase (LOX). Lipid peroxidation level can be determined using TBARS assay.

The complete membrane breakdown leads to an increase in ions efflux through membrane that is also manifest through enhancement in electrolyte leakage.

The increase in leakage of cellular solutes resulting from wound-induced degradation of membrane lipids has been observed in fresh-cut papaya accompanied by increase in lipid peroxidation catalyzed by LOX.[16]

Previous studies demonstrated that ethylene production is stimulated by slicing and the increase of the concentration of this hormone into packages can lead to undesirable effects on fresh-cut quality, like loss of firmness.[17] Ethylene plays a crucial role in the postharvest physiology for both climacteric and aclimateric products. In mature green tomato pericarp tissue, Saltveit demonstrated that applications of the ethylene inhibitor aminoethoxyvinylglycine (AVG) can reduce ethylene and protein biosynthesis without alteration for the rate of $CO_2$ production. [18] Moreover, Mao *et al.*[19] demonstrated increases in PLC and PLD activities in watermelon treated with exogenous ethylene and Fan *et al.*[20] concluded that *PLD* gene expression and PLD enzyme activity in leaves of *Arabidopsis thaliana* are enhanced by ethylene treatments. Interestingly, Pinhero *et al.*[14] suggested

the potential role of PLD in ethylene signal transduction with potentially distinct functions for ethylene receptors *SlETR*-1 and *SlETR*-2.[14,21]

More recently, researches were focused on the role of slicing in membrane deterioration for an ethylene mutant genotype (Gimar Never Ripe, Gimar Nr/Nr) and its wild type (Gimar nr/nr) in order to investigate the role of the hormone on electrolyte leakage, lipid peroxidation and phospholipases activities.[22] The Nr/Nr genotype showed higher membrane integrity (lower electrolyte leakage and lower lipid peroxidation) compared to its WT. Furthermore, the influence between temperature and the maturity stage of a tomato commercial variety (Jama) on physiological and biochemical parameters related to membrane alteration after slicing was reported.[23] In this research article, two different genotypes were used: the cultivar Jama and the Italian tomato landrace Canestrino at the full ripe stage of maturity stored at two different temperatures in order to investigate the role of slicing into membrane degradation and ethylene production. Fruits were collected at the full ripe stage of maturity because it is assumed to be closer to the senescence and then more sensitive to membrane alteration after slicing.[23] Furthermore, the use of the Italian local variety Canestrino as extra-genotype is related to its properties as a firm tomato with long shelf life also after slicing.

The objectives of the present research work were to: i) investigate the properties of Canestrino landrace as fresh-cut tomato compared to a commercial variety; ii) investigate phospholipases activities in the most susceptible variety; iii) validate the model of membrane degradation as suggested by Wang *et al.*[12] at two different temperatures in varieties with (putative) different attitudes for slicing.

## 2. Materials and methods

### *2.1 Plant materials and growing technique (fruit sampling, processing, and storage)*

Round-fruit tomato plants (*Solanum lycopersicum* L.) cv.s Jama and Canestrino were hydroponically grown in a glasshouse in Pisa (latitude 43°43'N; longitude 10°23'E; Italy) and the minimum (heating) temperature was set to 14°C.

Plants were grown in a semiclosed-loop hydroponic cycle (nutrient film technique system) and the recirculating nutrient solution was discharged after 3 weeks or whenever the EC was higher than 6 mS $cm^{-1}$. Indeed, the evapotranspiration was refilled using a nutrient solution with electrical conductivity (EC) 3.5 mS $cm^{-1}$ and pH 6.5. The substrate was rockwool slabs 1-m long with three single-stem plants to obtain a density of 3 plants $m^{-2}$.

Fruits were tagged at anthesis (defined as the time of petal drop and fruit set) and harvested on a single day at the colour stage full ripe that was achieved at around 50 days after anthesis corresponding to the stage 6 of ripeness classification described by Sargent and Moretti.[24] At harvest, fruits were selected according to colour, shape, size, and firmness.

After harvest and within a few minutes, tomatoes were washed in cold tap water, immersed for c.a. 1 minute in sodium hypochlorite solution (1 mg $L^{-1}$), rinsed in tap water and then dried with paper tissues. Fruits were cut into 10.0 mm thick slices from the stem end with a stainless-steel knife equipped with a smooth blade, but the outermost slices were removed. A single intact tomato (as control) and two slices derived from the equatorial portion of cut fruits were collected and stored according to the experimental plan into a 1.1 L plastic (polyethylene) fresh-cut produce container. Two slices of tomato fruit were used per each sample in order to avoid physiological disorders (e.g. translucency, flooding…) related to extra juice released after cutting and coming from stucked slices. [5] Refrigeration was conducted at 4.0 ± 0.5 °C and at 15.0± 0.5 °C in dark conditions for both intact tomatoes and slices. For all samples, the storage time was until 72 and 168 h after slicing for the storage at 15 and 4°C, respectively. The shorter storage time for the highest temperature was to avoid degenerative processes that could affect fruit metabolism and influence membrane degradation. The air exchanges rate of closed containers at both storage temperatures were determined using exogenous ethylene in preliminary experiments as tracer gas. Standard gas was

injected into containers and gas samples were collected at different storage time. No significant variation was detected over time and fresh-cut containers were airtight.

In total, the time points were: 0, 24 and 72 h for storage at 15°C and 0, 72, 120 and 168 h for the storage treatment at 4°C. This time point is significant to appreciate changes for gas (ethylene and $CO_2$) contents, enzyme activities, membrane degradation by EL and TBARS assays according to preliminary experiments.

To analyze lipid peroxidation and phospholipases activities, tomato portions were taken from the outer pericarp, frozen in liquid $N_2$ and stored at -80 °C until analysis. Electrolyte leakage was performed on freshly excised cubes from pericarp tissue at each measuring time.

*2.2 Cell membrane integrity: EL*

EL was analysed as described by Saltveit [9] with slight modifications. About 4 g of freshly excised cubes (1 cube of about 1 g FW) from the outer pericarp tissue were rinsed into approximately 200 mL of an isotonic 0.4 M mannitol solution to remove juice and cytosol liquid (due to slicing), which can interfere with the initial value of ion leakage reading. The cubes were put into 20 mL of 0.4 M mannitol solution in a 50 mL plastic tube and incubated for 3 h at 25 ± 1.0 °C. EC of the suspending solution was measured with a conductance meter after incubation to obtain the value of initial EC. The cubes of pericarp tissues were separated from the solution (stored at 4 ± 0.5 °C) and frozen overnight at -20 ± 1.0 °C in order to disrupt completely cellular organization. After storage, the total conductivity of the solution was measured according to the protocol described previously.

The tubes were gently shacked, and the EC probe was washed with deionised water between readings. Leakage data were expressed as a percentage of the total electrolyte reading.

*2.3 Membrane lipid peroxidation: TBARS*

Lipid peroxidation was performed using thiobarbituric acid reactive substances (TBARS) test also called malondialdehyde (MDA) test according to the procedure described by Health and Packer [25] with slight modification.

The content of peroxidized fatty acids was determined spectrophotometrically (UV-Vis Spectrophotometer, Lambda 35, Perkin Elmer, Massachusetts, USA). The basis of the biochemical assay was the malondyaldeide content that is released by peroxidation of free fatty acids due to thiobarbituric acid (TBA) added to the reaction mixture.

