European Food Research and Technology (2021) 247:475–484
https://doi.org/10.1007/s00217-020-03640-7

ORIGINAL PAPER

High pressure and thermal processing on the quality of zucchini slices

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Received: 2 September 2020 / Revised: 22 October 2020 / Accepted: 24 October 2020 / Published online: 9 November 2020

Abstract

In response to the market demand for low processed vegetables, high-pressure treatments (400,600 MPa; 1,5 min) were applied on zucchini slices and compared to a traditional blanching treatment. Histological observations, texture and color analysis, pectinmethylesterase (PME) and antioxidant (DPPH) activities were measured and compared to untreated samples. The histological observations revealed that the longer high-pressure treatments (5 min) led to more extended cell lysis and dehydration than the shorter ones (1 min) and blanching. High-pressure treatments resulted less effective than blanching on PME inactivation, with the best results obtained at 400 MPa for 1 min. Comparable texture parameters were observed for high-pressured and blanched samples. The negative correlation found between PME activity and the texture parameter 'distance of the first peak force' revealed an effect of PME on the texture recovery after treatments. High pressure led to a general browning of zucchini parenchyma and to DPPH drop. The correlations found between DPPH and color suggest the common nature of the phenomena. The influence of pressure and time on the studied parameters was revealed by two-way ANOVA. Principal component analysis clustered together the four high-pressure-treated samples, being clearly divided by blanched and untreated ones.

Keywords High pressure · Vegetables · Structure · Enzymatic activity · Antioxidant activity

Abbreviations

*a**	Greenness
ANOVA	Analysis of variance
2 W-ANOVA	Two-way ANOVA
*b**	Yellowness
BL	Blanching
C	Chroma
DistanceP1	Distance of the first peak force (mm)
DPPH	2,2-Diphenyl-1-picrylhydrazyl
F&V	Fruits and vegetables
Fp1	First peak force (N)
Fmax	Maximum puncture force (N)
h°	Hue angle
HP	High pressure
HPP	High-pressure processing
L	Lightness
PCA	Principal component analysis
PG	Polygalacturonase
PME	Pectinmethylesterase
POD	Peroxidase
PPO	Polyphenoloxidase
PVPP	Polyvinylpolypyrrolidone
TE	Trolox equivalents
TBO	Toluidine blue

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Introduction

Fruit and vegetables (F&V) consumption is mainly linked with a healthy diet [1]. Consumers, therefore, aim to purchase organoleptically inviting F&V with an adequate nutritional content [2]. Fresh F&V often meet this type of expectation, on the other hand, they have short shelf lives

and unpractical use, which do not fit well with the current lifestyles.

To meet the current market needs, novel non-thermal technologies are being studied [3, 4] with the aim to stabilize the products avoiding the detrimental effect of temperature [5]. In this context, high-pressure processing (HPP), applying pressure in the range between 100 and 1000 MPa for small periods of time, can deactivate pathogens and spoiling microorganisms, avoiding the detrimental effect of temperature [6].

In the field of F&V, HPP has mainly being applied on juices [7], beverages [8], purée [9] or sauces [10]. The effect of this technology on the quality of whole or pre-cut F&V is less debated.

Paciulli et al. [11], observed that high-pressure treatments at 200, 400 and 600 MPa for 5 min softened pumpkin cubes less than a thermal treatment at 85 °C for 5 min. Modifications of cell morphology, membrane integrity and pectin properties were considered responsible for this change. During the two months of storage, the same authors observed further texture loss for the HP-treated pumpkins, associated to residual pectinmethylesterase (PME) activity. The barotolerance of some F&V spoiling enzymes has been reported by many authors [12]. The main enzymes related to F&V consistency are polygalacturonase (PG) and PME, their synergistic activity leads to tissue softening during ripening. While PG appears to be one of the most pressure-labile enzymes, PME showed high-pressure resistance. Shook et al. [13] found no PG activity in tomato dices treated at 800 MPa for one minute, on the contrary, no inactivation of PME was observed in the same conditions. Polyphenol oxidase (PPO) and peroxidase (POD) are the two main enzymes responsible for color change in F&V, leading also to loss of some bioactive compounds [12]. Many of the PPOs investigated are extremely resistant to high-pressure inactivation, while PODs resistance varies depending on the source [12]. The relation between enzymatic activity and color has been highlighted by many authors, especially during storage [14]. However, as covalent bonds are not affected by pressure, HPP was found to be less detrimental than thermal processes to low-molecular-weight molecules like pigments. Krebbers et al. [15] showed that the green color of green beans becomes even more intense (decrease in L^*, a^* and b^* values) after HPP at 500 MPa for 1 min, however, at elevated temperature, the color shifted visibly to olive green. Paciulli et al. [16] showed that blanching affected blueberries color more than HPP at 400 and 600 MPa for 1 and 5 min. HPP may yield to more intense colors or bioactive activities, because of the leakage of small molecules, such as pigments or health-related compounds, from the broken cells. Castro et al. [17] applying HP to green and red peppers observed a better retention of ascorbic acid than on blanched samples, particularly for red peppers, that showed even an increase. Similarly, Paciulli et al. [18], found a better extraction of betanins, phenols and ascorbic acid from beetroots slices treated at 650 MPa from 3 to 30 min in comparison to blanched ones. On the other hand, McInerney et al. [19] did not find any difference in carotenoid content among untreated and HP-treated (400 and 600 MPa/2 min) carrots, green beans and broccoli.

