Journal of Food Measurement and Characterization
https://doi.org/10.1007/s11694-024-02401-4

ORIGINAL PAPER

Characterization of an optimized hot water treatment for eggplant as a non-chemical mean to maintain postharvest quality: validation of its effect on bioactive compounds and antioxidant capacity

Denisse Aurora Díaz-Corona[1] · Martha Edith López-López[1] · Lidia Elena Ayón-Reyna[1] · José Caro-Corrales[1] · Roberto Gutiérrez-Dorado[1] · Pedro de Jesús Bastidas-Bastidas[2] · Misael Odín Vega-García[1]

Received: 19 October 2023 / Accepted: 22 January 2024

Abstract

The objective of this study was to assess the effect of an optimized hot water treatment (OHWT) on the conservation of fresh eggplants (*Solanum melongena* L.), which was carried out through the Response Surface Methodology (RSM). Activity of pectin methylesterase (PME), color difference and firmness parameters, as well as the anthocyanin content were optimized according to a rotatable central composite design [process variables: temperature (30–50 °C) and time (5–15 min)]. The RSM results showed an activation on PME activity (1.333–2.922 U/g FW) along with a retention on the quality attributes and optimized conditions of 40 °C and 15 min. In addition, significant differences in bioactive compound contents were observed between treatments, where a greater amount of total phenolic (17.76 mg GAE/100 g) and chlorogenic acid (5.9715 µg CA/mg ME), and fewer anthocyanins were found at OHWT compared to control. Therefore, the application of the optimized conditions (40 °C, 15 min) would be the most suitable to maintain the quality of fresh eggplants.

Keywords Hydrothermal treatment · Optimization · *Solanum melongena* · Pectin methylesterase · Phenolic compounds

Introduction

Eggplant (*Solanum melongena* L.) is a member of the *Solanaceae* family which is generally cultivated as a vegetable in sub-tropical and tropical regions of the world [1]. It is an economically important crop with a wide diversity of shapes, sizes and colors [2]. According to diverse reports, eggplants are mostly distributed as fresh vegetables, so they are harvested and packaged individually in the field to reduce quality loss, accelerated softening and tissue darkening [3, 4].

To address these problems, some treatments have been used; such as low pH organic solutions [5], 1-methylcyclopropene (1-MCP) [6], calcium salts [7], among others. In particular, the application of hot water treatment (HWT) has shown to be effective as a physical, non-toxic and safe mean of maintaining postharvest quality for a range of horticultural products [8]. In addition, this treatment may affect ripening and protect against diverse physiological disorders and has been used as an alternative treatment for decay control [7]. However, excessive heat application can have a negative effect on ripening, due to increased activation of enzymes that degrade the cell wall such as pectin methylesterase (PME) (EC 3.1.11) and polygalacturonase (PG) (EC 3.2.1.15) [6]. PME and PG act in joint on the pectin to increase cell wall solubilization; however, de-esterification of cell wall galacturonans by PME may be required prior to hydrolysis by PG [7]. In particular, the PME enzyme is known to participate in several processes such as catalyzing the hydrolysis of methyl-ester groups of cell wall pectin, producing free acid, low degree of esterification pectin, and methanol [9].

Softening of the tissue is associated with alterations on the structure in the middle lamella, where pectin, cellulose, and

✉ Misael Odín Vega-García
mvega6@yahoo.com

[1] Posgrado en Ciencia y Tecnología de Alimentos, Facultad de Ciencias Químico-Biológicas, Universidad Autónoma de Sinaloa, Cd. Universitaria, Av. de las Américas y Josefa Ortiz S/N, cp, 80010 Culiacán, Sinaloa, Mexico

[2] Laboratorio Nacional para la Investigación en Inocuidad Alimentaria, Centro de Investigación en Alimentación y Desarrollo, A.C. Unidad Culiacán, Carretera a El Dorado Km 5.5, Campo el Diez, Culiacán, Sinaloa, Mexico

Published online: 28 February 2024

hemicellulose undergo structural modifications during ripening due to the activity of cell wall degrading enzymes. The activities of these enzymes leads to the solubilization of pectin from the cell wall. In addition to enzymes, there are other factors that can contribute to the softening of the fruit. These include differences in primary wall structures for different fruit varieties, which may be constituted by different proportions of polysaccharides and glycoproteins, ionic and covalently bound minerals, and phenolic esters [10].