For TBARS assay, each fruit sample (about 3 g FW) was homogenized with 1.15 mL buffer solution contained 0.1 % trichloroacetic acid (TCA), triturated with a mortar and pestle refrigerated with liquid $N_2$ and centrifuged at 15,000 x g for 10 min at 4 °C. For the measurement of TBARS content, 4 mL of 20 % TCA and 250 µL 0.5 % TBA was added to a 1 mL aliquot of the supernatant. The reaction mixture was heated at 95 °C for 30 min and then quickly cooled in an ice bath. The tube was centrifuged at 15,000 × g at 4 °C for 10 min and the absorbance of the supernatant was read at $A_{532}$ and $A_{600}$. The value for the non-specific absorption at $A_{\lambda 600\ nm}$ was subtracted from the $A_{\lambda 532\ nm}$ reading. The concentration of TBARS was calculated using MDA's extinction coefficient of 155 $mM^{-1}\ cm^{-1}$. [25]

*2.4 Ethylene and $CO_2$ concentrations*

Ethylene and $CO_2$ concentrations were measured by closing fruits samples in fresh-cut plastic (polyethylene) containers (1.1 L). Each sample consisted of an intact fruit (as control) or two tomato slices (as fresh-cut treatment). To analyze the level of accumulated ethylene or $CO_2$, gas samples (2mL each) were taken from the headspace of the containers with a hypodermic syringe at predefined times of storage at 4 °C. The ethylene and $CO_2$ concentrations in the container were measured by gas chromatography (HP5890, Hewlett-Packard, MenloPark, CA, USA) equipped with a stainless steel column (150 x 0.4 cm packed with hysep T), a flame ionisation detector (FID) for

ethylene and a thermal conductivity detector (TCD) for $CO_2$. Column and detector temperatures were 70 and 350 °C, for ethylene and 70 and 200°C for $CO_2$, respectively.

Results were expressed as absolute content (uL $L^{-1}$ and percentage) and as normalized content on fresh weight basis (pL $ml^{-1}$ $g^{-1}$ FW and μL $L^{-1}$ $g^{-1}$ FW) for ethylene and $CO_2$, respectively.

*2.5 Enzyme activities: PLC and PLD*

Activities of PLC and PLD were determined spectrophotometrically (UV-Vis Spectrophotometer, Lambda 35, Perkin Elmer) at λ400 nm. The assay is based on p-nitrophenol developed from p-nitrophenylphosphorylcholine (NPPC) added to the reaction mixture as enzymatic substrate.

PLC and PLD activites were analysed according to the procedure of Mao et al. [19] with slight modifications.

Pericarp tissue (about 6 g FW) was ground to a fine powder in liquid N2 with a mortar and pestle. Each 1 g of the homogenate was mixed with 1 mL of 50 mM Tris-HCl (pH 8.0) containing 10 mM KCl, 500 mM sucrose, and 0.5 mM phenylmethylsulfonylfloride (PMSF). After centrifugation at 15,000 × g at 4 °C for 30 min, the supernatant was assayed for PLC and PLD activities by using p-nitrophenylphosphorylcholine (NPPC, Fluka BioChemica - Sigma-Aldrich, Italy) as substrate. [26,27]

Reaction mixtures for PLC consisted of 1.0 mL of 0.25 M Tris–HCl (pH 7.2) with 20 mM NPPC 60% -sorbitol and 0.3 mL (about 300 μg protein) of the cell-free protein extracts.

For PLD, 0.9 mL of 50 mM Ca–acetate, pH 5.6 containing 27.4 mM NPPC was mixed with 0.1 mL (0.4 units) of acid phosphatase (Sigma-Aldrich, Italy) dissolved in 50 mM $Ca^{2+}$–acetate (pH 5.6) along with 0.3 mL of the cell-free protein extract. After 60 min incubations at 37 °C, 0.1 mL of 50 mM NaOH was added and p-nitrophenol content was determined at 400 nm.

Activities of PLC and PLD were expressed as pKat $mg^{-1}$ protein.

Protein content was measured using the Biorad protein assay method (Biorad, Munchen, D, UE) based on the differential colour change of a dye (Coomassie brilliant blue G-250) in response to various concentrations of protein with bovine serum albumin (Sigma) standard. [28]

*2.6 Statistical analysis*

Per each figure, two-way analysis of variance (ANOVA) was performed on data per each storage time and the factors of variability were: processing (intact fruit vs slice) and temperature (4 vs 15°C). Differences among means were determined by the least significant difference (LSD) multiple range test ($P \leq 0.05$).

Also multifactor analysis of variance was elaborated to define the significance of F values considering as factors of variability: genotype (Jama vs Canestrino), temperature (4°C vs 15°C) and processing (intact vs slice).

Correlation between biochemical and physiological parameters was calculated by Pearson product moment correlations between each pair of variables ($P \leq 0.05$).

All statistical analyses were conducted by using STATGRAPHICS Centurion XV.II (Stat Point, Inc., Herndon, VA, USA).

## 3. Results

Membrane alteration due to slicing and storage temperature were evaluated as EL and TBARS content in intact and sliced fruit stored at 4 and 15°C in Jama full ripe tomatoes (Fig. 1A, B). EL of sliced tomatoes sharply increased of 2 times after 24 h of storage at 15°C and of 1.5 times after 120h of storage at 4°C (Fig. 1A). Instead, in intact tomatoes, EL did not change over time for both storage temperatures (Fig.1B). As concerning membrane lipid peroxidation, during the storage at 15°C, after 24h from processing the TBARS content was 2-fold higher for sliced tomatoes

compared to the initial value at the beginning of storage and it slightly increased over time until 72h after slicing (Fig.1B). TBARS values of intact as well as of sliced tomatoes stored at 4°C did not change during the initial 72h of storage and only after 120 h the TBARS content significantly increased until the end of experiment for sliced tomatoes but not for intact fruits (Fig. 1B).

Interestingly, in Canestrino, slicing as well as storage temperature did not affect EL until 120h of storage but a consistent increase of 2.5-fold was found in sliced tomatoes compared to intact tomatoes only at the end of storage after 168h from cutting (Fig. 2A). Regarding membrane lipid peroxidation, in Canestrino TBARS content constantly increased over time during experiments at both storage temperatures (Fig. 2B). In particular, for tomatoes stored at 15°C, TBARS content reached the mean values of 2.34 and 1.83 umol $Kg^{-1}$ FW at 72h after storage for sliced. Also intact tomatoes, respectively and also in tomatoes stored at 4°C the values of TBARS content increased up to 3.08 and 1.58 umol $Kg^{-1}$ FW in intact and sliced tomatoes, respectively, at the end of storage (Fig. 2B).

The effect of slicing sharply increased ethylene absolute (Fig. 3A) and relative concentration (Fig. 3B) in intact as well as in sliced fruit stored at 15°C for cultivar Jama. At 15°C and after 72h of storage, the mean values for ethylene absolute content of sliced and intact tomatoes reached 114 and 29 µL $L^{-1}$ respectively, while the normalized contents were 1008 and 131 uL $L^{-1}$ $Kg^{-1}$ FW for processed and whole tomatoes, respectively. During storage at 4°C, the mean values of ethylene concentration increased for sliced tomatoes compared to intact fruits over time: 9.5 and 18.7 µL $L^{-1}$ for absolute content and 78.2 and 99.1 uL $L^{-1}$ $Kg^{-1}$ FW for the relative contents in sliced and intact fruit, respectively were detected after 168 h of storage.

