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Abstract

Pineapple cubes in sugar syrup were treated with high-pressure processing (HPP), conventional (DIM) heating and ohmic heating (OHM). Samples were compared in terms of microstructural, physical (total soluble solids, sieve analysis, texture and colour) and residual pectin methylesterase activity (PME) and total antioxidant capacity. OHM yielded relevant changes in cellular microstructure and electroporation of the cell wall. The HPP treatment favoured the presence of soluble solids in the syrup, and the samples were less damaged in terms of shape and microstructure. in the samples were harder following HPP than they were with OHM and DIM, while HPP showed the highest colorimetric (ΔE) differences compared with RAW samples. The PME residual activity was the lowest in pineapple treated by DIM, while the antioxidant capacity was comparable among treated samples.

Keywords: colour; high-pressure processing; microstructure; ohmic heating; pineapple cubes

1. Introduction

Among fruits, after banana and citrus, pineapple (*Ananas comosus* L.) is the third most important fruit in the world; it has a global production of 27.4 million tonnes (Mtons) in 2017 and Costa Rica (3.0 Mtons), the Philippines (2.7 Mtons) and Brazil (2.3 Mtons), which are the major pineapple-producing countries (Faostat, 2017). The European Union imported 1.4 Mtons of pineapples in 2017. Pineapple is an important source of sugars, organic acids, essential minerals, fibre and vitamins for human nutrition. In addition, pineapple is rich in health-promoting antioxidants such as ascorbic acid, flavonoids and carotenoid (Valderrain-Rodríguez, de Ancos, Sánchez-Moreno, & González-Aguilar, 2017). Considering the interesting nutritional properties of pineapple and the increasing awareness about the benefits of fruit consumption, the processed pineapple market is expected to grow in the future, driven by strong consumer demand for products with a better nutritional profile and fresh-like quality. This trend is confirmed by the great growth of the fresh-cut pineapple market in recent years. However, various foodborne pathogens have been linked to the consumption of fresh-cut fruits, and some of them may cause illness or even death among consumers (Feng et al., 2017); for this reason optimizing the use of alternative preservation methods is of great importance.

One of the most important thermally processed product manufactured from pineapple is fruit pieces in sucrose solution (Tumpanuvatr et al., 2015); however, even if the pasteurization is largely used for this kind of product, there is insufficient knowledge on the effect of stabilization treatments on the final qualitative and sensorial characteristics. In particular, spoilage and mycotoxin-producing heat-resistant fungi such as *Neosartorya fischeri* represent a major problem for fruit-processing industries as documented in many countries. Salomão, Slongo and Aragão (2007) researched pineapple juice and reported that high temperatures were essential for a significant elimination of ascospores but had adverse effects on the sensory and nutritional qualities.

Heating processes, due to non-enzymatic browning reactions and pigment destruction, can affect the quality of pineapple products, leading to consumer dissatisfaction. Rattanathanalerk, Chiewchan and

Srichumpoung (2005) studied the effect of thermal processing between 55 and 95 °C on the colour parameters (L, a, b and ΔE) of pineapple juice. They found that with increasing temperature and time the pineapple juice became darker, which corresponded to a decrease in L value. Saikia, Mahnot and Mahanta (2016) observed a general reduction of all nutritional parameters (total phenolic content, total flavonoid content, DPPH and FRAP) of pineapple juice after a treatment at 75 °C for 3 min, confirming the impact of mild thermal treatments.

New emerging thermal and non-thermal technologies, feasible for pineapple cubes in syrup production**,** need more investigation (Hounhouigan, Linnemann, Soumanou & Van Boekel, 2014). High-pressure and ohmic heating processing could be promising innovative technologies for pineapple products. High-pressure processing represents a cold technology, and it has been reported to better preserve the nutritional and organoleptic traits of fruits (Huang, Wu, Lu, Shyu & Wang, 2017; Oey, Lille, Van Loey & Hendrickx, 2008; Tewari, Sehrawat, Nema & Kaur, 2017). Previous studies have shown that high-pressure can preserve the overall sensory quality of pineapple and exotic fruits in general (Laboissière et al., 2007); moreover, high-pressure has been proposed as a treatment for extending the shelf-life of minimally processed fruit products with high quality and nutritional standards (Denoya et al., 2016b). Unfortunately, high-pressure treatments at ambient temperature not only cannot inactivate endogenous enzymes (Terefe & Buckow, 2017), in some cases they can cause an increase in their activity (Terefe, Buckow & Versteeg, 2014). Finally, in a recent paper HPP appeared to be more expensive than traditional thermal processes, but it had a lower environmental impact in almost all impact categories (Cacace, Bottani, Rizzi, & Vignali, (2020).

Ohmic heating, also known as dielectric heating, can overcome the limits of conventional heat transfer mechanisms in which liquids and solids warm up at different velocities and with important non-uniformity mainly for particulate food (Varghese, Pandey, Radhakrishna & Bawa, 2014). Only a few papers have reported results regarding ohmic heating applied to pineapple products. Pham, Jittanit and Sajjaanantakul (2014) successfully applied indirect ohmic heating for ready-to-eat (RTE) packed pineapple cubes with good visual appearance and firmness. Similarly, Tumpanuvatr et al.