To the best of our knowledge, the application of HPP on zucchini slices has been tested only once [20]. Due to the significant softening and quality degradation of the thermal treated controls, no comparison was possible, thus no deeper investigations were conducted.

This study aims to investigate the effect of HP treatments at 400 and 600 MPa for 1 and 5 min on the quality parameters of zucchini slides.

Materials and methods

Sample preparation

Zucchini (*Cucurbita pepo L*, cv. Nero di Milano) was purchased from a local market in Parma, Italy. Zucchini with a diameter of 30 ± 0.1 mm was washed and drained, the upper and the lower parts were removed, and the samples were cut, using a slicer, into slices of 8 mm thickness.

Treatments

Untreated zucchini (R) was subjected to five different treatments, as described:

Blanching (BL)

In accordance with Paciulli et al. [21], zucchini slices were immersed in a water bath (sample/water ratio of 1:5) at 90 ± 2 °C for 2 min. The treatment was conducted in triplicate.

High-pressure (HP) treatments

HP treatments at 400 and 600 MPa, both for 1 and 5 min (HHP400-1; HHP400-5; HHP600-1; HHP600-5) were carried out using a 300 L high-pressure plant (Avure Technologies Inc.), at "HPP Italia" of Traversetolo (Italy), using cold water (4 °C) as pressure medium. All the HP treatments were conducted at room temperature (~ 20 °C), considering a temperature increase due to compression, not higher than 2–3 °C/100 MPa. For the HP treatments, zucchini slices were vacuum-sealed in flexible (75 mm thickness) plastic pouches (Ultravac Solutions, Kansas City, MO, USA). Three pouches were processed and analyzed for each treatment condition.

Moisture content

Five g of untreated and treated ground samples was dried in a convection oven (ISCO NSV 9035, ISCO, Milan, Italy) at 105 °C for at least 16 h until constant weight [22]. The analysis was conducted in triplicate.

Histological analysis

As reported in detail by Paciulli et al. [16] for blueberries, untreated and treated zucchini slices were first fixed in FAA solution, then dehydrated with increasing alcohol concentrations solutions, thus included in methacrylate resin. The resulting blocks were sectioned in 4 µm thickness slices then stained with Toluidine Blue (TBO) or with a FeSO4 solution for the analysis of Tannins [23]. Four pieces of each vegetable and each treatment were analyzed.

Sections were observed with a Leica DM 4000B optical microscope (Leica Imaging Systems Ltd., Wetzlar, Germany) equipped with a Leica DMC 2900 digital camera (Leica Imaging Systems Ltd., Wetzlar, Germany). The measurements were done by the image analysis system LAS v4.10.0 (Leica Application Suite, Wetzlar, Germany) using a manual configuration. At least ten slides carried ten zucchini sections each, for each condition was observed.

Pectin methylesterase (PME) activity

The test was performed following the method described by Paciulli et al. [16]. The enzyme, extracted with a solution of 1 M NaCl containing 1% polyvinylpolypyrrolidone (PVPP), was mixed with 0.5% pectin, 0.01% bromothymol blue and water. The progressive discoloration of the blue solution, because of the enzymatic activity, was monitored every 15 s at 620 nm for two minutes by a Perkin Elmer UV–Visible spectrophotometer. PME activity was calculated from the slope of the linear segment absorbance–time, measuring the percentage variation of the treated samples in comparison to the untreated one. The analysis was conducted in triplicate.

Texture analysis

Texture was analyzed using a TA.XT2i Texture Analyzer equipped with a 25 kg load cell (Stable Micro Systems, Godalming, UK), at a trigger force of 0.01 N. Puncture test was performed using a 2 mm diameter stainless steel needle probe, driven up in a radial direction to the center of the samples at a speed of 3 mm s^{-1}, following the method of Paciulli et al. [21]. First peak force (F_{p1}, N) and maximum puncture force (F_{max}, N) were extracted from the force *vs* time curves. The distance of the first peak force ($Distance_{P1}$, mm), was measured as the space travelled by the needle probe from the contact with the epidermis up to the breaking point. These parameters were quantified using the application software provided (Texture Exponent for Windows, version 6.1 10.0). 10 vegetables were analyzed for each treatment.

DPPH free radical scavenging activity test

The test was performed according to Paciulli et al. [16]. The antioxidant molecules, extracted in methanol/water (70:30 v/v) solution, were mixed with the DPPH methanolic solution (0.2 mM) and kept in the dark for 30 min. The absorbance was recorded at 517 nm by a Perkin Elmer UV–Visible spectrophotometer. The radical scavenging activity (µmol Trolox eq./g dw) was calculated by fitting the values of I% (I% = [(Abs0 − Abs1)/Abs0] × 100, where Abs0 was the absorbance of the blank and Abs1 was the absorbance of the sample) in a standard curve absorbance *vs* concentration of Trolox methanolic solutions. Analyses were performed in triplicate.