Accordingly, for the landrace Canestrino the ethylene content (absolute and normalised values) for intact and sliced tomatoes increased over time at 15°C and 4°C (Fig.4A, B). At the highest temperature, at 72h after storage, the values of absolute ethylene content were 100 and 16 folds higher compared to time 0 for sliced and intact tomatoes. Furthermore, the normalised value were 1232 and 151 times higher in processed and whole tomatoes stored at 15°C after 72h. At 4°C, the

increases were registered over time, but of lower intensity compared to the experiment conducted at 15°C. Similarly to the pattern of ethylene for cultivar Jama, $CO_2$ absolute (Fig. 5A) and $CO_2$ relative (Fig. 5B) contents were affected by temperature and processing over time, with a sharply increased at the highest temperature. Indeed, at 15°C after 72h from slicing, the $CO_2$ contents in the boxes were around 9 and 3.5 times higher compared to the initial mean values in intact and sliced tomatoes in absolute terms (Fig. 5A). On the other hand, in relative terms, the $CO_2$ normalized contents were 5.2 and 3.5 folds higher in intact and processed tomatoes based on the measured value at the beginning of the experiment. At the lower temperature (4°C), the levels of $CO_2$ slowly increased upon storage and the highest concentrations were detected at the end of the storage (168h after slicing). In intact and sliced tomatoes, the absolute contents for $CO_2$ were 10 and 6.2-fold higher in intact and sliced tomatoes compared to the beginning of the storage (Fig. 5A). In relative terms, the $CO_2$ normalized contents increased of 10 and 3.8 times for intact and processed tomatoes at the end of the storage compared to the first detection (Fig. 5B). For the landrace Canestrino, the evolution of $CO_2$ and absolute normalized content over time are reported in Fig. 6A and Fig. 6B, respectively. Interestingly, for intact fruit samples stored at 15°C after 72h from processing, the $CO_2$ absolute contents were approximatively 12 and 6 times higher than in intact and sliced tomatoes compared to the beginning of storage. Beside these findings, at 168h after cutting with a temperature refrigeration of 4°C, $CO_2$ absolute contents were 7 and 2 times higher for intact and sliced tomatoes respect to the values detected at the beginning of storage (Fig. 6A). In normalized terms on fresh weight basis, $CO_2$ relative contents at 15°C and after 72h of storage were 10 and 5.5 times higher in intact and sliced tomatoes respect to the first detection. Furthermore, at the storage temperature of 4°C, at the end of the experiment (168h after cutting), the CO2 normalized values increased of 6.2 and 2.7 times in intact and sliced tomatoes compared to the beginning of storage (Fig. 6B).

The storage temperature and the processing had a pronounced effect on PLC enzyme activity in Jama tomatoes (Fig. 7). Already at 24 h of storage, the PLC catabolism was significantly higher for

tomato slices stored at 15°C compared to intact tomatoes stored at 4°C. Anyway, a relevant increase in PLC activity was registered at 168 h of storage for both intact and sliced tomatoes stored at 4 °C because the mean values were 2 and 4-fold higher compared to the beginning of storage, respectively for whole and processed tomatoes.

On the other hand, PLD activities did not significantly change over time for both temperatures until 72h of storage, but significantly increased at the end of the storage in sliced tomatoes stored at 4°C. Indeed, the mean values for PLD activities at 168 h of storage were around 5-fold higher in processed tomatoes compared to intact fruits.

The significance of F values for the factors of variability: genotype, storage temperature and processing were calculated in Table 1 for both cultivars referring to each physiological (electrolyte leakage, ethylene and $CO_2$) and biochemical (TBARS content) marker. Interestingly, the effect of genotype was significant for the parameters: electrolyte leakage and lipid peroxidation and not for the physiological processes related to ethylene and normalized carbon dioxide concentration. The effects of the storage temperature and processing were significant for almost all the parameters, except for $CO_2$ absolute content and EL (only for storage temperature).

Correlation analysis between all the physiological and biochemical parameters were reported in Table 2 for Jama tomatoes at 4°C. All the parameters: electrolyte leakage, TBARS contents, $C_2H_4$ (in absolute and normalized terms) and $CO_2$ (in absolute terms) were correlated among others at different degree of significance level. Only TBARS and $CO_2$ normalized content were not correlated. On the other hand, the biochemical activities of phospholipases (PLC and PLD) were highly correlated among them and with electrolyte leakage and TBARS used to estimate the level of membrane degradation.

## 4. Discussion

In fresh-cut industry, the most important issue is the loss of quality which is mainly related to membrane integrities of plasmalemma as well as of other cellular components (e.g. vacuoles). Therefore, membrane integrity is a very crucial subject in postharvest research because several factors can increase its catabolism. The loss of cell compartments enzymes induces several physiological disorders related to the impairment of biochemical processes. Fresh-cut vegetables are subjected to several stressing agents: i) slicing responsible of cell disruption; ii) low refrigeration temperature that induces chilling injury in the most sensitive products (e.g. tomatoes); iii) storage time which leads the tissue until senescence. [29,30,31]

Two overall but reliable index of membrane integrities are: electrolyte leakage and TBARS content. An increase of these parameters is therefore related to the increase in conductivity for the permeability of the membranes and lipid peroxidation, respectively. Our results clearly suggest that slicing deteriorates membrane integrity in both varieties at full ripe stage of maturity. In Canestrino, the electrolyte leakage did not sharply increase at 15°C, maybe because this physiological parameter is a general parameter for membrane catabolism which includes lipid peroxidation and other processes. All these findings suggested that the main damage in full ripe tomatoes is more related to slicing instead of chilling injury for the measured parameters, in opposition to our previous findings developed on pink tomatoes.[23] Data demonstrated that the cultivar Jama and the landrace Canestrino have different susceptibility to membrane degradation. Hodges and Toivonen [31] also stated that electrolyte leakage is in fresh-cut vegetables is more affected by chilling temperatures instead of slicing. Low storage temperatures are responsible for rearrangements (phase transition) in lipid composition that could enhance electrolyte leakage. Anyway, Artés *et al.* [2] reported that, in fresh-cut products, the storage is too short in time to appreciate the lipidic phase transition even in chilling injury sensitive fruits as tomato. Saltveit [32] deeply investigated ion leakage in chilling sensitive tissue of mature green tomato pericarp discs. Heat-shock treatments realized by floating Petri-dishes containing tomato pericarp in water bath at 45°C lead to a protection of membrane components. As a consequence, ion movement across the plasmalemma

into the apoplast was reduced compared to non-heat shocked tissue after exposure to chilling temperatures.

Moreover, our findings suggest that Canestrino could be used for further studies to investigate discrepancy between TBARS content and electrolyte leakage and, eventually, for breeding programs.