95 (2015) studied the effects of ohmic heating in a batch system compared with a conventional method
96 on pineapple cubes in a sucrose solution. The reported results indicated that ohmic heating has the
97 potential to provide higher quality products than conventional heating based on colour and texture if
98 the same heating rate is applied.
99 Based on the aforementioned encouraging results, a better understanding of applying innovative and
100 commercially available technologies on pineapple is needed; thus, the aim of this paper was the study
101 of the physical, chemical and microstructural effects of ohmic heating and high-pressure processing
102 and emerging and green technologies on pineapple cubes in syrup.
103

104 **2. Materials and Methods**

105 *2.1 Samples, preparation and storage*

106 Fresh pineapples, (untreated, RAW) *Ananas comosus* L. var. Smooth Cayenne, were of commercial
107 maturity (average weight 1.8±0.3 kg) and were obtained from a producer in Thailand **harvested in**
108 **the Prachuap Khiri Khan province**, washed under running tap water, peeled and cut by machine
109 **into 10±0.5 mm c**ubes and immersed in an isotonic solution **(16 g/100g sucrose)** with a ratio of 70/30
110 solid/liquid. Then, 0.1% potassium metabisulphite was added to the samples as a preservative for
111 stabilizing the product during the journey from Thailand to Italy. Three pasteurization techniques
112 were investigated: two thermal treatments (conventional and ohmic) and one non-thermal treatment
113 (high-pressure processing); all treatments are currently available technologies for fruit product
114 stabilization **with regard to quality parameters such as colour, texture and nutritional aspects**
115 **as well as microbial ones**. Thermal treatments for high-acid products can achieve commercial
116 sterility while high-pressure processing requires refrigerated storage: the choice of the right
117 technology depends on the compromise between the final quality of the product and constraints for
118 the product shelf-life.
119 All trials were performed on an industrial plant in triplicate; for each trial approximately 400 kg of
120 product was used as follows:

- Conventional thermal treatment (DIM - **Sterideal DT ®, John Bean Technologies, Chicago, Illinois, United States) at John Bean Technology facility of Parma (Italy)**: the treatment section consisted of a stainless steel 304 dimpled tube with an internal diameter of 55 mm and a total length of 12 m. The flow rate was set to 1600 l/h. The tube presented dimples on the outer surface and protrusions on the inner surface that work as gentle vortex generators, which resulted in better fluid/solid mixing near the wall and higher turbulence. **Pineapple cubes presented a pH lower than 4.2 and the recommended lethality was calculated by considering mesophilic spores in acid foods in the pH range 4.0 – 4.6: the reference temperature of 93.3 °C and the z value of 8.9 °C was used for calculating a sufficient $F_{93.3}^{8.9}$ equal** to 5 min at the slowest heating point (SHP) corresponding to the centre of the cube (NFPA, National Food Processor Association in USA). This value corresponds to more than 6D reduction of ascospores of *Neosartorya fischeri*, one of the most frequently reported heat-resistant moulds causing spoilage in fruit products (Salomão, Slongo, & Aragão, 2007). By means **of preliminary tests, the syrup temperature profile at the end of the holding section measure by means of a resistance temperature detector Pt100 with diameter of 3 mm (Endress+Hauser AG Reinach BL, Switzerland was used for calculating the heat penetration in cubes through mathematical modelling and sterilizing value, as consequence** (Cordioli, Rinaldi, Copelli, Casoli & Barbanti, 2015). The time required for reaching the holding temperature at the SHP was approximately 40 s. After thermal treatment, the samples were cooled and aseptically packed in 10 L bags (**Goglio Spa, Milano, Italia - PE/MET/PE, thickness 77 µm)** by means of **commercial plant (AF200 Classic Aseptic Filler, John Bean Technologies, Chicago, Illinois, United States).**

- High hydrostatic pressure (HPP): treatments were conducted in a 300 L high-pressure plant **(Avure Technologies Inc., Erlanger, Kentucky, United States)** at the "HPP Italia" facility of Traversetolo (Italy). Samples were packed in PET bottles with an internal volume of 250 ml (**wet bag method**). **Indirect method for generation of high isostatic pressure by means of cold water (4 °C) was**

146 **used**, and the temperature increase due to compression was not higher than 2-3 °C/100 MPa. HHP
147 treatments were conducted at 600 MPa for 3 min, which is considered to be economically and
148 microbiologically safe at the pasteurisation level coupled with refrigerated storage. These conditions
149 were chosen based on industrial practises for fruit products and on the good results obtained on the
150 same kind of samples (Oey et al., 2008). The treated samples were then stored at a refrigerated
151 temperature (+4 °C).

