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Effect of pulsed electric field coupled with vacuum infusion on quality parameters of frozen/thawed strawberries

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Abstract

In this study, pulsed electric field (PEF) was coupled with vacuum infusion (VI) to impregnate strawberries with cryoprotectants. Electroporation of fruits was reached with 5 bi-polar, rectangular pulses of 100 μs width with a nominal electric field strength of 850 V/cm. After PEF treatment, the strawberries were vacuum infused with a cryoprotective solution (12 g/100 g trehalose and 0.2 g/100 g acclimated winter wheat extract containing antifreeze proteins) for 14 min. The strawberries were frozen in liquid nitrogen and thawed in air at 20 °C. Cell survival, texture and color were evaluated before and after freezing and thawing cycle. The fruit pre-treated with PEF prior to VI exhibited higher cell viability in epidermal layer and 30% more red color retention compared to just VI samples. However, no further improvement on strawberry quality in terms of drip loss and texture was observed upon the application of PEF.

Keywords: vacuum infusion; pulsed electric field, cryoprotectants, freezing, strawberry, quality parameters

1. Introduction

Strawberries are among the most consumed summer fruits due to their potential benefits for human health coming from their high content of bioactive compounds with antioxidant, anticancer, anti-inflammatory and anti-neurodegenerative properties (Gamboa-Santos et al., 2014). They are also highly appreciated for their texture, flavor (organic acids and soluble sugars content) and the vivid red color, which are predominant for their quality and consumption (Velickova et al., 2013b; de Bruijn & Bórquez, 2014). Even though strawberries are very attractive fruits for the consumers, due to their active metabolism and easy deterioration, they are available mainly in the processed form as an ingredient to food products or as a frozen product (Janowicz et al., 2007).

Freezing is one of the techniques often used for preservation of fruits. The structural integrity of frozen plant tissue could be preserved in a best way using high freezing rates due to small ice crystal formation, less water migration, less breakage of cell walls and consequently less texture deterioration (Van Buggenhout et al., 2008). Recently, Velickova et al. (2013a) improved the resistance of strawberries to freezing damage by vacuum impregnation of the fruits with the cryoprotective substances, trehalose and antifreeze proteins (AFPs). Vacuum impregnation or infusion of fruits with components of interest is already proven to be highly suitable method for enhancing the fruit's quality properties during freezing or drying (Phoon et al., 2008; Cruz et al., 2009; Velickova et al., 2013a). It is based mainly upon rapid hydrodynamic mass transfer and allows, within a few minutes, the occluded air initially contained in the fruit pores to be replaced by the impregnating solution. Strawberries' structure consists of compact outer layer with epidermal cells, a second layer composed of hypodermal cells and third layer of cortical cells with larger intercellular spaces (Suutarinen et al., 1988). Hence, VI placed the cryoprotective substances in the extracellular space, mostly in the second and third layer. It is believed that

even better cryoprotection might be achieved if these substances could be introduced in the cells themselves, because it will allow cryoprotection of all layers, no matter how compact they are (Phoon et al., 2008).

During recent years, emerging technologies have been proposed to reduce the limitations related to conventional freezing (Suutarinen et al., 2000; Moraga et al., 2006; Velickova et al., 2013a). As a new strategy in freezing of fruit and vegetables, the application of PEF represents a promising alternative (Phoon et al., 2008; Ben Ammar et al., 2010; Wiktor et al., 2012; Parniakov et al., 2015). PEF treatment is based on the application of short, high voltage pulses to a food material placed between two or more electrodes (Wiktor et al., 2015). Under the effect of PEF with low to medium electric field strength of 0.5–5 kV/cm plant cell membranes become electroporated and permeable for small molecules or even some macromolecules (Wiktor et al., 2015). Two types of electroporation can be obtained, reversible or irreversible, based on the optimization of the electric field parameters such as intensity, frequency, pulse width and shape (Parniakov et al., 2015; Dellarosa et al., 2016; Tylewicz et al., 2017). Reversible electroporation creates transient pores that can be sealed after some time, which enables entrapment of materials of interest inside the cell membranes. Irreversible electroporation destroys the cells by permanent membrane damage and it is usually used in the microbial inactivation processes and to increase extraction yield (Teissié et al., 2005; Dukić-Vuković et al., 2017).

Phoon et al. (2008) coupled the VI with PEF to impregnate the cells of spinach leaves with trehalose as cryoprotectant. They showed improvement of the freezing tolerance of spinach, in terms of cell survival after thawing. In the present study, combination of trehalose and antifreeze protein was used as cryoprotectant. The benefits of their vacuum infusion into the strawberry were described in our previous paper (Velickova et al., 2013a). Viable cells were detected from the 2nd mm from the surface, while cells

from the 1st mm of the tissue, considered outer epidermal layer, did not survive the freezing process and resulted in dead cells. Therefore, this research was aimed to couple the PEF with vacuum infusion prior to freezing in order to create transient pores in all cells, including the outer epidermal cells, that will be able to entrap the cryoprotectants inside them and survive the freezing. The quality of pretreated frozen/thawed strawberries was evaluated in terms of drip loss, cell viability, color and texture retention.

2. Materials and Methods

2.1. Raw material handling

Strawberries (10.5 ± 1.6 °Brix, 11.6 ± 0.9 % dry weight and $\rho = 0.896 \pm 0.004$ g/cm^3) were purchased daily in Lund (Sweden) during summer, from the same supplier at the local market. The Atago PAL-1 digital refractometer was used for measuring the soluble solids of the strawberries at 20°C and they were expressed as °Brix. Dry weight (%) was determined gravimetrically by drying the samples at 70°C until a constant weight was achieved (AOAC, 2002). The bulk volume of fresh strawberries was measured using the liquid displacement technique (Velickova et al., 2014). Measurements were made as quickly as possible (less than 30 s) to avoid water uptake by samples. The bulk density (g/cm^3) was expressed as the ratio of the sample to its bulk volume. Five randomly chosen strawberries from every purchase were used for determination of the soluble solids, dry weight and density. The fruits were washed, hand stemmed and selected according to size (height: 30 ± 2 mm; width: 27 ± 3 mm), firmness and similar visual ripening.