Tomato is a climacteric fruit, thus ethylene plays a crucial role in tissue maturity and regulates physiological process like respiration. In the present research, we used absolute and normalized values on fresh weight bases for ethylene and $CO_2$ contents inside the containers during storage time in order to estimate the real content inside the tray and to evaluate gas accumulation (derived from tissue biosynthesis) per weight unit. Ethylene increase is also a response to abiotic stresses and in general represent a wound induced response.[33] According to our previous studies [22] in wild type ethylene sensitive tomatoes (Gimar), ethylene concentrations significantly increased in fresh-cut boxes over storage for sliced tomatoes more than for intact fruits. Full ripe tomatoes of the cultivar Jama and the landrace Canestrino positively responded to slicing with a huge increase in ethylene production that was more accelerated for containers stored at 15°C. It is well-known that wounds induce ethylene biosynthesis, and the hormone is able to enhance the ethylene production by auto-catalytic way. Also Vilas-Boas and Kader demonstrated in kiwifruit, as extremely sensitive fruit to ethylene, but also in mango and persimmon that cutting hasten ethylene production after processing and 1-Methylcyclo-propene (1-MCP) treatments can decrease the autocatalytic biosynthesis of the hormone within a short time of storage at 5°C. [34] Marrero and Kader in pineapple revealed that wounding trigger a permanent increase in ethylene production at 10°C of storage and a peak at 7.5 and 5 °C within a short time from processing. [35]

The $CO_2$ concentration also increased over time in our research, showing a similar trend detected for ethylene, as wound response in climacteric fruit.[36,37]

Membranes are mainly constituted by lipids (phospholipids and glycolipids) and the alteration of its component affects electrolyte leakage and lipid peroxidation. The variety Jama showed higher

values for both parameters over storage and it could be considered as sensitive variety for slicing. For these reasons, further investigation on PLC and PLD activities were focused on the variety Jama. In the experiment conducted at 4°C, PLC activity significantly increased at the end of storage for both intact and sliced tomatoes, but at 15°C no significant difference was measured at 72h after slicing in disagreement with the peak in electrolyte leakage and TBARS. Therefore, it is possible to assume that electrolyte leakage and TBARS are only partially related to the lipid degradation catabolized by PLC, since the PLC activities were significantly differentiated in the treatments after 24 h when the membrane integrity indexes also showed changes.

PLD activity increased only in sliced tomatoes at 168h after processing at 4°C according to a high significant increase of electrolyte leakage and TBARS content. On the other hand, no changes were detected at 15°C, despite the increases in electrolyte leakage and TBARS content.

Combining these findings with our previous study on tomato mutant for ethylene sensitivity [22], it could be inferred that some points of the working model described by Wang *et al.* [12] on *Arabidopsis* would apply to the membrane catabolic process observed in fresh-cut tomatoes. Wounding activates phospholipases, mainly PLD and PLC which release signalling messengers (e.g. phosphatic acid) responsible for the activation of a downstream catabolism leading to membrane peroxidation and electrolyte leakage.

## 5. Conclusions

Our results demonstrate a partial involvement of phospholipases in the loss integrity of cell membrane during storage of fresh-cut tomatoes for the cultivar Jama. Moreover, the present research provides evidence that the processes of membrane degradation and lipid peroxidation evaluated as EL and TBARS content are strictly affected by the genotype whilst no influence by the genotype. Indeed, tomato fruits of the landrace Canestrino showed significant increase on EL only at the end of storage compared to cultivar Jama. Our findings suggest that Canestrino has a different

structural composition for the main membranes and this genotype could be useful in breeding programs focused on fresh-cut tomato.

Accordingly, further investigations should take in consideration other lipids such as glycolipids instead of phospholipids are catabolized as consequence of slicing. Glycolipid catabolism provide fatty acids suitable for peroxidation and subsequent membrane disruption that is detected as increase in electrolyte leakage and TBARS.



## 6. Ackonwledgements

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**Tables**

**Table 1:** Significance of F values for the genotypes: cultivar Jama *vs* landrace Canestrino; A), temperature (4°C *vs* 15°C; B), processing (intact *vs* slice; C) and their interactions according to multifactor ANOVA of dependent variables of different evaluated parameters. Sliced and intact full ripe tomatoes of Jama and Canestrino cultivars were stored in 1L fresh-cut plastic containers at +4 and +15°C in dark conditions after slicing.

| **Parameter** | **A** | **B** | **C** | **AB** | **AC** | **BC** | **ABC** |
|---|---|---|---|---|---|---|---|
| EL | *** | n.s. | *** | * | * | n.s. | n.s. |
| TBARS | *** | *** | *** | *** | n.s. | n.s. | *** |
| $C_2H_4$ absol. | n.s. | *** | * | n.s. | n.s. | *** | n.s. |
| $C_2H_4$ normal. | n.s. | *** | *** | n.s. | n.s. | *** | n.s. |
| $CO_2$ absol. | n.s. | n.s. | n.s. | n.s. | *** | n.s. | n.s. |
| $CO_2$ normal. | *** | *** | *** | ** | *** | ** | ** |

*, **, *** show significant differences at level of significance P≤0.05, 0.01 and 0.001, respectively; ns, not significant effect.

Abbreviations: EL, electrolyte leakage; TBARS, thiobarbituric acid reactive substances; $C_2H_4$ and $CO_2$ absol., ethylene and carbon dioxide absolute content respectively; $C_2H_4$ and $CO_2$ normal., ethylene and carbon dioxide normalized content respect to sample fresh weight; PLC, phospholipase C; PLD, phospholipase D.

**Table 2:** Overall evaluation of correlation between several qualitative parameters describing the effect of slicing on sliced and whole full ripe tomatoes of the cultivar Jama. Samples were stored in 1L fresh-cut plastic containers at +4°C in dark conditions after slicing for 0, 24, 72, 120 and 168 h.

| **Correlated parameters** | EL | TBARS | $C_2H_4$ absol. | $C_2H_4$ normal. | $CO_2$ absol. | $CO_2$ normal. | PLC | PLD |
|---|---|---|---|---|---|---|---|---|
| EL | - | | | | | | | |
| TBARS | **0.58** (***) | - | | | | | | |
| $C_2H_4$ absol. | **0.39** (*) | **0.42** (*) | - | | | | | |
| $C_2H_4$normal. | **0.36** (*) | **0.36** (*) | **0.94** (***) | - | | | | |
| $CO_2$ absol. | **0.39** (*) | **0.38** (*) | **0.75** (***) | **0.67** (***) | - | | | |
| $CO_2$ normal. | **0.40** (*) | **0.33** n.s. | **0.74** (***) | **0.83** (***) | **0.78** (***) | - | | |
| PLC | **0.71** (***) | **0.60** (***) | **0.23** n.s. | **0.22** n.s. | **0.33** n.s. | **0.38** (*) | - | |
| PLD | **0.64** (***) | **0.59** (***) | **0.26** n.s. | **0.23** n.s. | **0.28** n.s. | **0.31** n.s. | **0.92** (***) | - |

r, Pearson coefficient is reported in bold; P, level of significance is reported in brackets (n=24)

*, **, *** show significant differences at level of significance P≤0.05, 0.01 and 0.001, respectively; n.s., not significant effect.

Abbreviation.s.: EL, electrolyte leakage; TBARS, thiobarbituric acid reactive substances; $C_2H_4$ and $CO_2$ absol., ethylene and carbon dioxide absolute content respectively; $C_2H_4$ and $CO_2$ normal., ethylene and carbon dioxide normalized content respect to sample fresh weight; PLC, phospholipase C; PLD, phospholipase D.