152 - Ohmic heating (OHM, **Sterideal DT ®, John Bean Technologies, Chicago, Illinois, United**
153 **States**): a temperature increase up to 90 °C was obtained by means of an ohmic heater at a flow rate
154 of 1,900 l/h; then, the product was sent to the same holding section used for DIM tests obtaining the
155 same sterilizing effect reported above, **obtained by means of mathematical modelling**. The flow
156 rate was increased to prevent overheating in the ohmic heater section and the required electrical power
157 was **automatically precalculated by** the ohmic machine by considering the electrical conductivity
158 of pineapple cubes (0.31 Sm^{-1} at 25 °C and 0.55 Sm^{-1} at 50 °C) measured on a homogenized sample
159 using a digital conductivity metre and in accordance with reported values (Amiali, Ngadi, Raghavan
160 & Nguyen 2006). **The actual electrical power was obtained by means of a feedback control on**
161 **product temperature measured at the outlet of the ohmic section. The product used for the**
162 **voltage setting was discarded.** The calculated time required for reaching the holding temperature at
163 the SHP was approximately 3 s. After thermal treatment, the samples were cooled and aseptically
164 packed in 10 L bags (**Goglio Spa, Milano, Italia - PE/MET/PE, thickness 77 µm).**

165

166 *2.2 Histological analysis*

167 The samples were fixed in FAA solution (formalin: acetic acid: 60% ethanol solution, 2:1:17 v/v)
168 (Ruzin, 1999). After two weeks, they were dehydrated with gradual alcohol concentrations and
169 included in a methacrylate resin (Technovit 7100, Heraeus Kulzer & Co., Wehrheim, Germany). The
170 resulting blocks were sectioned at 3 µm thickness (transversal cuts) with a semithin Leitz 1512

171 microtome (Leitz, Wetzlar, Germany). The sections were stained with a toluidine blue (TBO) solution
172 (Ruzin, 1999) to evaluate the structural variation after each treatment. The sections were observed
173 under a Leica DM 4000 optical microscope (Leica Imaging Systems Ltd., Wetzlar, Germania)
174 equipped with a digital camera Leica DMC 2900 (Leica Imaging Systems Ltd., Wetzlar, Germania).
175
176 *2.3 Physical analyses*
177 The total soluble solids of the syrup were determined by a refractometer (Model 2WAJ, Optika, Italy)
178 at a temperature of 25 °C and expressed as °Brix. The pH **of the homogenized whole product** was
179 measured with a pH metre (Model 3150, Jenway, UK); **cubes and syrup were blended and made**
180 **into paste prior to pH determination.**
181 A sieve analysis of cubes was performed using a laboratory vibratory sieve shaker (Giuliani
182 Tecnologie srl, Torino, Italy). The sieve **square** mesh sizes used were 9.5, 8.0, 5.6, 3.35 and 2.36 mm
183 **(ASTM E11-95 - Standard Specification for Wire Cloth and Sieves for Testing Purposes).**
184 **Analyses were carried out at a frequency of 5 Hz for 5 miuntes.**
185 The texture of all samples (RAW, DIM, HPP and OHM) was analysed by a TPA double-compression
186 test using a TA.XT2i Texture Analyzer **(Stable Micro Systems, Godalming, United Kingdom)**
187 equipped with a 35 mm diameter cylindrical aluminium with a pre-test, test and post-test speed of 1
188 mms^{-1} up to 40% of the original sample height as measured by a Vernier calliper. Each test was
189 performed on a single cube previously drained on a metal strainer and without evident structural
190 damages. **The textural parameters considered were hardness, cohesiveness, resilience,**
191 **springiness and chewiness (Bourne, 1978). Ten** samples from each trial were analysed.
192 Colour determination was performed using a Minolta Colorimeter (CM 2600d, Minolta Co., Osaka
193 Japan) equipped with a standard illuminant D65. The assessments were conducted on two sides of
194 eight pineapple cubes. L* (lightness, black = 0, white = 100), a* (redness >0, greenness <0), b*
195 (yellowness, b* > 0, blue <0) were quantified on each sample using a 10-degree position of the
196 standard observer. Sixteen samples from each trial were analysed.

197

198 *2.4 Pectin methylesterase (PME) activity assay and DPPH free radical scavenging capacity test*