2.2. Cryoprotectant solutions

The following cryoprotectants were prepared as aqueous solutions: trehalose (Cargill*Ascend 16400, Denmark) and spray dried, unpasteurized cold-acclimated winter

126 wheat grass extract (AWWE) as a source of antifreeze protein (AFP) (Microstar Biotech
127 Ltd., Zhuhai, China; AWWE contained 12 % proteins). The prepared cryoprotective
128 solution contained 12 % (w/w) trehalose and 0.2 % (w/w) AWWE, based on previous
129 experiments (Velickova et al., 2013a).
130
131 2.3. Treatments
132 2.3.1. Pulsed electric field treatment (PEF)
133 PEF treatment was performed using a generator Arc Aroma Pure (Lund, Sweden).
134 A digital oscilloscope (Fluke 123, Washington) was connected to the system to monitor the
135 delivery of the pulses to the sample. Six different protocols were tested in preliminary trials
136 displayed in Table 1. Only two of the protocols permitted the electroporation and survival
137 of the cells, one of them was chosen because of the highest percentage of electroporated
138 cells, measured by using fluorescent microscope. This protocol enabled electroporation of
139 whole strawberries with bi-polar, rectangular pulses with a nominal electric field strength of
140 850 V/cm, in a chamber with volume of 400 cm^3 (100 x 100 x 40 mm) as shown in Figure
141 1. The chamber was filled with tap water with conductivity of 226 ± 87μS/cm and the
142 temperature of 20 °C. The temperature of the samples was the same and it did not rise more
143 than 5 °C during the treatment. PEF treatment consisted of 5 pulses of 100 μs duration and
144 of 1000 μs interval between the pulses. The specific energy input was calculated by
145 equation 1 (Zhang, Barbosa-Canovas, & Swanson, 1995):

146 $$Q = \frac{V^2 \cdot t}{R \cdot m} \quad (1)$$

147 where, V is the voltage of the square pulses (kV), R is the effective resistance (Ohm), t is
148 the treatment time (s) and m the mass of the sample (kg). The employed field strength had
149 specific energy input of 213 J/kg.

Table 1, Figure 1

2.3.2. Vacuum infusion (VI)

Vacuum infusion was carried out immediately after PEF treatment, at 20°C in a chamber connected to a vacuum pump. Whole strawberries (70 g) were immersed in the solutions for a total time of 14 min. This duration comprised a gradual decrease of pressure for 4.5 min, a holding time of 5 min at 86 kPa (absolute pressure) and a gradual increase of pressure for 4.5 min. After the infusion, the strawberries were immediately taken out of the solution and their surface was blotted with a tissue paper to remove the excess of solution. Trehalose, being only 45% of the sucrose sweetness, is not likely to adversely influence the taste of the strawberries, neither is the low concentration of AWWE (Velickova et al., 2013a).

2.3.3. Freezing and thawing

Freezing was performed in liquid nitrogen immediately after VI process. Control (untreated) and infused fruits were placed on a metal basket with square wire mesh of 5 mm. The basket was placed in liquid nitrogen for 25 s, time needed to reach -18 °C. An extra sample was prepared to monitor the temperature drop during the freezing process using a thermocouple inserted in the middle of the strawberry to follow the freezing temperature reached over time with a data logger (USB TC-08, Pico Technology, Cambridgeshire, UK). When the fruit reached the temperature of -18 °C at the centre, the samples were immediately thawed at room temperature (20 °C) for 2 h and let to drip overnight at 4 °C. For thawing, a quantity of 70 g strawberries was placed in a plastic funnel and covered with parafilm to avoid evaporation. All the experiments were repeated

three times with three different batches of strawberries for each treatment, resulting in total of nine replicates.

2.4. Analyses

2.4.1. Mass gain

The mass gain (m_{gain}, %) of the VI strawberries was calculated from the following equation 2 (Tylewicz et al., 2012):

$$m_{gain} = \frac{m - m_0}{m_0} \cdot 100\% \quad (2)$$

Where, m is the mass of the infused strawberries and m_0 is the initial mass of the fresh ones. The measurement was repeated 3 times with total of 15 strawberries per batch.

2.4.2. Drip loss

The strawberries were weighed after freezing and 20 h after thawing on an analytical balance (Precisa Instruments Ltd, Switzerland). The drip loss (d_{loss}, %) was calculated from the equation 3 (Xie & Zhao, 2014):

$$d_{loss} = \frac{m_1 - m_2}{m_1} \cdot 100\% \quad (3)$$

Where, m_1 is the mass of the frozen strawberries and m_2 is the mass of the strawberries after thawing. The measurement was repeated 3 times with total of 15 strawberries per batch.

2.4.3. Microscopic observations

The viability of the cells was evaluated by using fluorescein diacetate (FDA, Sigma-Aldrich, USA, λex=494 nm, λem=521 nm), as described by Gómez Galindo et al. (2005). The strawberries were cut longitudinally in 2 mm thick slices using sharp razor blades. From every longitudinal slice, a rectangular piece with the following dimensions: length =

198 15 mm, width = 5 mm and thickness = 2 mm, was transversely cut as described by
199 Velickova et al. (2013a). The rectangular piece was incubated for 5 min in a 0.5 M sucrose
200 solution containing 10^{-6} M FDA in the darkness at room temperature. Stained sections were
201 rinsed thoroughly in distilled water for 1 min and examined under fluorescent light in a
202 Nikon upright microscope (Eclipse Ti-U, Nikon Co, Japan) equipped with a Nikon digital
203 video camera (digital sight DS-Qi1Mc, Nikon Co, Japan) at a magnification of 4x. The
204 microscopic plate was placed along a graduated meter and slid slowly, mm by mm, to
205 evaluate the viability of the cells along the whole length (15 mm) of the rectangular piece.
206 Undamaged, viable cells could be easily identified by a bright fluorescence. The analysis
207 was carried out on fresh, VI, PEF + VI and frozen/thawed fruits. Nicon's imaging software
208 NIS-Elements (Nikon, Japan) was used for counting and measuring cells.
209 Cell electroporation in the strawberry tissue was evaluated with propidium iodide
210 (PI; Sigma-Aldrich, USA, λex=535 nm, λem=617 nm), a commonly used test molecule for
211 membrane electroporation that binds to DNA in the cell. The sample was placed in the
212 electroporation chamber, which was filled with 250 µM PI aqueous solution with
213 conductivity of 130 µS/cm. After application of pulses, rectangular pieces cut from the
214 treated samples were rinsed with deionized water and immediately examined under the
215 microscope. Again, the microscopic plate was slid slowly, mm by mm, and micrographs
216 were captured alongside rectangular piece with magnification of ×10. The number of PI
217 focal binding sites in the pictures, assumed to be DNA in the nuclei, were counted using
218 ImageJ (Wayne Rasband, MD, USA) software. The tissue was considered electroporated
219 (Table 1) if the measurement showed more than 90% of electroporated cells.
220
221 2.4.4. Texture analysis