Eggplant is particularly rich in a large number of bioactive compounds, especially phenolic compounds such as flavonoids (anthocyanins) and phenolic acids (chlorogenic acid) which are predominantly found in the peel and pulp, respectively, and which have been linked to various health benefits [11]. Anthocyanins are glycosides of polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrylium cation salts, which not only contribute to the color appearance of fruit and vegetables (tissue darkening), but have various health-related benefits, such as antioxidants, anti-cardiovascular, anti-diabetic, anti-tumor, anti-inflammatory and anti-cancer [2]. Anthocyanins are unstable compounds that degrade easily, and this degradation can be affected by various exogenous and endogenous factors, such as proteins, oxygen, light, pH, metal ions, but especially heat treatments. When this degradation occurs, polymeric compounds can also be produced [12].

The application of a hot water treatment has previously been carried out in eggplant fruits [5, 6]. However, no mathematical model has been carried out to find the appropriate application conditions for said treatment. Therefore, the present study aims to optimize the application of the factors of a HWT (temperature and time) in eggplant fruit while discovering its impact on quality and biochemical attributes. For which a characterization of various parameters was carried out, once the optimal hot water treatment (OHWT) was obtained.

Materials and methods

Experimental design

This study was divided into two parts: in the first (optimization process), different temperatures and times were applied to obtain an OHWT evaluating four responses; while in the second (chemical characterization), the effect of the OHWT was studied and characterized.

Optimization process

Response modeling

For this experiment, the effect of hot water treatment (HWT) was tested and modeled in purpose of PME activity, total color difference (ΔE), firmness, and anthocyanin content of eggplant fruit through the use of Response Surface Methodology (RSM) and through a Central Composite Design (CCD). The total number of experiments needed (N) of the CCD was determined using Eq. (1) as is shown:

$$N = 2^k + 2k + N_0 \quad (1)$$

where k corresponds to the number of variables studied, in this study were temperature (T) and time (t) (k = 2). Meanwhile 2^k, 2k and N_0 are the cubic, axial and center point runs, respectively. Each variable was examined at five different levels ($-\alpha$, -1, 0, 1, and $+\alpha$) as follows: T = 25.86, 30, 40, 50 and 54.14 °C; whereas t = 2.93, 5, 10, 15 and 17.07 min. The selection of the levels was determined based on literature review (in cherry tomato [13]; in cucumber [14]; in tomato [15]) and preliminary trials. A total of 13 treatments were conducted (Table 1).

It was assumed that a mathematical function existed between the responses studied according to both variables related to HWT processing given in Eq. (2).

$$Y = f(T, t) \quad (2)$$

A second order model with two factors was used to predict the experimental behavior of each response, using the following Eq. (3):

$$Yi = b_0 + b_1X_1 + b_2X_2 + b_1^2X_1^2 + b_2^2X_2^2 + b_1b_2X_1X_2 \quad (3)$$

where Yi is the response studied, X_1 is temperature, X_2 is time, and b_0, b_1, b_2, b_1^2, b_2^2, and b_1b_2 are the regression coefficients. Experimental data were analyzed through the response surface methodology, using the statistical package Design-Expert 7.0 software (Stat-Ease Inc., Minneapolis, MN, USA). The desired goals for each variable and response were chosen. All the independent variables were kept within their respective ranges while the responses were maximized.

Plant material and treatment application

Eggplant fruits harvested at commercial maturity were provided by farmers from Sinaloa, Mexico. Eggplants cultivar "Classic" with exportation quality were selected based on peel color (deep purple-black color and elongated oval shape) uniformity and absence of physical damage. The fruits were washed with soap and disinfected with sodium hypochlorite (600 mg/L) for 3 min, and then they were divided into 13 groups for the application of the different temperature/time combinations (Table 1). Three fruits per treatment (a total of 39 fruits) received the HWT by complete immersion in a water bath (Cole Parmer, Vernon Hills, IL, USA) according with the optimization experimental design.