199 The PME residual activity was evaluated following the procedure reported by Vicente, Costa,
200 Martínez, Chaves and Civello (2005). In brief, 2 grams of fruit were ground with 6 ml of **1 mol/L**
201 **NaC**l and 8 g l^{-1} **polyvinylpolypyrrolidone** (PVPP). The obtained suspension was stirred for 4 h and
202 then **centrifuged at 10,000 g for 30 min** at room temperature. The supernatant was collected,
203 adjusted to pH 7.5 with 0.**01 mol/L NaOH** and used for assaying enzyme activity. The activity was
204 assayed in a mixture containing 1,200 µl of 0.5 **g/100ml pectin from apple with a degree of**
205 **esterification of 50-75 % (Sigma Aldrich, Merck KGaA, Darmstadt, Germany),** 300 µl of 0.01
206 **g/100ml** bromothymol blue pH 7.5, 100 µl of water pH 7.5 and 200 µl of enzymatic extract. The
207 mixture was incubated at 37 °C, and the reduction of optical density at 620 nm was followed every
208 15 s. The results were expressed as the percentage variation compared with the raw sample using the
209 values of the slope of a linear segment in the absorbance-time curve (Adams, Brown, Ledward &
210 Turner, 2003). The analyses were performed in triplicate.
211 The total antioxidant capacity was determined using DPPH assay (2,2-diphenyl-1-picrylhydrazyl free
212 radical) following the procedure reported by Moon and Shibamoto (2009). The samples were
213 centrifuged **at 10,000 g for** 15 min at 4 °C. Then, the supernatant was collected for further analysis,
214 and 0.2 mL of 10-fold diluted supernatant was mixed with 4.0 mL of **a 70% methanolic solution** of
215 DPPH (0.14 mmol/L). The analyses were performed in triplicate, and the absorbance of the solution
216 was measured at 517 nm after an incubation time of 30 min in the dark at room temperature. All data
217 were then expressed as Trolox equivalents (µmol/100 g pineapple pulp), and the total antioxidant
218 capacity was referred to as the Trolox equivalents antioxidant capacity (TEAC).
219

220 *2.5 Statistical analysis*

221 The means and standard deviations were calculated with SPSS (v. 25.0, SPSS Inc., Chicago, USA),
222 and the same software was used to perform one-way analysis (ANOVA) to evaluate the significant

223 differences (p <0.05) followed by Tukey's test (p < 0.05) for a comparison among the different
224 treatments.
225
226 **3. Results and discussion**
227 *3.1 Histological analysis*
228 The raw material appeared to comprise large parenchymatic cells with a thin cell w**all ranging from**
229 **1.8 to 4.6 μm** (Figure 1a). The tissue was characterized by diffuse intercellular spaces throughout the
230 structure. Vascular bundles were dispersed in the parenchyma tissue. **The cell membrane appeared**
231 **with low turgor pressure (flaccid) and the cells appeared slightly dehydrated; this phenomenon**
232 **was due to the dehydration treatment of the sample prior to inclusion in the resin**.
233 After DIM treatment, the tissue was modified. In fact, the cells were no longer cohesive, and large
234 intercellular spaces appeared in the structures (Figure 1b). Cell detachment after heat treatment has
235 been widely discussed by several authors (Paciulli et al., 2016; Sila, Doungla, Smout, Van Loey &
236 Hendrickxet, 2006). **Cell wall thickness ranged from 2.7 to 7.8 μm.**
237 For the pineapple samples treated with HPP, the structure remained apparently unchanged (Figure
238 1c). The cells showed signs of dehydration (Figure 1c). Denoya, Nanni, Apóstolo, Vaudagna and
239 Polenta (2016a) described **few** structural changes for peach cube samples after HPP treatments. In
240 that study, the authors showed that peach cubes treated at 600 MPa for 5 min kept their microstructure
241 almost unaltered. The cells appear slightly dehydrated. The most important change that occurred in
242 our samples concerns the thickening of the cell wall: it appeared thicker in some areas due to swelling
243 (Figure 1c) **with a significant increase in range compared to RAW (7.7-3.0.0 μm).** Cellular
244 swelling in plant biology is a change in the cell wall that occurs in response to external stimuli (stress),
245 and it results in increased cell wall thickness due to an accumulation of liquid. In particular, several
246 papers have reported an increase in cell wall thickness/swelling after HP treatments on different fruits
247 and vegetables such as carrots (Araya et al., 2007), berries (Hilz et al., 2006) and peaches (Denoya et
248 al., 2016a). In our study the HPP appeared to induce the mechanism suggested by Christensen (1967)

249 in which uptake of liquid and vapour involves two stages (wetting or adsorption at capillary surfaces
250 followed by molecular penetration and swelling of the solid phase).
251 The OHM treatment appeared to cause the most important changes to the anatomical pineapple
252 structure, especially at the cell wall level. The treatment involved damage to the cell wall, which
253 showed an irregular thickening overall surface of the cell (Figure 1d) as well as electroporation points.
254 **Cell wall thickness presented great dissimilarities with the highest distribution from**
255 **electroporation zones (5.1 μm) to swelled ones (38.2 μm).** According to some authors (Galindo,
256 Vernier, Dejmek, Vicente & Gundersen, 2008; Ganeva, Galutzov & Teissieet, 2014; Mahnič-
257 Kalamiza, Vorobiev & Miklavčict, 2014), electroporation can influence the permeability of the cell
258 wall**. As reported by Lebovka et al. (2005) relaxation curves of potato and apple tissues evidently**
259 **showed softening of the tissue as a result of ohmic treatment combined to holding at high**
260 **temperature.** The results obtained by these authors could explain the effect observed in our study, in
261 which a structural modification of the cell wall was observed (Figure 1d).