222 The texture of the fresh and treated strawberries before and after freezing/thawing
223 cycle was measured at 20 °C using a Universal Instron testing machine (series 442H1004,
224 UK) with a 100 N load cell. Crosshead speed was set at 60 mm/s and the penetration depth
225 was 20 mm. A single, whole, strawberry was placed on a flat platform with the longer axis
226 normal to the testing probe. A sharpened cork borer (d=5 mm) was used to cut through the
227 strawberry tissue till 80% of the total fruit width, as described by Velickova et al. (2013a).
228 The textural parameters measured on the resulting force-distance curves were firmness and
229 slope of the peaks. The mean value of six replicates, expressed respectively in N and
230 N/mm, were reported.
231
232 2.4.5. Color evaluation
233 The color of the strawberry surface and the strawberry inner flesh was evaluated on
234 fresh and treated fruits before and after freezing/thawing cycle using a colorimeter (Konica
235 Minolta, Sensing, Spectro photo meter CM-700d, CM-A177, Japan) to obtain the L^*
236 (lightness), a^* (redness) and b^* (yellowness) values as mean of three readings, from each
237 batch of strawberries, resulting in total of 27 measured fruits. Surface color was measured
238 by placing the whole fruits against the colorimeter, while for the inner flesh, the
239 strawberries were cut in halves and measured. The light source was D65 with 2° standard
240 observer (black and white tile) and the measuring area was 0.5 cm^2.
241 Moreover, the following color parameters were calculated using the following equations:
242 1) Total color difference (ΔE):
243 $$\Delta E = \sqrt{(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2} \quad (4)$$
244 Where, ΔL*, Δa*, and Δb* are the differences of mean L*, a* and b*parameters,
245 respectively, between untreated and treated samples (Wiktor et al., 2015).
246 2) Saturation (C)

$$C = \sqrt{(a*)^2 + (b*)^2} \quad (5)$$

3) Hue angle (h°)

$$h° = tan^{-1}\frac{b*}{a*} \quad (6)$$

Where, a* (red–green) and b* (yellow–blue) are parameters of color measurement (Tylewicz et al., 2017).

2.4.6. Statistical analysis

Significant differences between the treatments were assessed by one-way analysis of variance (ANOVA, 95% significance level) with Tukey's comparison test using STATISTICA 6.0 (Statsoft Inc., Tulsa, UK).

3. Results and Discussion

3.1. Effect of PEF and VI treatment on weight gain and drip loss

The mass gain of VI strawberries with the cryoprotectants, trehalose and AWWE, was 19 ± 3 g/100 g_{fw} which did not differ significantly ($p < 0.05$) from 18 ± 3 g/100 g_{fw} for the strawberries treated with the combination of PEF and VI (PEF+VI). In previous work, the strawberries mass gain after VI ranged from 9 to 19, in relation to the different combination of cryoprotectants used (Velickova et al., 2013a). The high uptake of the solution end external solutes into the pores of strawberry is due to action of hydrodynamic mechanism (HDM) and deformation–relaxation phenomena (DRP), which lead to the filling of intracellular capillaries (Fito et al., 1996).

The effect of the different pre-treatments on the drip loss of strawberries after freezing/thawing cycle is shown in Figure 2. The data obtained from this analysis showed a significantly lower water loss for the treated strawberries when compared to the untreated

272 frozen/thawed fruits. Both pre-treatments proved to be effective for reducing the drip loss
273 of thawed strawberries, but the combination of the pre-treatments did not further improve
274 the drip loss of the samples. Similar results were observed by Xie and Zhao (2014),
275 showing the reduction of drip loss of frozen-thawed strawberries from 38 % (untreated
276 samples) to 20 and 30% (vacuum impregnated samples with high fructose corn syrup and
277 high methoxyl pectin-HMP, respectively).

278 Figure 2.

279

280 3.2. Microscopic observations after freezing and thawing

281 The microscopic observations of the tissue treated using the selected protocol are given in
282 Figure 3. Fig 3A shows, by way of example, the electroporated cells in hypodermal tissue,
283 which are indicated by arrows, however the whole tissue behaved in the same way. Fig. 3B
284 instead, exhibits microscopic observation obtained using fluorescein diacetate (see 2.4.3.
285 paragraph) to identify cell viability of electroporated tissue. From this last picture, it is
286 evident that cell viability, indicated by bright fluorescence, was well preserved after PEF
287 treatment along the whole length of the strawberry.

288 Figure 4 shows the micrographs of the frozen/thawed strawberries. As expected the
289 untreated sample did not survive the freezing and thawing cycle and exhibited no live cells
290 throughout the tissue (Fig. 4a). In our previous study (Velickova et al., 2013a), we proved
291 that when strawberries were infused with cryoprotectants, the cryoprotection effect was
292 influenced by the heterogeneity of the strawberry tissue and cell survival was observed only
293 in the cortical tissue and the pith, while the viability of cells in the more compact tissue
294 (epidermal, the outer layer, approximately 2 mm from the surface of the strawberry) could
295 not be preserved. Fig. 4b exhibits the viability of the outer layer of the vacuum infused
296 samples, prepared in this study. As can been seen, there is a partial viability of the cells in

297 this layer compared to the control sample (Fig. 4a). The cell viability of the electroporated
298 tissue is presented in Fig. 4c. The number of viable cells, their type and size are given in
299 Table 2. Neither treatment enables survival of the 1st mm, but it is evident that the
300 combination of PEF + VI prior to freezing enhances the viability of the outer layer after a
301 freezing/thawing cycle. The viable cells were visible starting from 2 mm from the
302 strawberry surface. There was a significantly higher number of live cells in PEF+VI tissue
303 in comparison to those from VI tissue (Table 2). Therefore, it can be assumed that this
304 improvement is due to the electroporation of cells located in the outer layer, which due to
305 its dense cell packing could not be completely protected with the only vacuum infusion.
306 However, the protection of the cell viability was not possible in the 1st mm of the tissue,
307 this is probably due to nature of the epidermal cells. In fact, they are round, densely
308 stacked, with very little extracellular space. Therefore, probably there was not enough
309 cryoprotective solution in between the cells to enable solution to enter into the cells during
310 electroporation and thus to promote cryoprotection.
311 Tylewicz et al. (2017) observed that the cell survival in strawberry tissue strongly depended
312 on the strength of the applied electric field applied. In their work, the application of PEF at
313 100 V/cm partially preserved cell viability of strawberries, while the increase of the electric
314 field strength (200 and 400 V/cm) caused a complete loss of cell viability, showing also a
315 greater structural damage. Even though the electric field strength applied in the present
316 study (850 V/cm) was higher, the number of pulses was lower, leading also to the lower
317 total energy input in comparison to the samples treated with 200 and 400 V/cm. Moreover,
318 Tylewicz et al. (2017) worked on organic strawberries, which could result in higher
319 sensitivity to the PEF treatment.
320 Figure 3, Figure 4, Table 2
321

322 3.3. Texture analysis

323 The results of texture analysis carried out on strawberry samples after the pre-
324 treatments and freezing/thawing cycle are respectively given in Figs 5 and 6. In Fig 5A, as
325 an example, a typical force-distance curves of fresh and treated strawberry tissue sample
326 has been reported, where three different peaks can be distinguished. The first peak indicates
327 the point of internal fracture and it is taken as the parameter of flesh firmness with values of
328 3.0-3.5 N and 1062 ± 32 N/mm for the firmness and its slope, respectively, for fresh
329 strawberry fruits. The second peak gives the firmness of the second firmer layer of the flesh,
330 the core (pith) with values around 2.0-2.5 N, corresponding to a slope of 562 ± 17 N/mm.
331 The third peak shows the firmness of the cortical tissue and it has firmness similar to the
332 first peak 3.0-3.5 N, with slope of 484 ± 15 N/mm. After the pre-treatments there were no
333 significant changes in the firmness of the strawberry tissue. The values for the first and the
334 third peak were in the range of 3.1-3.3 N, while for the second they varied from 2.0 to 2.2
335 N (Fig 5B). The slope of the first peak was around 985 ± 35 N/mm, 539 ± 20 N/mm for the
336 second and 475 ± 25 for the third (Fig 5C).