**Figures**

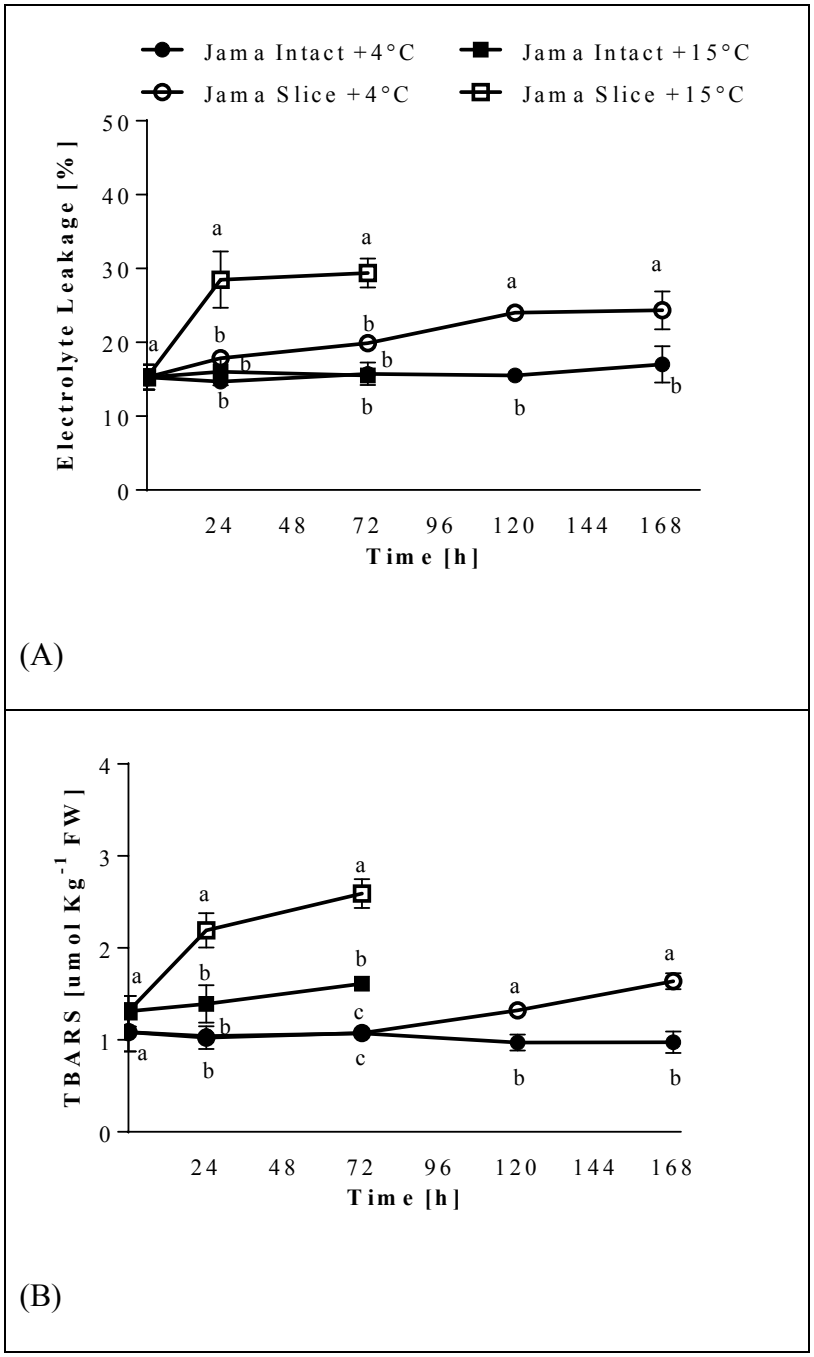


**Figure 1.** Effect of slicing on electrolyte leakage (A) and TBARS content (B) on sliced and intact full ripe tomatoes of the cultivar Jama. Samples were stored in 1L fresh-cut plastic containers at +4 and +15°C in dark conditions after slicing. Data are mean values (n=4) with standard error; means within each storage time with different letters are significantly different at $P \leq 0.05$.

Electrolyte leakage data [%] have been subjected to angular transformation [rad] before ANOVA.