262

263 *3.2 Physicochemical analyses*

264 The total soluble solids content of the syrup was 16.6 ± 0.2, 15.8 ± 0.4, 17.6 ± 0.2 and 16.3 ± 0.3
265 **°Brix** for RAW, DIM, HPP and OHM, respectively, with a significantly higher solid content in the
266 syrup for HPP compared with the other treatments. This could be caused by the increase in the
267 diffusivity of water and solute in the high-pressure treated pineapple slices as previously reported by
268 Rastogi and Niranjan (1998) and due to cell wall breakage or swelling as observed in the histological
269 analysis (Figure 3). The increase in cell wall thickness was probably due to swelling, as already
270 observed in vegetables and fruits (Araya et al., 2007; Denoya et al., 2016a); it could have caused
271 absorption of water from the isotonic solution with an increase in sugar content in the syrup as a
272 consequence. In contrast, the pH values of the homogenized whole product were in the range of 3.4-
273 3.6 with no significant differences among all treated samples.

274 The sieve analysis results (Figure 2) showed significant differences among the samples after
275 treatments. Regarding the first class (> 9.5 mm), only HPP samples presented a significantly higher
276 percentage compared with the other samples with no differences among them. The HPP samples also
277 showed cubes with higher dimensions compared with RAW, and this fact could be explained by the
278 wide cell walls swelling in the HPP samples due to water absorption leading to a volume increase.
279 The most damaged samples appeared to be OHM (Figure 2), which the highest frequencies in the
280 latter classes (5.6, 2.36 and 3.35 mm). This result probably depended on cell membrane
281 electroporation as observed in the histological analysis and was linked to damages due to the
282 movements imposed by the pumping operation and to the higher flow rate compared with DIM. In
283 addition, the electroporation rate was reported to be directly related to product temperature (Lebovka,
284 Praporscic, Ghnimi, & Vorobiev, 2005). Thus, damages at the expense of the OHM samples could
285 be attributed both to the **faster** temperature increase of the solid particulates in ohmic heating
286 compared with DIM and to the high number of impacts between solids due to the high solid/liquid
287 ratio of the sample.
288 The textural parameters for all samples are reported in Table 1. The highest hardness values were
289 obtained for RAW samples as expected, followed by HPP and OHM and finally by DIM.
290 Tumpanuvatr et al. (2015) reported similar results on ohmic-treated pineapple with a good retention
291 of firmness compared with raw samples. In the same way, high-pressure processing was also reported
292 to reduce firmness of vegetable tissue due to turgor loss and cellular changes but to a lower extent
293 compared with thermal treatment (Oey et al., 2008). The textural data confirmed the results obtained
294 from the histological analyses. The DIM samples showed turgor loss and cell separation as well as
295 cell wall disruption which probably caused the lowest hardness value (Table 1) **(Li, Zhu, & Sun,**
296 **2018).** The chewiness values presented the same trend of hardness while other textural parameters
297 did not show any significant difference.
298 The colour parameters (Table 2) showed differences among samples for L* and b*, while no
299 differences were observed for a*. In particular, the samples treated by high-pressure showed the

300 lowest L* values compared with the other treatments. This finding was in agreement with that of
301 Denoya, Vaudagna, and Polenta (2015) and Denoya et al. (2016a), who observed an increase in the
302 cell permeability of HPP-treated peach with the consequent movement of water out of the cells,
303 resulting in translucent or watery characteristics. This fact, confirmed by the microstructural analyses
304 (Figure 1c), could explain the low values of L* in the HPP-treated samples (Table 2). The thermal
305 (DIM) and ohmic-treated samples (OHM) showed no significant difference compared with RAW in
306 terms of lightness. Thus, both DIM and OHM can be confirmed as suitable technologies for treating
307 food products with pieces in syrup and preserving the L* colour attribute. In addition, the b* values
308 of DIM and HPP samples significantly differed from RAW with lower values, leading to less
309 yellowness of the pineapple cubes. Although the heat exchange in the DIM treatment was enhanced
310 by protrusions, the time-temperature combination for obtaining the goal F-value at the centre of the
311 cubes probably played a role in the b* colour coordinate. For the HPP samples, the observed changes
312 in b* could be linked to the watery aspect reported above. Finally, the total colour differences (Table
313 2) showed that the highest ΔE was obtained for HPP, this parameter being the sum of differences of
314 the considered colour indicators (L*, a* and b*). The best technology for food products with
315 particulates appears to be the ohmic treatment, as previously reported (Varghese et al., 2014), due to
316 the very high and uniform heating rate. **However, the presence of metabisulphite could have**
317 **played an important role and further tests without any additive must be carried out**.
318
319 *3.3 PME residual activity and DPPH free-radical-scavenging capacity test*
320 The PME residual activity results directly linked to the extent of the applied treatment. DIM presented
321 the lowest residual activity as expected, with a mean value of 14.2 ± 1.1%, followed by OHM and
322 HPP (40.9 ± 3.6% and 81.7 ± 5.3%, respectively)(Figure 3).
323 The PME in pineapple was reported to be very resistant to traditional heat treatments (Castaldo et al.,
324 1997), and a residual activity ranging from 1.0×10^{-3} to 3.9×10^{-3} U/g was observed in pineapple cubes
325 in syrup. Thermal inactivation of PME in pineapple **cubes** was calculated by Cautela, Castaldo and