Color

Color of zucchini slices was measured both on skin and parenchyma by means of a Minolta Colorimeter (CM2600d, Minolta Co., Osaka, Japan) equipped with a standard illuminant D65 and 10° position of the standard observer. The instrument was calibrated before each analysis with white and black standard tiles. L*, *a**, *b**, C, and *h*° [24] were quantified on each sample. ΔE of the treated zucchini was calculated in comparison to the untreated ones. 10 determinations were performed for each treatment.

Statistical analysis

SPSS statistical software (Version 25.0, SPSS Inc., Chicago, IL, USA) was used to perform one-way analysis of variance (ANOVA) among all the different treated samples and two-way (2 W) ANOVA among the HPP treated samples, using pressure and time as independent variables. LSD post hoc test at a 95% confidence level ($p \leq 0.05$) was used to further identify differences among treatments. Pearson correlation was performed to evidence relations among the variables ($p < 0.05$; $p < 0.01$).

Principal component analysis (PCA) was performed using normalized variables, as reported by Medina-Meza et al. [25]. Only parameters with factor loadings higher than 0.70 were used for this analysis.

Results and discussion

Histological analysis

Untreated zucchini

In Fig. 1a and b, the microstructure of untreated zucchini is shown. The external parenchyma of *Cucurbita pepo* L., cv. Nero di Milano is composed by a single layer of epidermis tissue (mean thickness: 21.1 µm) in which, the cells are covered by an abundant cuticle. Hypoderm (the sub-epidermal tissue) is instead composed by isodiametric, thickened cells, with few intercellular spaces.

Figure 1a and b show the structure of zucchini mesocarp. External mesocarp resulted composed by cells of different dimensions, from the smaller in subderm (12.5 µm), to the larger in central mesocarp (38 µm). The external mesocarp showed high degree of cell-to-cell contact throughout the tissue, with scarce intercellular spaces. The cells of the middle mesocarp had tiny walls and were more separated from each other than in the external mesocarp. Inner mesocarp consisted of a parenchyma composed of larger cells (more than 65 µm). Endocarp resulted organized in large irregular cells; those observations were in accord with the study of Paciulli et al. [21], on zucchini.

In untreated samples, the observation of transverse section stained with Tannins Solution, did not show the presence of tannin inclusions.

Blanched zucchini

After blanching (Fig. 1c and d), the structure showed only slight variations in comparison to untreated; inner parenchyma cells showed weak dehydration (Fig. 1d). In Fig. 1d, it is possible to observe the presence of wide intercellular spaces and cell separation due to the thermal treatment. The same phenomenon was also observed on other vegetables both blanched [21] or differently cooked [26]. Focusing on tannins, as for untreated samples, after staining with Tannins Solution, their presence was not observed.

HPP400-1

Zucchini slices treated at 400 MPa for one minute did not show significant histological changes, compared to the untreated samples (Fig. 2a). From Fig. 2a, it is possible to observe swelling of cellular walls (in epidermis and subdermis) and a mild dehydration. Dehydration was also observed by Araya and collaborator [27] after HPP treatments at 100, 200 and 300 MPa on carrots, with an initial temperature of 20 °C. The use of Tannins Solution stain on zucchini sections, highlighted the presence of scarce quantities of tannins mainly in the endocarp and near the seeds.

HPP-400-5

HPP treatment at 400 MPa for 5 min, did not damaged significantly the zucchini microstructure (Fig. 2b). The