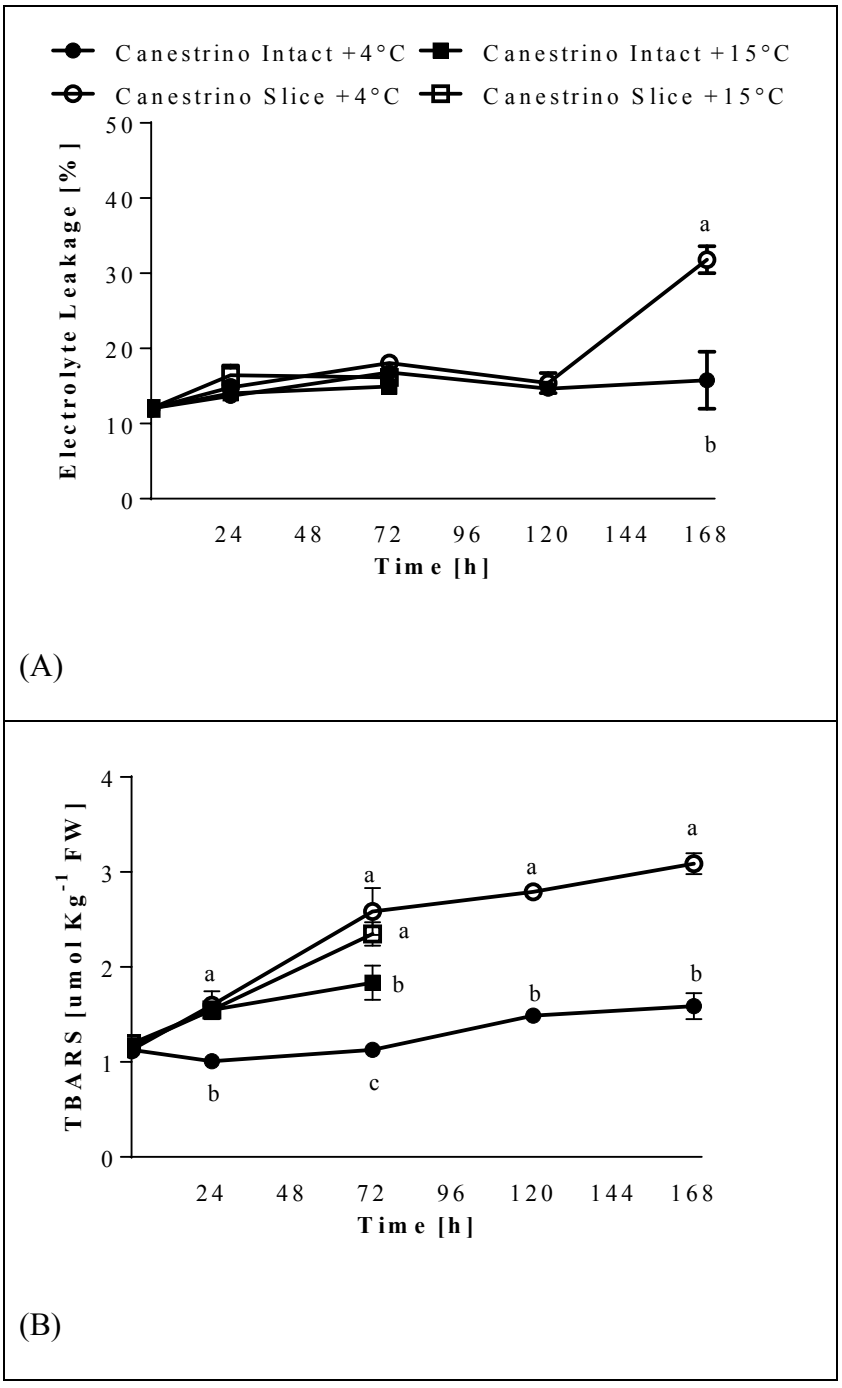


**Figure 2.** Effect of slicing on electrolyte leakage (A) and TBARS content (B) on sliced and intact full ripe tomatoes of the landrace Canestrino. Samples were stored in 1L fresh-cut plastic containers at +4 and +15°C in dark conditions after slicing. Data are mean values (n=4) with standard error; means within each storage time with different letters are significantly different at $P \leq 0.05$.

Electrolyte leakage data [%] have been subjected to angular transformation [rad] before ANOVA.

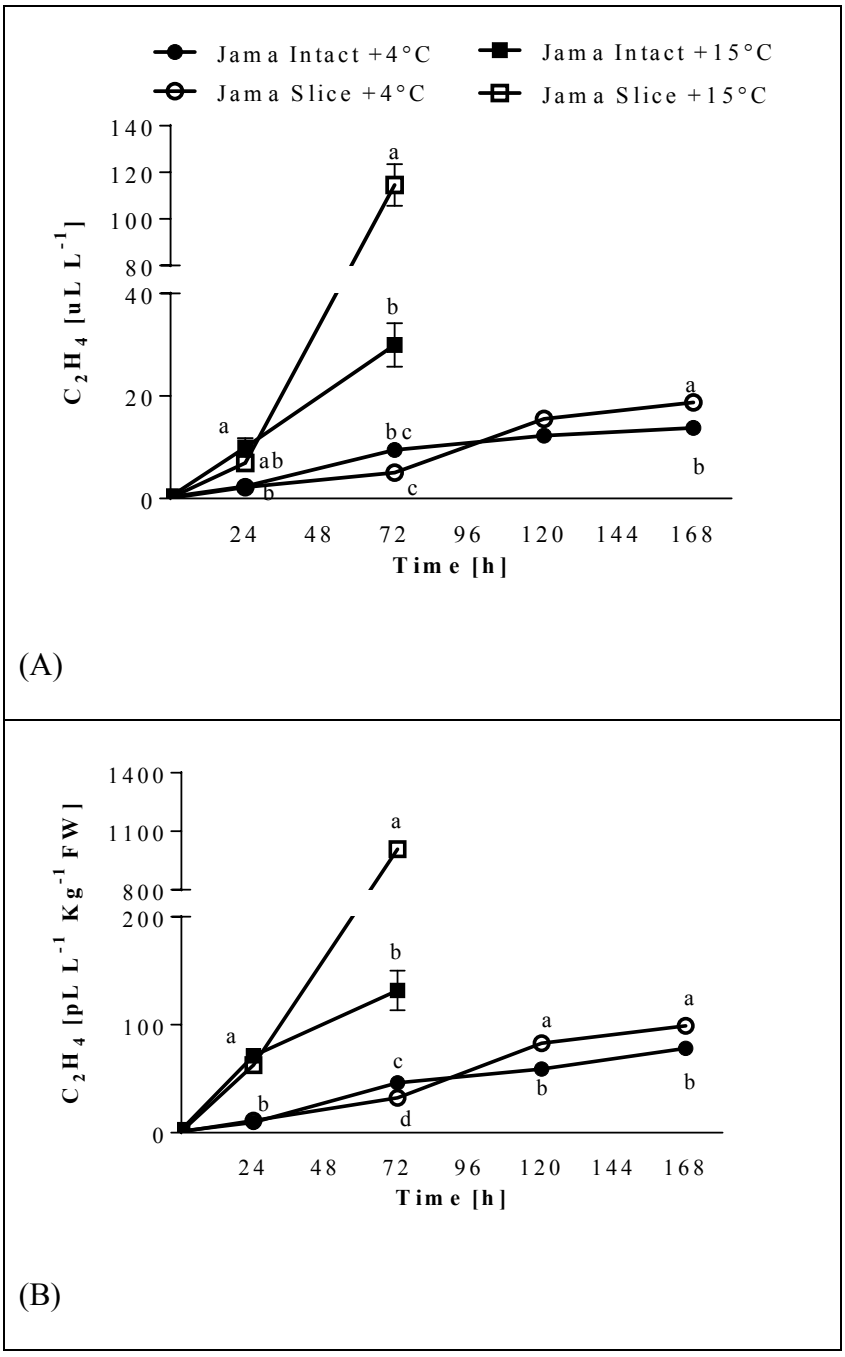


**Figure 3.** Effect of slicing on absolute $C_2H_4$ content (A) and normalized $C_2H_4$ content (B) on intact and sliced full ripe tomatoes of the cultivar Jama. Samples were stored in 1L fresh-cut plastic containers at +4 and +15°C in dark conditions after slicing. Data are mean values (n=4) with standard error; means within each storage time with different letters are significantly different at $P \leq 0.05$.