326 Laratta (2018) on pineapple juice; according to the reported D-value of D_{95}^{36}=45 s, the OHM treatment
327 yielded an inactivation equal to half of that given by DIM, consistent with the data in Figure 3.
328 Regarding the very low inactivation of PME in the HPP samples, the data are in accordance with
329 Terefe et al. (2014) reporting that PME forms are highly resistant to HPP and are at most partially
330 inactivated under commercially feasible pressure treatment conditions. The inactivation level of PME
331 in DIM can be considered adequate in accordance with Cautela, Castaldo and Laratta (2018) who
332 recommended a residual activity of approximately 10% in cloudy pineapple juice (1 decimal
333 reduction of PME activity). For the OHM samples, residual activity could represent a limiting factor
334 in product shelf-life even if further studies are required to evaluate the effects of PME residual activity
335 on cube firmness. Finally, even HPP could not reach the required inactivation; refrigerated storage is
336 reported to decelerate PME activity and to obtain an acceptable shelf-life for fruit pieces
337 (Dermesonlouoglou, Angelikaki, Giannakourou, Katsaros & Taoukis, 2019). However, the observed
338 residual activities in OHM and HPP could be leveraged by adding calcium to the syrup for enhancing
339 the firmness of fruit pieces (Anthon, Blot & Barrett, 2005).
340 The measures of total antioxidant capacity using the DPPH method were 23.2 ± 1.0 for RAW, 29.6
341 ±0.1 for DIM, 27.8 ± 0.2 for OHM and 26.5 ± 0.3 for HPP; the only significant difference was
342 observed between RAW and DIM, where there was an increase of antioxidant capacity after thermal
343 treatment probably **due to an higher diffusion of metabisulphite in fruit cubes thanks to**
344 **structural damages**. In general, all samples appeared to be quite stable to technological treatments
345 thanks to the metabisulphite solution which might have a protective effect for antioxidant compounds
346 (Aydin & Gocmen, 2015).
347

4. Conclusions

In this paper, the effects of high-pressure and ohmic heating on pineapple cubes in syrup were compared with those of conventional heat treatment. From the histological and sieve analysis, the OHM treatment seemed to be more related to cells and cell wall damage as well as a consistent worsening in the dimensional class distribution (higher presence of smaller pineapple pieces). Moreover, it resulted in a similar solid content of the syrup as the conventional treatment. Conversely, the HPP samples showed good technological results by better preserving both the original dimension and shape as well as the hardness of pineapple cubes. HPP also showed the highest colour modifications compared with the untreated samples, followed by conventional and ohmic treatments. Moreover, the relevant residual pectin methylesterase activity observed in OHM and HPP samples could be leveraged to improve the textural attributes of pineapple cubes. Further studies should be conducted under different treatment conditions to obtain a favourable balance of positive effects on pineapple cubes.

Declaration of competing interest

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502 **Table 1.** Textural parameters of raw and treated pineapple cubes.

	Hardness (N)	*Cohesiveness*	*Resilience*	*Springiness*	*Chewiness (N)*
RAW	69.2 (5.0) a	0.26 (0.06) a	0.115 (0.025) a	0.470 (0.043) a	9.64 (2.66) a
DIM	16.9 (3.6) c	0.21 (0.03) a	0.093 (0.017) a	0.438 (0.058) a	1.47 (0.31) c
OHM	29.0 (4.5) b	0.21 (0.08) a	0.108 (0.003) a	0.447 (0.037) a	2.31 (0.42) b
HPP	29.2 (3.3) b	0.22 (0.04) a	0.102 (0.021) a	0.430 (0.047) a	2.20 (0.74) b

503

504 [a, b, c] Same letters within each column do not significantly differ (n = 10; $p < 0.05$); standard deviation

505 given in parenthesis.

506

507 **Table 2.** Colorimetric parameters of raw and treated pineapple samples.

	*L**	*a**	*b**	*ΔE*
RAW	58.6 (1.5) a	-2.95 (0.27) a	15.5 (1.3) a	-
DIM	56.9 (0.7) a	-3.29 (0.21) a	12.7 (2.5) b	4.52 (0.31) b
OHM	57.7 (0.6) a	-3.11 (0.21) a	14.0 (2.6) ab	2.18 (0.50) c
HPP	53.2 (1.4) b	-3.13 (0.40) a	12.5 (1.6) b	6.71 (0.95) a

508

509 [a, b, c] Same letters within each column do not significantly differ (n = 16; $p < 0.05$); standard deviation

510 given in parenthesis.