337 Figure 5.

338 As previously shown in Figs. 3 and 4, after the freezing/thawing cycle the untreated
339 fruit was completely damaged, with high drip loss and dead cells; this behavior influenced
340 the texture of this sample in which it was not possible to detect the three distinguished
341 peaks. The untreated strawberry sample had only one prolonged peak with reduced
342 firmness of 1.3 N (Fig 6A and 6B) and slope with no steepness of 280 ± 20 N/mm (Fig 6C).
343 For the both, VI and PEF+VI samples, the second and the third peak could be measured
344 (Figs 6B and 6C) after surpassing the prolonged compression due to partial loss of vitality
345 and turgidity in the outer layer (Fig. 4). As showed in Figs 6B and C the firmness and slope
346 of the peaks were not significantly different among the differently pretreated strawberry

347 samples. It seems that the higher firmness of pretreated samples, compared to the untreated
348 one, is due to the VI rather than PEF effect. Even though the drip loss and texture are the
349 parameters usually used to verify the tissue response to the freezing/thawing cycle, it
350 should be noticed that these measurements can detect only the macroscopic changes,
351 therefore non-always they are sensible enough to evidence the differences after different
352 treatments, as it was observed by microscopic observation of viable cells (Fig 4).

353 Figure 6.

354 Several authors studied the effect of the PEF pre-treatment on the freezing tolerance of
355 potato, carrot and apple (Jalte et al., 2009; Shayanfar et al., 2013; Shayanfar et al., 2014;
356 Parniakov et al., 2015). Jalte et al., (2009) stated that PEF treatment prior to freezing
357 accelerates freezing process and such frozen samples require higher cutting force in
358 comparison with the traditionally frozen samples. Shayanfar et al. (2014) also investigated
359 the effect of PEF pre-treatment with trehalose on freezing tolerance of carrot and proved
360 that PEF-treated carrot discs significantly retained their firmness after defrosting. Parniakov
361 et al. (2015) tested the PEF pre-treatment with glycerol on the stability of frozen/thawed
362 apple discs. They reported data on prevention of tissue softening after defrosting by
363 application of combined PEF with osmotic treatment. It is worth mentioning that freezing
364 can be affected by many factors such as shape of samples, size and shape of cells (Lopez-
365 Leiva & Hallstrom, 2003, Velickova et al., 2013a). The intensively compact structure of
366 carrot cells could be responsible for the higher resistance than the softer potato cell walls
367 against PEF damaging effects (Shayanfar et al., 2013). It can be assumed that although
368 electric pluses lead to pore generation in cell membranes, the high integrity of dense plant
369 cell walls could withstand the cell wall destruction (Shayanfar et al., 2014). The strawberry
370 itself has heterogeneous structure, with dense, external, epidermal layer which could be the
371 reason for the good texture retention after VI and PEF+VI treatments.

372 3.4. Color changes

373 Color is a major quality index for the fresh and processed fruits, because consumers
374 initially base their decision on good looking products, which for the strawberries would
375 mean fruits with intense red color. Therefore, it is highly important to implement the least
376 invasive technique that will enable the best color retention. The L*, a*, b* values and the
377 calculated ΔE, C and h° representing the color of strawberries' surface and inner region of
378 fresh and pre-treated samples (VI, PEF+VI), are shown in Table 3. In the strawberry
379 surface, luminosity and redness did not significantly changed in the treated samples, while
380 yellowness significantly increased at the same level in VI and PEF+VI samples. This has
381 resulted also in an increase of ΔE and h°. The more evident changes of color were observed
382 in the inner part of fruit tissue, in particular the PEF treatment significantly reduced the a*
383 and b* parameters, and consequently also the h° parameters. This reduction is expressed as
384 a typical dark red color. Ribeiro et al. (2007) also related the dark red color of coated
385 strawberries with the decrease in b* values. PEF impact on color can have dual effect. On
386 one side it causes the leakage of intracellular content and enhances activity of some
387 enzymes, but on the other side it also inactivates certain enzymes enabling better color
388 retention (Wiktor et al., 2015). Zhao et al. (2009) and Odriozola-Serrano et al. (2009)
389 reported that PEF treatment did not cause significant changes in green tea extract color and
390 carrot juices, respectively. Other researchers stated that PEF treatment can cause slight
391 browning effect in apples (Grimi et al., 2010). Concerning the total color difference ΔE, it
392 was observed that PEF + VI treated strawberries had significantly higher ΔE values, in
393 comparison to the one just VI. According Tiwari et al. (2010), the values of ΔE higher than
394 2 indicate that such color change could be visible by a consumer with the naked eye.

395 Table 3

396 The measured and calculated color parameters of untreated, VI and PEF+VI
397 strawberry samples after freezing and thawing are given in Table 4. The color of strawberry
398 surface changed in a similar way in both treated samples after thawing in terms of reduction
399 of L*, ΔE and increasing of a*, b* and h° parameters as compared with the untreated
400 thawed sample. Concerning the inner tissue of the strawberries, on the other hand, it was
401 evident that PEF+VI treatment promoted an increase of all considered color parameters that
402 reached values similar to that of the fresh one (Table 4). The ΔE of PEF+VI was much
403 lower (4.6 ± 0.6) in comparison to the untreated one and just vacuum infused (14.8 ± 2.1
404 and 18.6 ± 1.6, respectively). Ngo et al. (2007) stated that freezing of strawberries at -37 °C
405 in air blast freezer, promoted the changes of berry surface due to ice crystallization and
406 increase of fruit volume, which provoked more discontinuities in the cuticle layers. The
407 authors reported significantly higher L* values for the thawed berries in comparison to the
408 fresh samples, suggesting that the increased lightness might be due to the physical changes
409 resulting from collapse of thawed berry structure.

410 Table 4

411

412 **4. Conclusion**

413 The strawberries pre-treated with PEF prior to VI enabled higher cell viability and
414 significantly better inner flesh color retention, in terms of a vivid red color, in thawed
415 strawberries, comparable to that of fresh samples. However, the applied conditions of PEF
416 were not adequate to promote an improvement of strawberry quality in terms of drip loss
417 and texture in comparison to the samples treated only with VI.

418 Obtained results demonstrate potentiality of PEF application to enhance the effect
419 of cryoprotectants, probably because this technique delivers the used substances inside the
420 cells, in improving the quality of fruits.