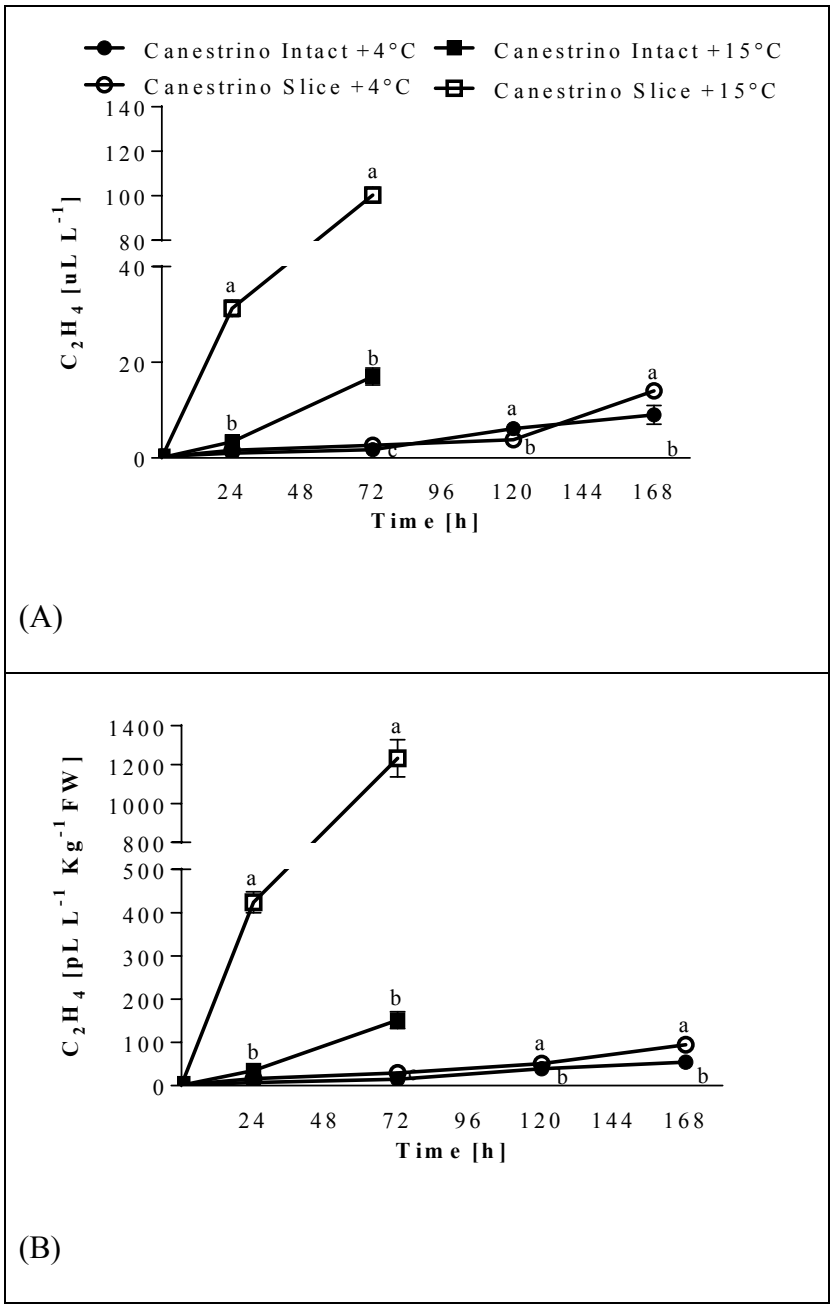


**Figure 4.** Effect of slicing on absolute $C_2H_4$ content (A) and normalized $C_2H_4$ content (B) on intact and sliced full ripe tomatoes of the landrace Canestrino. Samples were stored in 1L fresh-cut plastic containers at +4 and +15°C in dark conditions after slicing. Data are mean values (n=4) with standard error; means within each storage time with different letters are significantly different at $P \leq 0.05$.

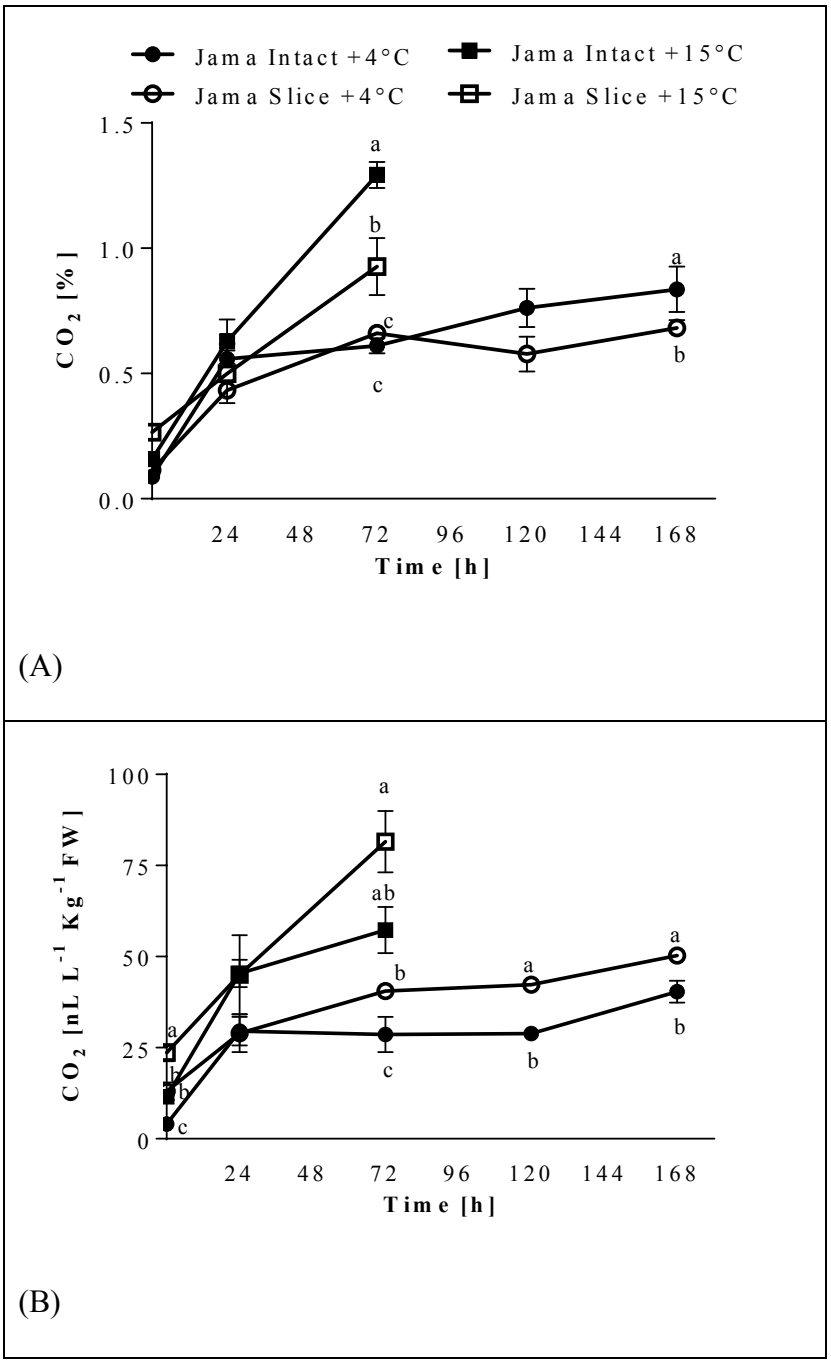


**Figure 5.** Effect of slicing on absolute $CO_2$ content (A) and normalized $CO_2$ content (B) on intact and sliced full ripe tomatoes of the cultivar Jama. Samples were stored in 1L fresh-cut plastic containers at +4 and +15°C in dark conditions after slicing. Data are mean values (n=4) with standard error; means within each storage time with different letters are significantly different at $P \leq 0.05$.