Captions to figures

Figure 1. Transverse sections of pineapple samples subjected to different treatments and stained with Toluidine Blue: **A. raw (20x); B. DIM (20x); C. HPP (40x); D. OHM (100x).**

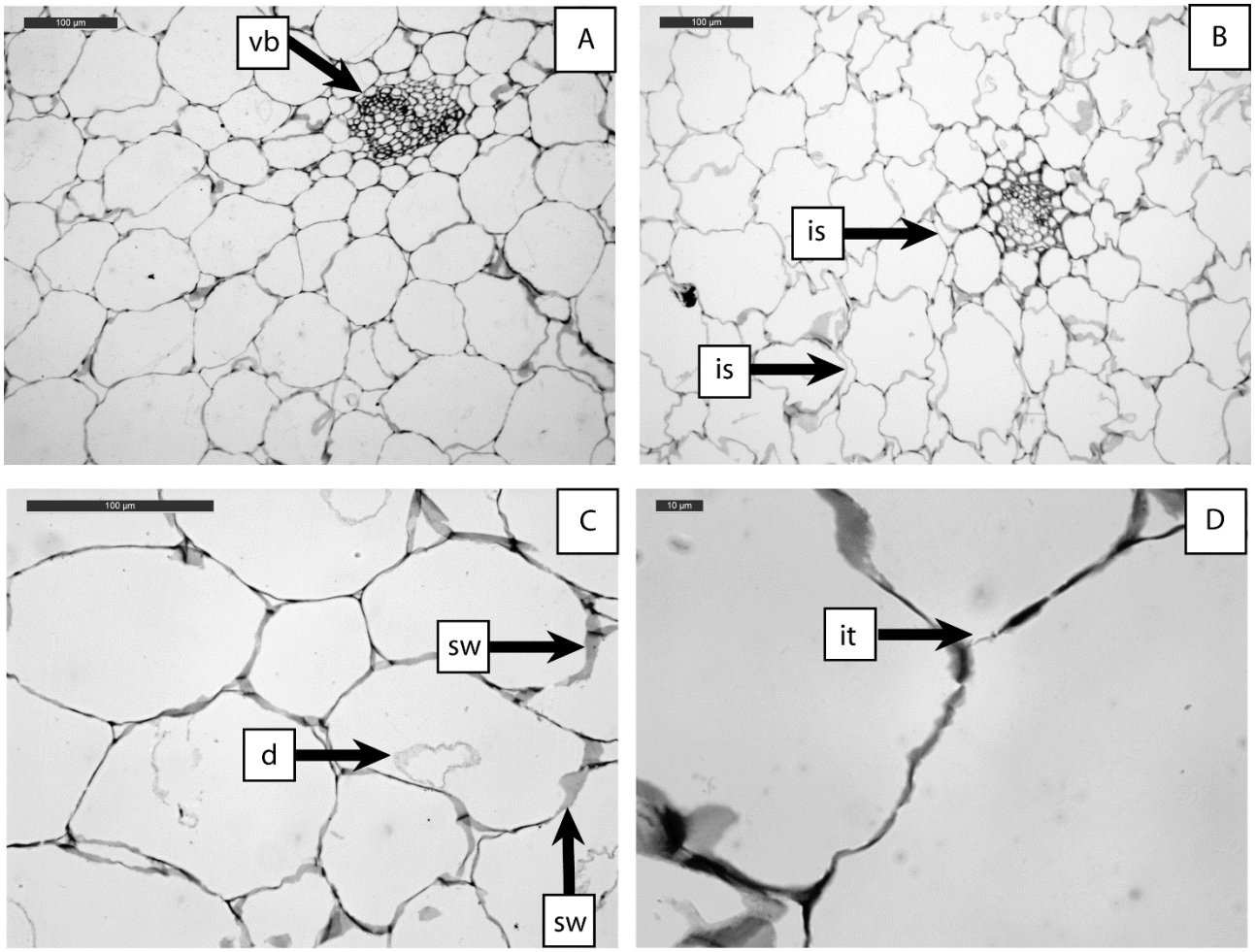
Legend: d: cellular dehydration; is: intercellular spaces; it: irregular thickness of the cell wall; sw: swelling; vb=vascular bundles.

Figure 2. Sieve analysis of RAW, DIM, OHM and HPP samples with relative abundances (%) for each dimensional class (2.36, 3.35, 5.6, 8.0 and 9.5 mm). **Different letters among the same dimensional class denote significant differ between samples ($p < 0.05$). Maximum RSD: 25.3, 5.4, 11.3, 20.8 and 12.5 % for 2.36, 3.35, 5.6, 8.0 and 9.5 mm sieve, respectively.**

Figure 3. PME activity (%) for DIM, OHM and HPP treated samples. Different letters significantly differ ($p < 0.05$).