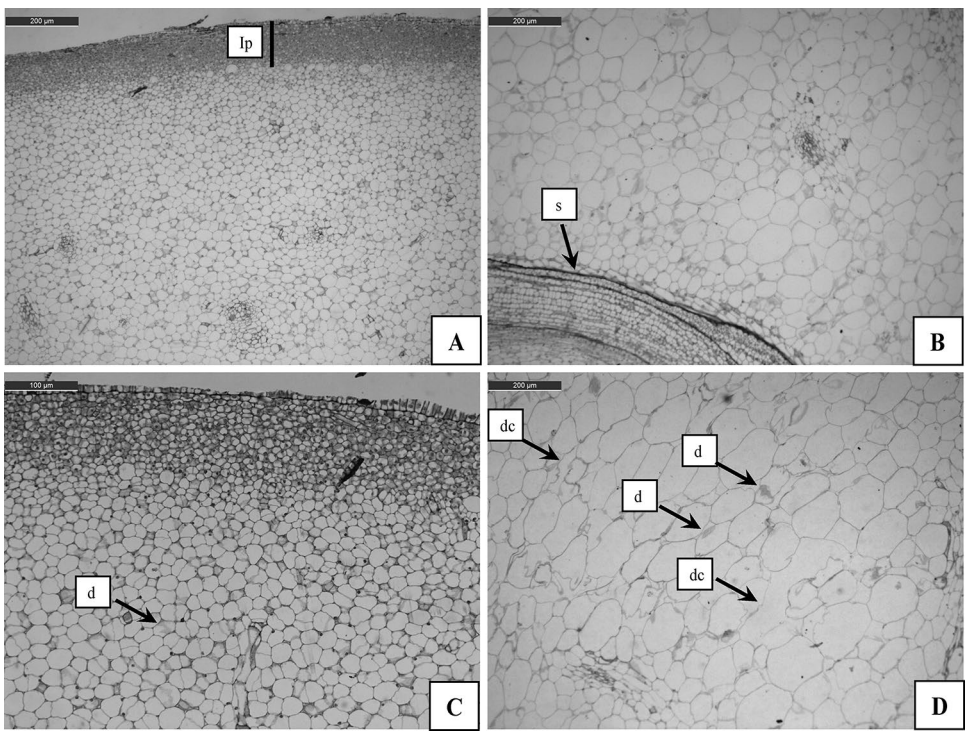

Fig. 1 Transverse sections of zucchini samples stained with Toluidine Blue O: **a** magnification of external tissue (epi- and hypodermis) of untreated/uncooked samples; **b** magnification of internal tissue (mesocarp and endocarp) of untreated/uncooked samples; **c** magnification of external tissue (epi- and hypodermis) of blanched samples; **d** magnification of internal tissue (mesocarp and endocarp) of blanched samples. Ip = hypodermis; s = seed; d = dehydrated cells; dc = detached cells

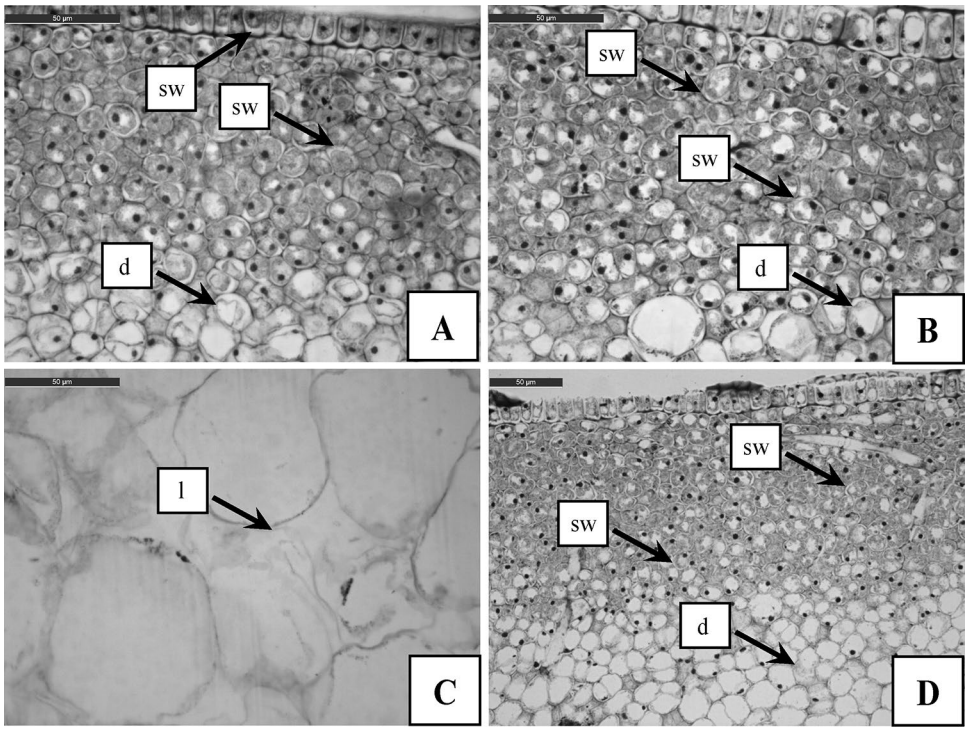

Fig. 2 Transverse sections of zucchini samples stained with Toluidine Blue O: **a** magnification of external tissue (epi- and hypodermis) of sample treated with 400 MPa for 1 min; **b** magnification of external tissue (epi- and hypodermis) of sample treated with 400 MPa for 5 min; **c** magnification of endoderm tissue of sample treated with 400 MPa for 5 min; **d** magnification of external tissue (epi- and hypodermis) of sample treated with 600 MPa for 5 min. d = dehydrated cells; sw = Swelling of cell walls; l = lacuna in the tissue due to the HPP treatment

changes were similar to those observed after 1 min treatment (swelling of cellular wall in epidermis and subdermis tissues), even if a more intense dehydration than HPP 400-1 was observed, being also more uniformly distributed in all the tissues. Inner parenchyma showed the major damages in comparison with the other tissues, with evidence of cellular walls lysis (Fig. 2b and c). Tannin Solution stain evidenced the presence of tannins inclusion only near zucchini's seeds, as observed for HPP400-1.