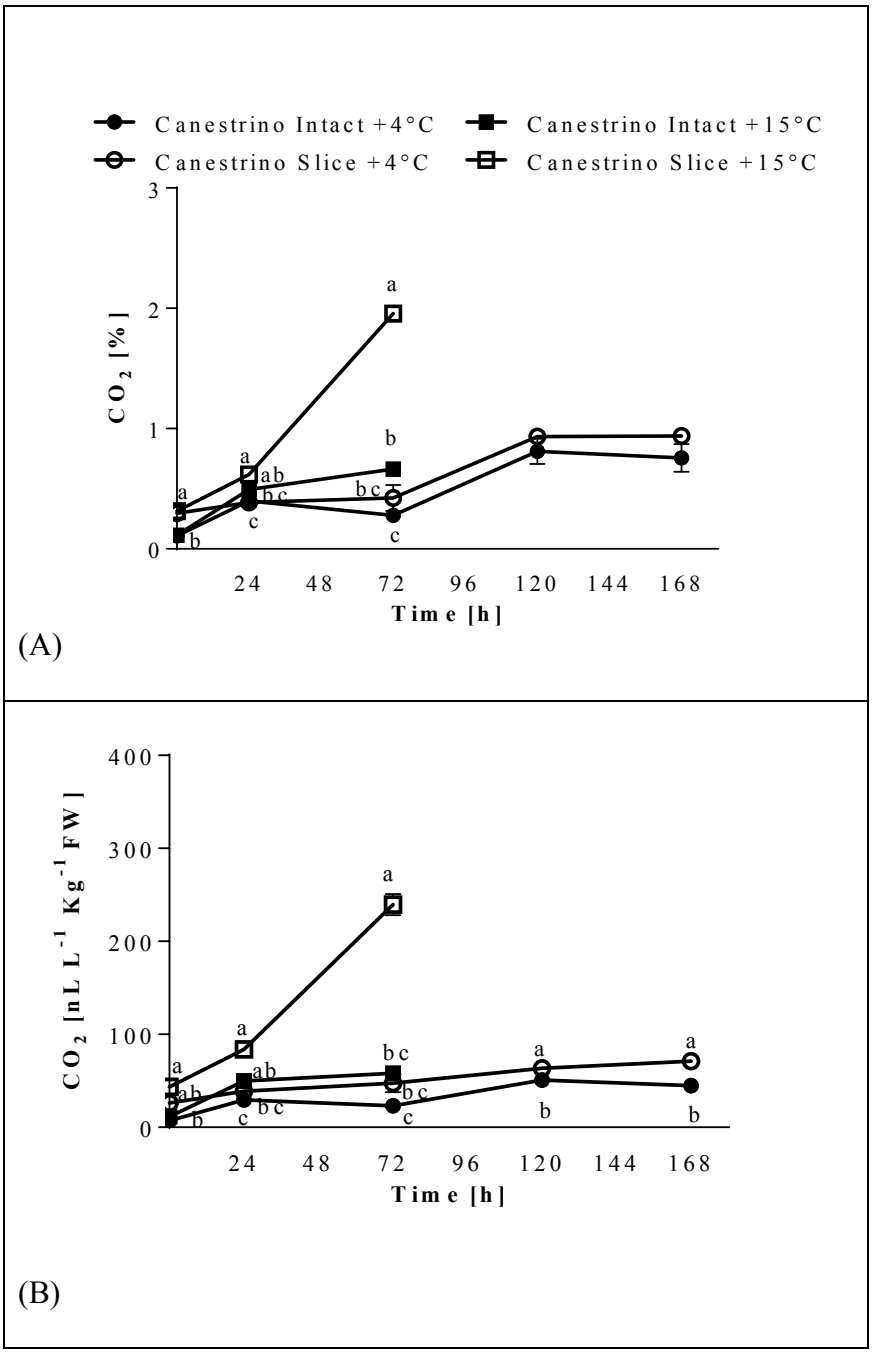


**Figure 6.** Effect of slicing on absolute $CO_2$ content (A) and normalized $CO_2$ content (B) on intact and sliced full ripe tomatoes of the landrace Canestrino. Samples were stored in 1L fresh-cut plastic containers at +4 and +15°C in dark conditions after slicing. Data are mean values (n=4) with standard error; means within each storage time with different letters are significantly different at $P \leq 0.05$.

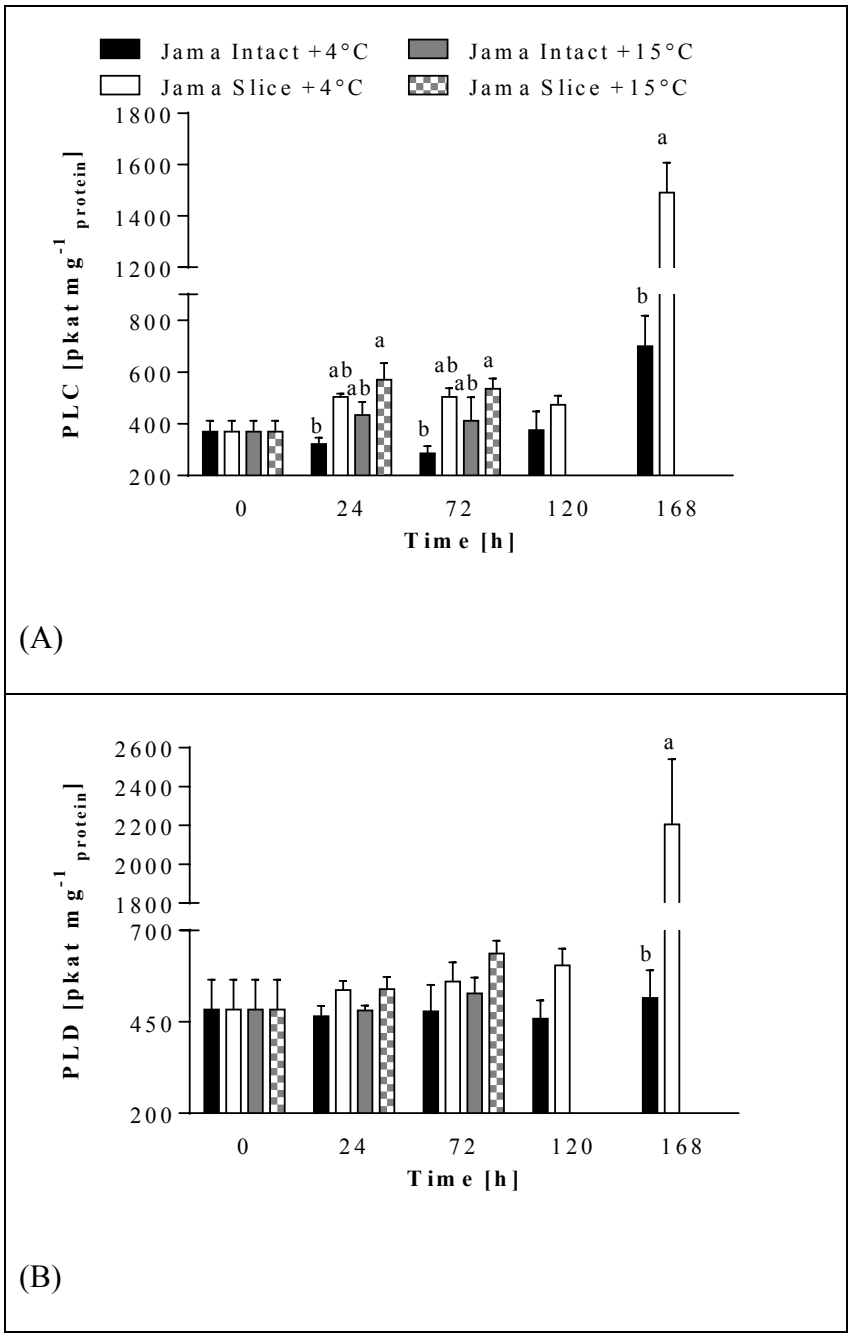


**Figure 7.** Effect of slicing on PLC (top) and PLD (bottom) activities on sliced and intact full ripe tomatoes of the cultivar Jama. Samples were stored in 1L fresh-cut plastic containers at +4°C in dark conditions after slicing. Data are mean values (n=4) with standard error; means within each storage time with different letters are significantly different at $P \leq 0.05$.

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