537

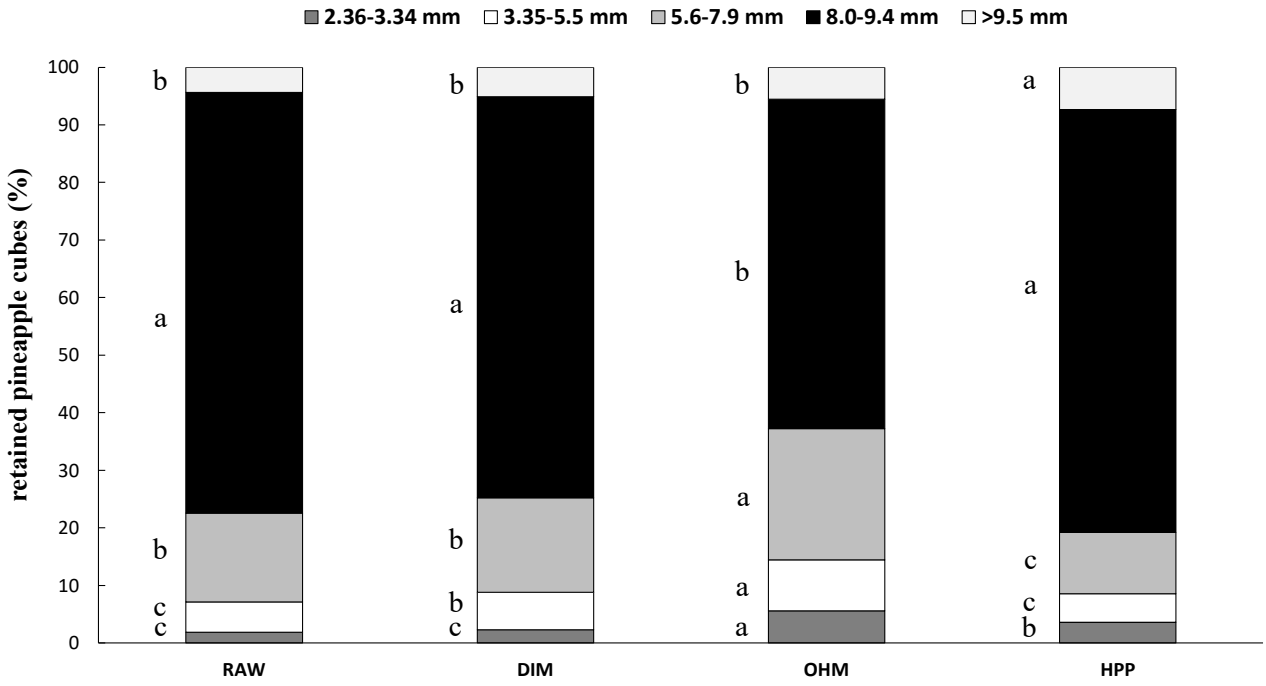
538 **FIG.1**



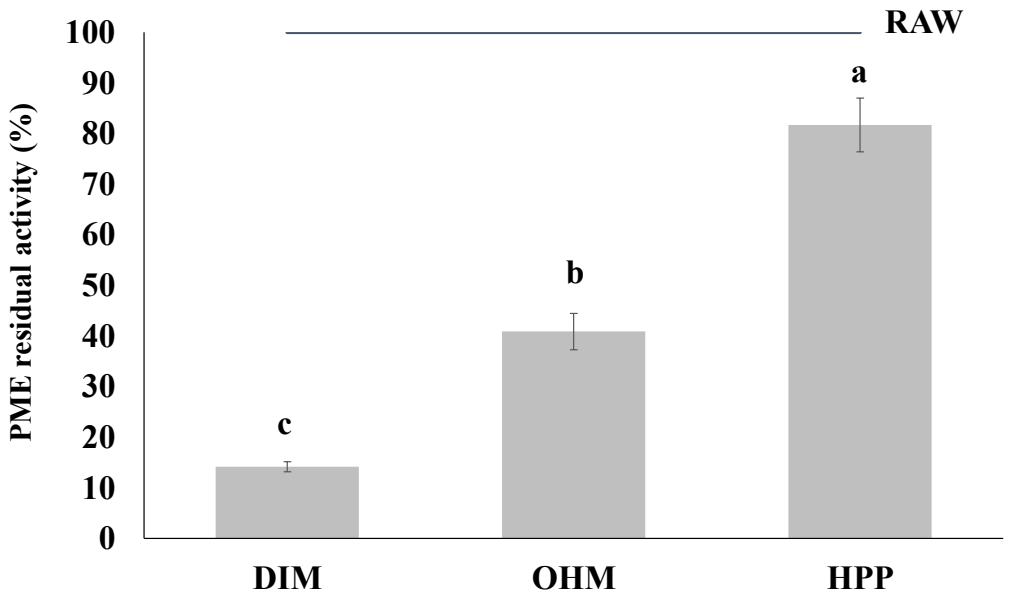
539

540

FIG. 2


2.36-3.34 mm
3.35-5.5 mm
5.6-7.9 mm
8.0-9.4 mm
>9.5 mm
retained pineapple cubes (%)
100
90
80
70
60
50
40
30
20
10
0
b
a
b
c
c
b
a
b
b
c
b
b
a
a
a
a
c
c
b
RAW
DIM
OHM
HPP

557 **FIG. 3**



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