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Figure Captions

Figure 1. Schematic presentation of the pulsed electric field treatment

Figure 2. Drip loss of untreated and treated samples after freezing/thawing process. The bars with different letters differ significantly ($p < 0.05$). The mean values of 15 replicates, expressed in %, are reported.

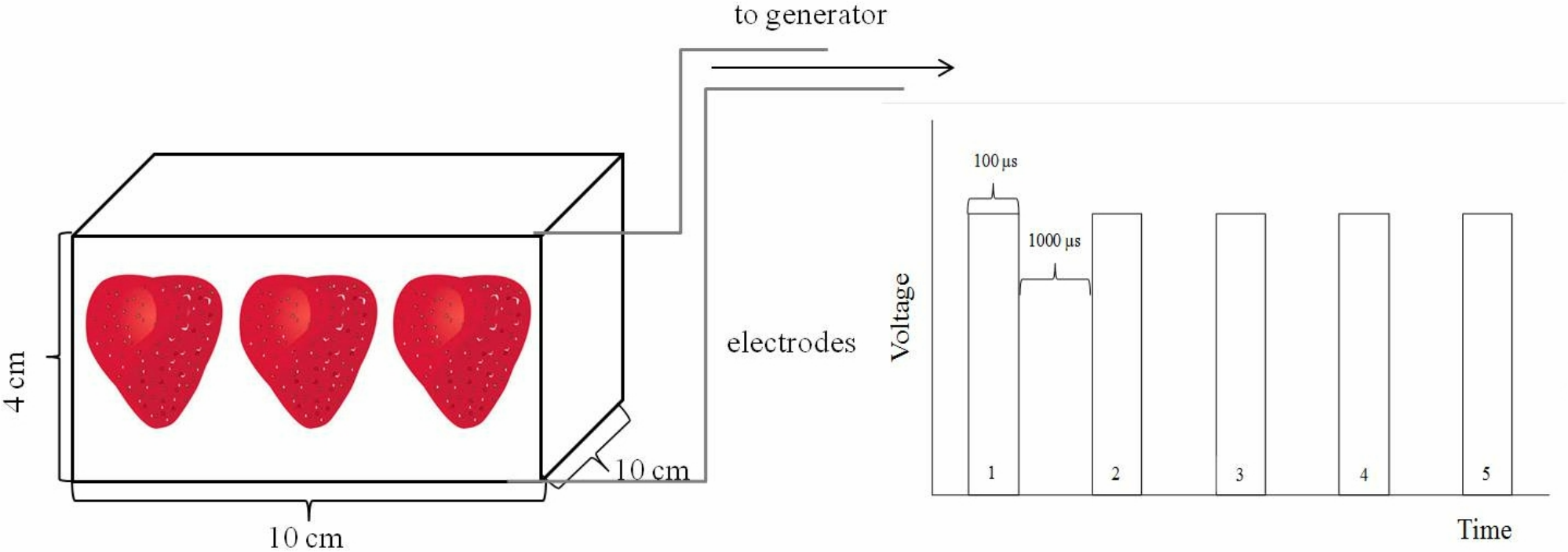
Figure 3. Micrographs of electroporated and viable strawberry tissue: A) electroporation is indicated by the penetration of propidium iodide into the hypodermal cells and the staining of their nuclei, which can be clearly seen in the pictures as bright circles inside the cells; B) living tissue stained with fluorescein diacetate is indicated by bright green cells. The scale bar represents 100 µm.

Figure 4. Cell viability test of strawberries after one freezing/thawing cycle: A) untreated strawberry, B) vacuum infused; C) PEF and vacuum infused. Micrographs of the different layers of the strawberries. The scale bar represents 100 µm.

Figure 5. Texture analysis of strawberries after the pre-treatment: A) typical force-distance curve for whole strawberry, B) hardness; C) peak slope. Bars of same peak with different letters are significantly different ($p < 0.05$). The fruits were infused as described in the Materials and Methods section. The mean values of six replicates, expressed in N and N/mm, are reported.

Figure 6. Texture analysis of strawberries after the freezing/thawing cycle: A) typical force-distance curve for frozen/thawed samples; B) hardness; C) peak slope. Bars of same peak with different letters are significantly different ($p < 0.05$). The mean values of six replicates, expressed in N and N/mm, are reported.

- Pulsed electric field (PEF) treatment was coupled with vacuum infusion (VI)
- Combined PEF and VI treatment prior to freezing was tested
- The PEF and VI treatment improved the color retention of thawed strawberries
- No further improvement on texture and drip loss by application of PEF prior VI