HPP-600-1

The treatment at 600 MPa for 1 min reflected the same changes observed for HPP400-1, both for the microstructure of tissues and for the presence of tannins.

HPP600-5

Zucchini treated at 600 MPa for 5 min showed similar effects on microstructure than those observed after the treatment at 400 MPa for 5 min. From the microscopic observations (Fig. 2d), swelling of the cellular walls and cell walls breakage of the inner parenchyma larger cells was observed. Moreover, diffuse dehydration of the tissue was highlighted. The analysis of tannins showed the same trend of HPP400-1.

PME activity

From Table 1, it is visible how blanching reduced zucchini PME activity to 18%, in comparison to untreated samples. This result confirms the strong effect of thermal treatments on this class of enzymes. High-pressure treatments resulted less effective than blanching on PME inactivation (Table 1). In particular, HPP400-1 and HPP400-5 resulted, respectively, the most and less effective among the studied treatments, leading, respectively, to 27% and 14% PME inactivation. Moreover, while longer treatment time at 400 MPa led to lower PME inactivation, at 600 MPa the opposite trend was observed: the longer the exposure time, the higher the enzyme inactivation. Therefore, the 2 W-ANOVA showed a significant interaction between treatment time and applied pressure (Table 1). The high PME baroresistance was already reported in other studies, showing a high species-specific behavior. Paciulli et al. [16], treating blueberries at 400 and 600 MPa for 1 and 5 min observed the same trend of this study, with HHP400-1 as the most effective high-pressure treatment, leading to a PME inactivation of around 35%. Sila et al. [28], treating shredded carrots with HPP, observed an increase of PME activity up to 400 MPa and a further decrease up to 600 MPa. This behavior has been related to reversible configuration of the enzyme and/or substrate. The PME activity led to the formation of Low Methoxyl Pectin, known to form stable gels under high-pressure treatments [14]. The swollen cell walls, observed by the

histological analysis on the high-pressure-treated zucchini, may be related with the formation of this gel network.

Texture

The texture parameters are reported in Table 2. The differences observed between samples cannot be attributable to water loss, since the water content was not affected by the treatments (Table 1). They can be related to tissues modifications and residual enzymatic activity (Paragraphs 3.1 and 3.2).

The first peak force (F_{P1}), related to the resistance opposed by the external cell layers to needle penetration, was not affected by BL in accordance with the histological observations (Fig. 1c and d; Par. 3.1) and with a previous study conducted in the same conditions [21]. Among the HP treatments, HPP400-1 resulted the most influencing one, with F_{P1} drop of around 15% in comparison to R. The 2 W-ANOVA, conducted among the HP-treated samples, revealed a dependence of F_{P1} from pressure and time, with HPP600-5 showing the highest values (0.49 ± 0.03 N).

$Distance_{P1}$ underwent a significant increase compared to *R*, for all the studied treatments. An increase of the distance may be described as a general decrease of the tissue crispiness [29]. BL, HPP400-1 and HPP600-5 increased $Distance_{P1}$ of around 78% in comparison to *R*, while for HPP400-5 and HPP600-1, the increase was around 72%. A negative correlation was found between $Distance_{P1}$ and PME activity ($R = -556$; $p < 0.05$). These results confirmed the effect of PME on texture retention after treatments, due to the tendency of demethylated pectin to form crosslinks in presence of divalent cations, such as calcium [30]. $Distance_{P1}$ is influenced not only from epidermis strength, but also from the integrity of the subdermis cells; the swollen cell walls observed after HPP treatments (Par. 3.1), probably due to the PME gelling phenomena, may have F_{max}, which represents the resistance opposed by the inner tissues to the needle penetration, was reduced of around 70% after all the studied treatments in comparison to R, despite the different phenomena were observed after blanching (cell separation) or HPP treatments (cell lysis) on the inner parenchyma.

Color

Color of zucchini, mainly related to chlorophylls and carotenoids, resulted affected by all the studied treatments, more on parenchyma than epidermis, as revealed by the higher ΔE values (Table 3).

Blanching enhanced the green epidermis color (lower a^*, higher *C* and $h°$) in comparison to *R*, keeping almost unaltered the color of parenchyma. Same results were observed by Mazzeo et al. [31] on zucchini slices blanched in the same conditions. The color changes were attributed to modifications of the surface reflecting properties, due to air expulsion and its replacement with water and cell juices.