to generator
electrodes
4 cm
10 cm
10 cm
100 µs
1000 µs
Voltage
Time
1
2
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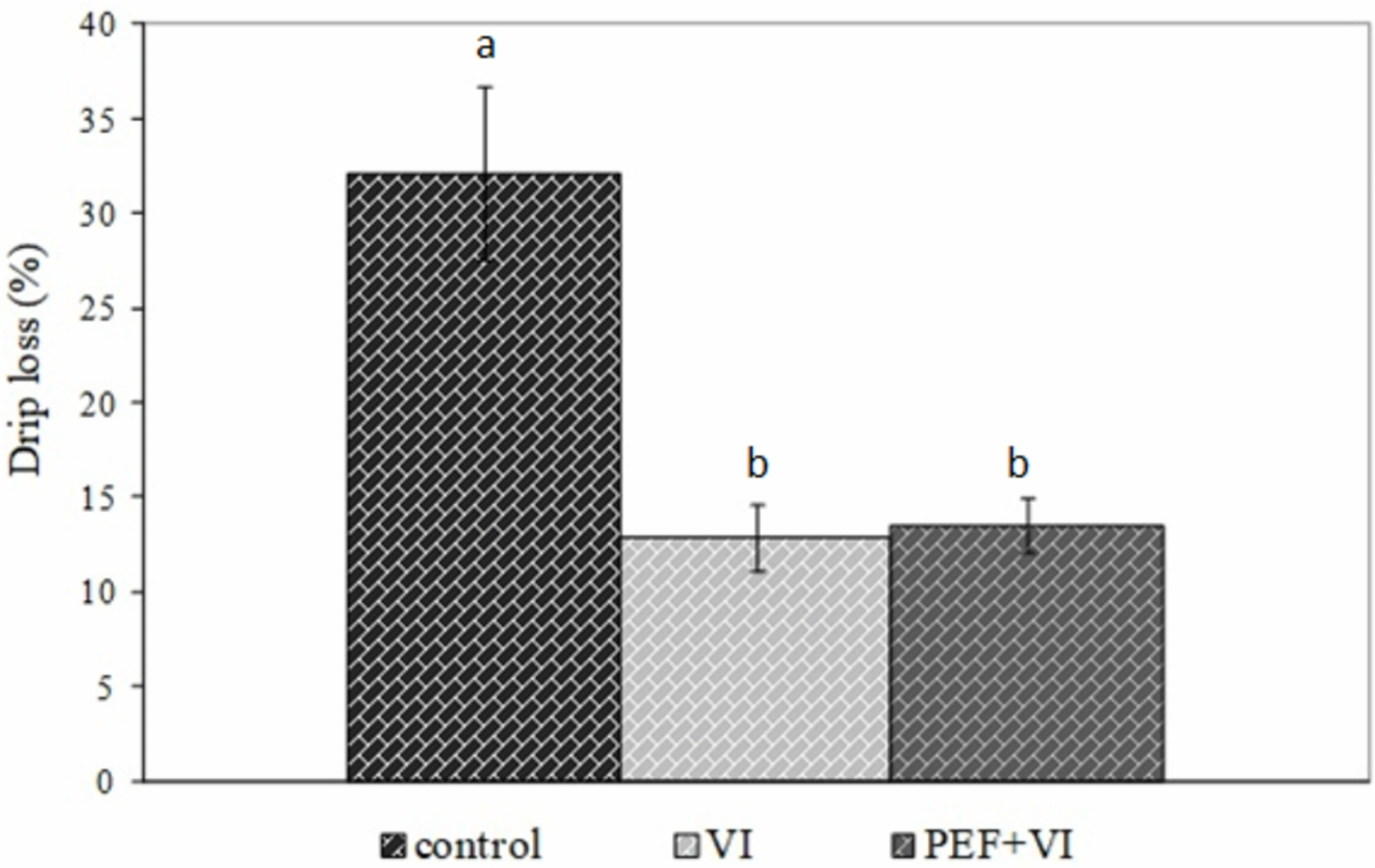
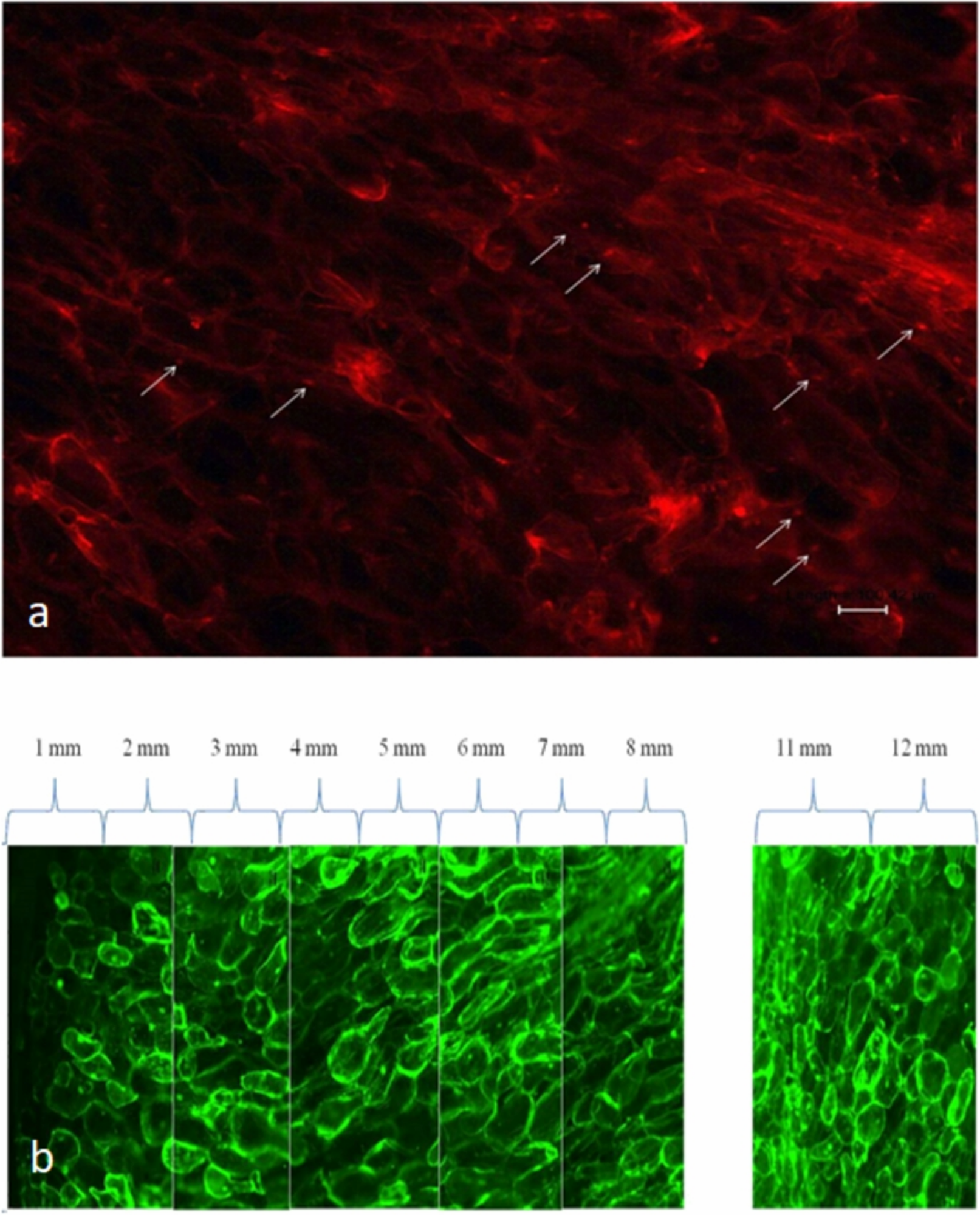
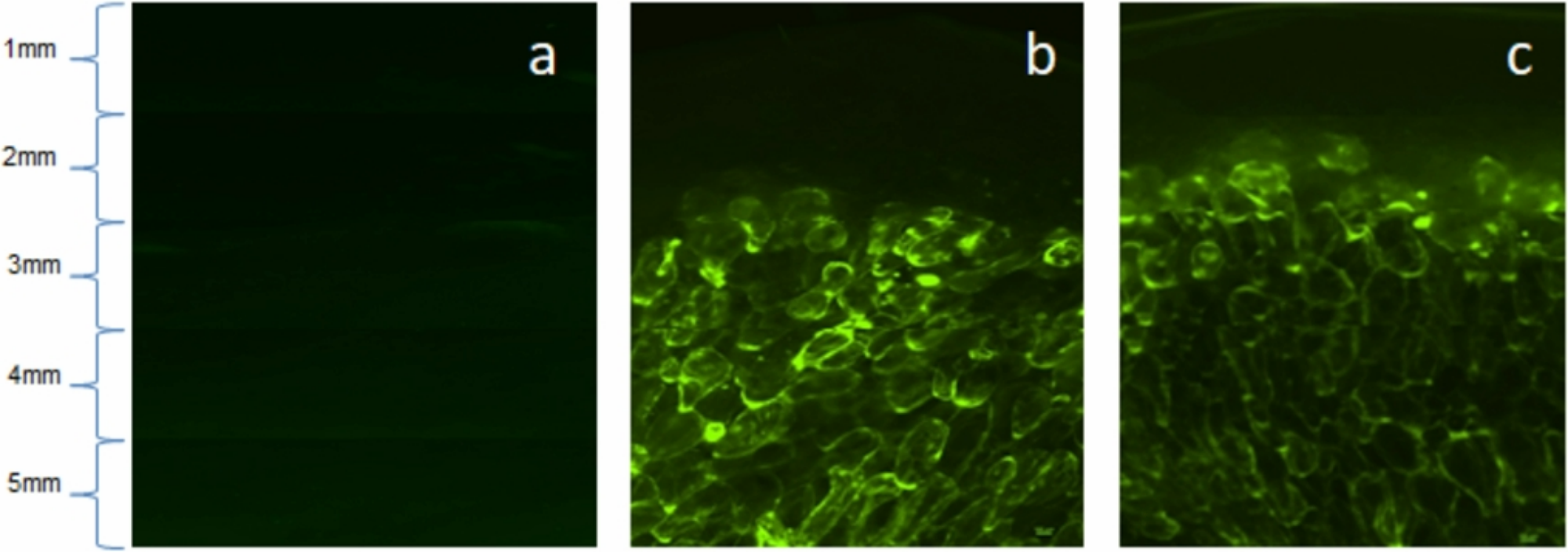
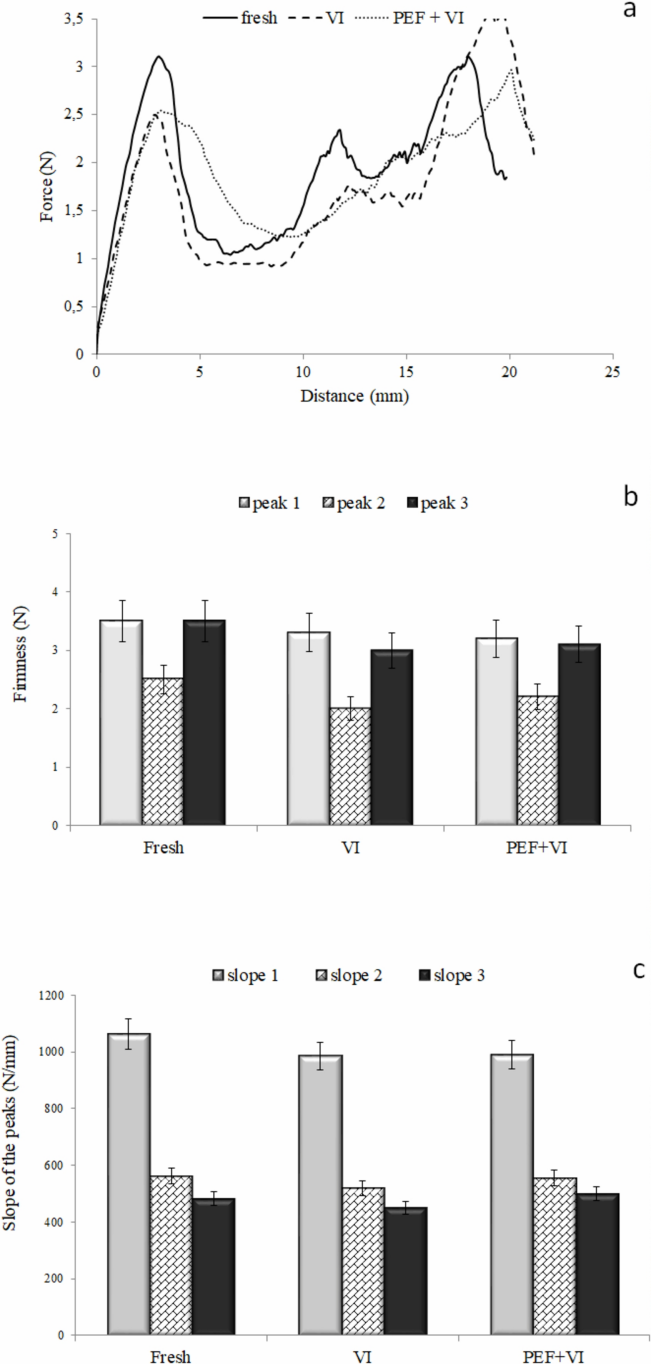
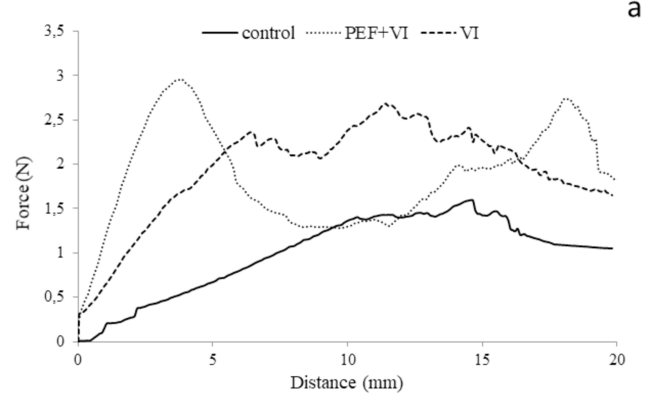
5


40
35
30
25
20
15
10
5
0
Drip loss (%)
a
b
b
control
VI
PEF+VI


a
1 mm
2 mm
3 mm
4 mm
5 mm
6 mm
7 mm
8 mm
11 mm
12 mm
b


1mm
2mm
3mm
4mm
5mm
a
b
c


a
fresh
VI
PEF + VI
Force (N)
Distance (mm)
b
peak 1
peak 2
peak 3
Firmness (N)
Fresh
VI
PEF+VI
c
slope 1
slope 2
slope 3
Slope of the peaks (N/mm)
Fresh
VI
PEF+VI


a
control
PEF+VI
VI
Force (N)
Distance (mm)
3,5
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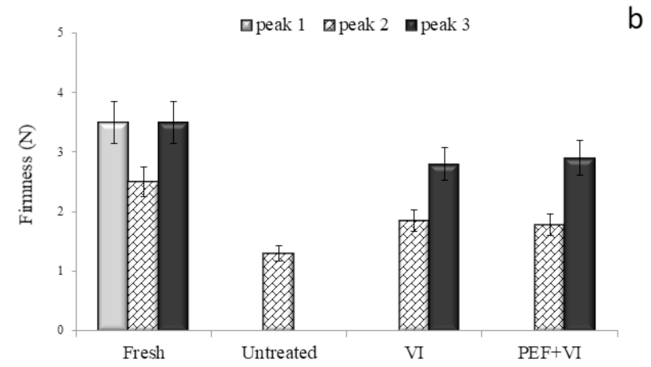
2,5
2
1,5
1
0,5
0
0
5
10
15
20