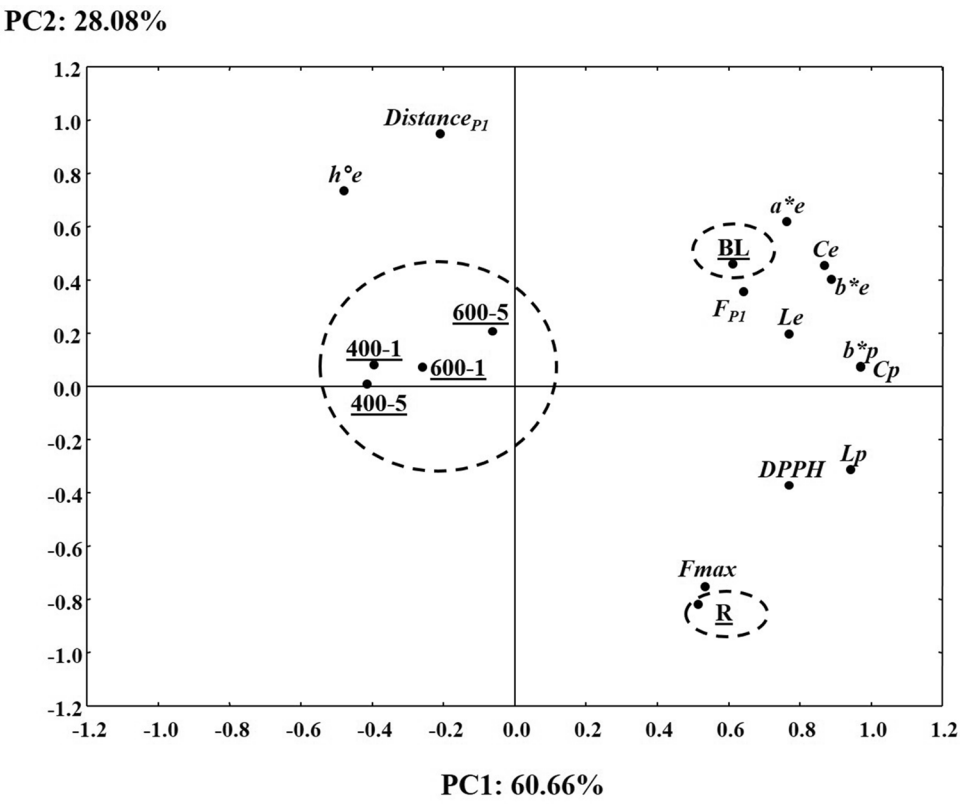

Fig. 3 Principal Component Analysis (PCA) results. Projection of the variables and of the cases on the factor plane (1 × 2). Abbreviations: R = untreated/untreated; BL = blanched; 400-1 = HHP at 400 MPa for 1 min; 400-5 = HHP at 400 MPa for 5 min; 600-1 = HHP at 600 MPa for 1 min; 600-5 = HHP at 600 MPa for 5 min; F_{P1} = first peak force (N); $Distance_{P1}$ = distance of the first peak force (mm); F_{max} = absolute maximum force (N); Lp = lightness parenchyma; Le = lightness epidermis; a^*e = greenness epidermis; b^*p = blueness/yellowness parenchyma; b^*e = blueness/yellowness epidermis; Cp = chroma parenchyma; Ce = chroma epidermis; $h°$e = hue angle epidermis; PC = Principal Component

HPP treatments led to general darkening both on zucchini epidermis and parenchyma, as revealed by the lower *L*, *b** and *C* values other than the higher *h*°, in comparison to *R*. The *a** parameter resulted not affected by the HPP treatments. Looking at Δ*E* values, the parenchyma color resulted much more affected by HPP than blanching. Moreover, the 2W-ANOVA showed an influence of the pressure level on almost all the color parameters, with 600 MPa resulting less affecting than 400 MPa. F&V browning was already reported by other authors and related to the still high, or even enhanced, PPO activity after HPP [32]. Phenolic compounds are substrates of plant PPO, enzyme that produces brown polymers, generally leading to a decrease in visual and healthy quality.

DPPH

The radical scavenging activity of zucchini is mainly attributable to the presence of polyphenols, ascorbic acid and carotenoids, such as lutein [33]. The DPPH assay revealed how blanching did not affected the antioxidant activity of zucchini, while HPP led to a significant reduction in comparison to *R* (Table 1). The retention of bioactive molecules after zucchini blanching was already observed by Mazzeo et al. [31].

The significant DPPH reduction after HPP may be related to still high enzymatic activities of the oxidative enzymes PPO and POD, as already reported by other authors [34]. The phenomenon is the same that leads to color browning, as already discussed in the previous paragraph. In support of this hypothesis, high correlations between *L*, *b**, *C* and *h*° with DPPH were found, both on epidermis and parenchyma (*L* epidermis $R=0.554$ $p<0.05$, parenchyma $R=0.849$ $p<0.01$; *b** epidermis $R=0.636$ $p<0.01$, parenchyma $R=0.645$ $p<0.01$; *C* epidermis $R=0.596$; $p<0.01$; parenchyma $R=0.645$; $p<0.01$; *h*° parenchyma $R=-0.564$; $p<0.05$), suggesting oxidation both of pigments and antioxidant molecules as effect of the oxidative enzymes.

Table 1 Water content and antioxidant activity of untreated and treated zucchini

	C	BL	400-1	400-5	600-1	600-5	*P*	*t*	Pxt
PME (%)	100 ± 0.0 a	18.5 ± 3.9 d	73.1 ± 1.1 c C	86.0 ± 7.5 b A	83.8 ± 1.7 bc AB	79.0 ± 6.7 bc B	n.s	n.s	*
Water content (%)	94.4 ± 0.0 a	94.9 ± 0.2 a	95.0 ± 0.4 aA	94.5 ± 0.0 aB	94.6 ± 0.0 aB	94.6 ± 0.1 aB	n.s	n.s	n.s
DPPH (μmol TE g^{-1}_{dw})	6.84 ± 1.24 a	6.29 ± 1.65 a	3.08 ± 0.63 b A	1.96 ± 0.59 b B	1.71 ± 0.38 b B	3.22 ± 0.43 b A	n.s	n.s	*

[a]Data are expressed as means ± standard deviations of 3 samples. Means in rows followed by different lowercase letters are significantly different ($p \le 0.05$) according to the one-way ANOVA ($p \le 0.05$). Means in rows followed by different uppercase letters (only for high pressure treated samples) are significantly different according to 2W-ANOVA ($p \le 0.05$), performed considering pressure and time as independent variables. Abbreviations: *R*, untreated/untreated; BL, blanched; 400-1; HHP at 400 MPa for 1 min; 400-5, HHP at 400 MPa for 5 min; 600-1, HHP at 600 MPa for 1 min; 600-5, HHP at 600 MPa for 5 min; TE, Trolox equivalents

Table 2 Texture parameters for untreated and treated zucchini

	F_{P1} (N)			Distance$_{P1}$ (mm)			F_{max} (N)		
R	0.47 ± 0.05 a			0.20 ± 0.03 c			4.61 ± 0.49 a		
BL	0.47 ± 0.03 a			0.88 ± 0.09 ab			1.25 ± 0.11 b		
HHP400-1	0.40 ± 0.03 b C			0.90 ± 0.19 a A			1.38 ± 0.16 b A		
HHP400-5	0.45 ± 0.03 ab B			0.72 ± 0.11 b B			1.34 ± 0.11 b A		
HHP600-1	0.44 ± 0.06 ab B			0.72 ± 0.15 b B			1.41 ± 0.22 b A		
HHP600-5	0.49 ± 0.03 a A			0.85 ± 0.18 ab A			1.33 ± 0.17 b A		
	P	*t*	Pxt	*P*	*t*	Pxt	*P*	*t*	Pxt
	*	*	n.s	n.s	n.s	*	n.s	n.s	n.s

Data are expressed as means ± standard deviations of 10 samples. Means in columns followed by different lowercase letters are significantly different according to the one-way ANOVA ($p \le 0.05$). Means in columns followed by different uppercase letters (only for high pressure treated samples) are significantly different according to 2 W-ANOVA ($p \le 0.05$), performed considering pressure and time as independent variables. The p values were corrected for multiple comparisons use LSD method. Abbreviations: *R*, untreated; BL, blanched; HHP400-1; HHP at 400 MPa for 1 min; HHP400-5, HHP at 400 MPa for 5 min; HHP600-1, HHP at 600 MPa for 1 min; HHP600-5, HHP at 600 MPa for 5 min; F_{P1}, maximum force first peak; Distance$_{P1}$, distance at the maximum of the first peak; F_{max}, absolute maximum force; *P*, pressure; *t*, Time; n.s., non-significant

Table 3 Color parameters for untreated and treated zucchini

	L			*a**			*b**			*C*			*h*°			Δ*E*		
Zucchini epidermis																		
R	36.35 ± 2.82 a			− 4.77 ± 1.05 a			8.96 ± 2.59 b			10.15 ± 2.78 b			118.50 ± 2.33 c					
BL	35.93 ± 1.98 b			− 8.19 ± 1.20 b			12.94 ± 2.34 a			15.33 ± 2.56 a			122.50 ± 2.18 b			5.69 ± 2.40 a		
400-1	33.49 ± 2.62 b A			− 4.59 ± 0.96 aAB			6.81 ± 1.98 cAB			7.97 ± 2.37 cAB			126.04 ± 3.19 aA			4.72 ± 1.08 aAB		
400-5	33.51 ± 3.29 b A			− 4.02 ± 0.72 aA			5.42 ± 1.12 cB			6.75 ± 1.32 cB			126.74 ± 1.78 aA			5.61 ± 1.06 aA		
600-1	32.56 ± 2.04 b A			− 4.71 ± 1.17 aAB			6.65 ± 2.22 cAB			8.16 ± 2.49 cAB			125.82 ± 2.11 aA			5.17 ± 1.16 aA		
600-5	33.78 ± 2.36 b A			− 5.04 ± 0.36 aB			7.27 ± 1.56 cA			8.85 ± 1.75 cA			125.02 ± 2.29 bA			3.86 ± 1.66 aB		
	P	*t*	Pxt	*P*	*t*	Pxt	*P*	*t*	Pxt	*P*	*t*	Pxt	*P*	*t*	Pxt	*P*	*t*	Pxt
	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	*
Zucchini parenchyma																		
R	85.47 ± 1.19 a			− 2.63 ± 0.28 a			22.81 ± 1.52 a			22.96 ± 1.52 a			96.58 ± 0.70 cd					
BL	77.02 ± 3.68 b			− 2.28 ± 0.55 a			24.45 ± 1.68 a			24.57 ± 1.69 a			95.32 ± 1.16 d			8.78 ± 3.86 c		
400-1	50.93 ± 3.17 d C			− 2.40 ± 0.49 a AB			13.29 ± 1.72 c B			13.51 ± 1.74 c B			100.25 ± 1.83 a A			35.90 ± 2.76 aA		
400-5	51.29 ± 3.49 d BC			− 2.06 ± 0.42 a B			14.28 ± 2.56 c B			14.43 ± 2.58 c B			98.22 ± 1.12 b B			35.33 ± 3.38 aA		
600-1	53.80 ± 2.65 c C			− 2.54 ± 0.39 a A			17.44 ± 2.26 b A			17.64 ± 2.23 b A			98.41 ± 1.67 b B			32.19 ± 2.73 bB		
600-5	57.20 ± 4.33 c A			− 2.29 ± 0.46 a AB			19.10 ± 2.46 b A			19.24 ± 2.46 b A			96.87 ± 1.24 cd C			28.63 ± 4.23 bC		
	P	*t*	Pxt	*P*	*t*	Pxt	*P*	*t*	Pxt	*P*	*t*	Pxt	*P*	*t*	Pxt	*P*	*t*	Pxt
	*	n.s	n.s	n.s	*	n.s	*	n.s	n.s	*	n.s	n.s	*	*	n.s	*	n.s	n.s