b
peak 1
peak 2
peak 3
Firmness (N)
Fresh
Untreated
VI
PEF+VI

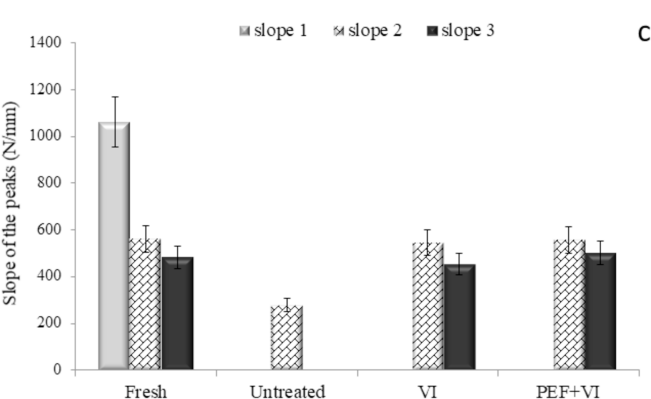

c
slope 1
slope 2
slope 3
Slope of the peaks (N/mm)
1400
1200
1000
800
600
400
200
0
Fresh
Untreated
VI
PEF+VI

Table 1. Optimization of pulse electric field conditions with fixed electric field strength of 850 V/cm to obtain viable and electroporated strawberry tissue.

Treatment conditions		**Propidium iodide**	**Fluorescein diacetate**
Pulse duration	500 μs	no electroporation	dead cells
Number of pulses	1		
Time between pulses	1000 μs		
Pulse duration	200 μs	electroporation	live cells
Number of pulses	1		
Time between pulses	1000 μs		
Pulse duration	200 μs	no electroporation	dead cells
Number of pulses	5		
Time between pulses	2 s		
Pulse duration	100 μs	no electroporation	live cells
Number of pulses	1		
Time between pulses	1000 μs		
Pulse duration	100 μs	**electroporation**	**live cells**
Number of pulses	5		
Time between pulses	1000 μs		
Pulse duration	100 μs	no electroporation	dead cells
Number of pulses	10		
Time between pulses	1000 μs		

Table 3. Effect of the pre-treatment on the color parameters of strawberries'surface and inner region. The mean values of 27 replicates, expressed as L*, a*, b*, ΔE, C and h° parameter, are reported.

Color parameters	Sample's surface			Sample's inner region		
	Fresh	**VI**	**PEF+VI**	**Fresh**	**VI**	**PEF+VI**
L*	20.3 ± 0.4[a]	20.2 ± 0.2 [a]	19.9 ± 0.4 [a]	33 ± 1[a]	31.9 ± 0.2 [a]	33 ± 1 [a]
a*	26.6 ± 0.3 [a]	26.1 ± 0.3 [a]	26.3 ± 0.3 [a]	45 ± 1 [a]	46.2 ± 0.2 [a]	28 ± 1 [b]
b*	14.7± 0.1 [b]	18.5 ± 0.2 [a]	18.3 ± 0.2 [a]	37± 1 [a]	37.8 ± 0.5 [a]	21 ± 1 [b]
ΔE	-	3.9 ± 0.4 [b]	4.6 ± 0.5 [a]	-	1.8 ± 0.1 [b]	3.1 ± 0.3 [a]
C	33.4 ± 0.3 [a]	32 ± 0.4 [b]	32.9 ± 0.2 [a]	58.6 ± 0.8 [a]	59.7 ± 0.6 [a]	61.2 ± 0.9 [a]
h°	28.8 ± 0.2 [b]	35.3 ± 0.3 [a]	34.8 ± 0.1 [a]	39.2 ± 0.9 [a]	39.3 ± 1 [a]	37.1 ± 0.8 [b]

Values in the same row with different letter are significantly different ($p<0.05$).

Table 4. Effect of the pre-treatment on the color parameters of frozen/thawed strawberries'surface and inner region. The mean values of 27 replicates, expressed as L*, a*, b*, ΔE, C and h° parameter, are reported.

Color parameters	Sample's surface			Sample's inner region		
	Untreated	VI	PEF+VI	Untreated	VI	PEF+VI
L*	25.9 ± 0.8[a]	19.7 ± 0.6[b]	19.9 ± 0.4[b]	27.2 ± 0.9[b]	28.2 ± 0.8[b]	33.3 ± 0.5[a]
a*	20.3 ± 0.5[b]	28.7 ± 0.7[a]	26.3 ± 0.3[a]	36.4 ± 0.8[b]	33.5 ± 0.4[b]	47.5 ± 1.5[a]
b*	11.5± 0.7[c]	15.5 ± 0.6[b]	18.3 ± 0.2[a]	27.1± 0.6[b]	23.7 ± 0.5[b]	40.8 ± 2.0[a]
ΔE	9.2 ± 1.1[a]	2.5 ± 0.5[b]	2.7 ± 0.8[b]	14.8 ± 2.1[b]	18.6 ± 1.6[a]	4.6 ± 0.6[c]
C	23.3 ± 0.5[b]	32.7 ± 0.4[a]	32 ± 0.7[a]	45.4 ± 0.8[b]	41 ± 0.6[c]	62.6 ± 0.9[a]
h°	29.5 ± 0.2[b]	28.4 ± 0.3[b]	34.8 ± 0.1[a]	36.7 ± 0.9[b]	35.3 ± 1[b]	40.7 ± 0.8[a]

Values in the same row with different letter are significantly different ($p<0.05$).

Table 2. Number, type and size of viable cells of fresh and frozen/thawed strawberries' tissue in the outer 5 mm layer. The types of cells are: E-epidermal, H-hypodermal and C-cortical. The size of cells is given in μm. The mean values of observation from 15 fruits, are reported.

Samples	**Viable cells' number, type and size**									
	1 mm		**2 mm**		**3 mm**		**4 mm**		**5 mm**	
	number	**type and size**	**number**	**type and size**	**number**	**type and size**	**number**	**type and size**	**number**	**type and size**
fresh	32±3[a]	E:277.7±28.8[a]	34±6[a]	E:315.0±42.2[a]	24±4[b]	H:492.7±69.1[a]	24±6[a]	H:613.0±34.5[a]	34±7[a]	C:582.6±72.2[a]
untreated	/[b]	/[b]	/[c]	/[b]	/[c]	/[b]	/[b]	/[b]	/[b]	/[b]
VI	/[b]	/[b]	19±2[c]	E:291.6±11.9[a]	35±4[a]	H:460.7±38.7[a]	34±7[a]	H:623.0±64.5[a]	40±4[a]	C:604.0±52.1[a]
PEF+VI	/[b]	/[b]	25±3[b]	E:256.9±28.3[a]	31±3[ab]	H:428.9±83.4[a]	30±5[a]	H:567.7±31.6[a]	33±5[a]	C:547.7±62.2[a]

Values in the same column with different letter are significantly different ($p<0.05$).