[a]Data are expressed as means ± standard deviations of 10 samples. Means in columns followed by different lowercase letters are significantly different according one-way ANOVA ($p \leq 0.05$). Means in columns followed by different uppercase letters (only for high pressure treated samples) are significantly different according to 2W-ANOVA ($p \leq 0.05$), performed considering pressure and time as independent variables. Abbreviations: *R*, untreated; BL, blanched; 400-1; HPP at 400 MPa for 1 min; 400-5, HPP at 400 MPa for 5 min; 600-1, HPP at 600 MPa for 1 min; 600-5, HPP at 600 MPa for 5 min

A combined effect of pressure and time was showed by the 2W-ANOVA, indicating HPP400-5 and HPP600-1 as the most affecting treatments, which led to more than 70% antioxidant activity reduction.

Principal component analysis

Figure 3 shows the results obtained from the Principal Component Analysis (PCA). Among all the studied dependent variables, the factor analysis excluded first peak force (FP1), parenchyma color parameters a^* and h° and PME activity values, having score values lower than 0.7. Eleven variables were selected and the first two principal components (PC) explained 88.74% of the total variance. The projection on the factorial space enabled the discrimination among the treatments: *R*, BL and HPP. *R* samples with negative factor loadings on PC2, resulted especially described by higher F_{max}. BL samples, which clustered alone with positive factor loadings on PC2, resulted well described from the epidermis color parameters. On the other hand, the parenchyma color parameters, especially Lp, and the DPPH values resulted to join R and BL samples. All the HP-treated samples clustered together, showing negative factor loadings on PC1, being however located in a middle zone on PC2. The variables that better described the HP treatments were epidermis color parameter h° and texture parameter $Distance_{P1}$. From the PCA results, on the base of the selected variables and processing conditions, HP-treated samples may be considered a middle way between the raw and blanched ones.

Conclusion

The effect of HP on zucchini slices is discussed here for the first time. In comparison to blanching, the use of HP at 400 and 600 MPa for 1 and 5 min had a stronger impact on zucchini quality. The 2W-ANOVA, conducted among the four HP treatments, showed a combined effect of pressure and time on almost all the studied parameters. These results revealed as less advantageous the treatments 400-5 and 600-1, except for texture, which showed a better retention in those conditions, in relation to the high PME activity. The parenchyma color parameters resulted instead more affected by pressure, turning to darker color the zucchini treated with the strongest conditions. In view of these considerations, and being the tissue damages more extended on the 5 min HP-treated samples, HPP 400-1 resulted best solution for the HP treatment of zucchini. On the other hand, PCA did not find any difference among the four HP treatments, clustering them in the same group. Starting from these findings, a future investigation for the treatment of zucchini slices may be the combination of low pressures and short times using temperature as additional variable.

Author contributions MP: Conceptualization; Data curation; Investigation; Methodology; Writing—original draft;. TG: Conceptualization; Formal analysis; Investigation; Methodology; Writing—review & editing. IGMM: Conceptualization; Data curation; Methodology; Writing—review & editing. MR: Conceptualization; Methodology; Supervision; Validation. MR: Formal analysis; Investigation; Methodology. MM: Conceptualization; Methodology; Resources; Supervision. EC: Conceptualization; Project administration; Writing—review & editing.

Funding Open access funding provided by Università degli Studi di Parma within the CRUI-CARE Agreement. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Compliance with ethics requirements This study does not contain any studies with human or animal subjects.

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