Table 1 Central composite design arrangement for the optimization process and means for each response variable

Run number	Process factors			Response variables		
	Temperature (°C)	Time (minutes)	PME (U/g FW)	ΔE	Firmness (N)	Anthocyanin (Eq. of cyanidin-3-glucoside/g FW)
1	30	5.00	1.333	1.063	31.9	5.128
2	50	5.00	2.305	1.125	27.56	2.935
3	30	15.00	1.706	1.076	31.9	4.928
4	50	15.00	2.788	1.152	26.3	2.324
5	25.86	10.00	1.353	1.059	32.75	5.375
6	54.14	10.00	2.922	1.149	26.2	2.417
7	40	2.93	1.422	1.075	31.8	4.924
8	40	17.07	2.236	1.14	28.75	3.472
9	40	10.00	1.638	1.093	31	4.578
10	40	10.00	1.624	1.101	32	4.873
11	40	10.00	1.597	1.098	31.5	4.67
12	40	10.00	1.668	1.091	30.9	4.873
13	40	10.00	1.59	1.107	31.2	4.696

The first column does not correspond to the running order of the treatments. Values correspond to mean ± standard deviation of data. Different letters in the same line indicate a significant difference (Fisher $p < 0.05$)

PME pectin methylesterase; *ΔE* total color difference

PME activity

The enzyme was extracted according to the method reported by Díaz-Corona [7] with some modifications. About 10 g of eggplant and 12.5 mL of 1 M NaCl (4 °C) were homogenized using an Ultra Turrax (T18 basic, IKA Works, Inc. Wilmington, NC, USA) for 1 min. The homogenate was centrifuged at 17,000×*g* for 45 min at 4 °C and filtered with Whatman No. 4 paper. The supernatant was saturated at 80% with ammonium sulfate ($(NH_4)_2SO_4$) and stirred for 1 h under refrigeration to precipitate the protein, followed by a second centrifugation at 17,000×*g* for 20 min. The precipitate containing the protein was resuspended in 15 mL of distilled water at 4 °C and was used to measure the enzymatic activity.

PME activity was determined based on the change of color of a pH indicator during the reaction catalyzed by PME [7]. Due to the hydrolyzation of pectin ester bonds, acid groups are produced, and pH suffers a decline, causing the indicator dye to change its color. This change was continuously monitored (every min during 5 min) with a spectrophotometer (UNICO SQ 2800, New Jersey, USA). Two mL of citrus pectin (0.5%) were mixed with 0.5 mL of bromothymol blue (0.01%) in potassium phosphate buffer (0.003 M) and 4 mL of distilled water. The mixture was adjusted to pH 7.5 and the reaction was started by adding 100 µL of the enzymatic extract. The reaction was carried out at 30 °C and was monitored every minute for 5 min at 620 nm. A standard curve of galacturonic acid (0.5%) was used. The PME activity was expressed as PME units per gram of fresh weight (U/g FW). Four readings per replicate were obtained of three fruits per treatment.

Color

This parameter was determined using a chroma meter (Minolta CR-200 Chroma meter, Osaka, Japan) and L^* (lightness), a^* (green-red tonality), and b^* (blue-yellow tonality) variables were recorded. Furthermore, to better assess the overall color change among samples during storage, the following Eq. (4) was used:

$$\Delta E = \left(\left(L_{initial} - L_{final}\right)^2 \left(a_{initial} - a_{final}\right)^2 \left(b_{initial} - b_{final}\right)^2 \right)^{0.5} \quad (4)$$

Color variables were measured at six locations per sample.

Firmness

A penetrometer (Chatillon DFE-100, AMETEK Inc, Largo, FL) equipped with a flat tip of 11 mm of diameter at a constant penetration rate (50 mm/min, and 5 mm penetration) was used to evaluate this parameter [7]. Both sides of the eggplant fruit were separated, the tip was placed on the surface of the halfway point, and both ends, obtaining six points per fruit. Results were reported as Newtons (N).

Anthocyanin content

The method described by Abdel-Aal [16] was used to quantify anthocyanin content. The anthocyanin extracts were prepared with 0.1 g of lyophilized fruit and acidified cold ethanol (95% methanol and 1 N HCI, 85:15, v/v). After that, the sample was centrifuged (Model 5415D; Eppendorf AG, Hamburg, Germany) at 3000×*g* for 10 min, and the supernatant was collected. The absorbance of the sample was measured immediately at 520 nm in a microplate reader (Model xMark TM; Bio-Rad, CA, USA). Total anthocyanin content per sample (mg/kg fresh weight) was calculated as equivalents of cyanidin-3-glucoside using the following Eq. (5):

$$C = (A/£) \times (vol/1000) \times \mathrm{MW} \times (1/\text{sample weight}) \quad (5)$$

where C is concentration of total anthocyanin (mg/kg), A is absorbance reading, £ is molar absorptivity (cyanidin-3-glucoside = 25,965 cm^{-1} M^{-1}), *vol* is total volume of anthocyanin extract, and MW is molar weight of cyanidin-3-glucoside (449.2 g).

Chemical characterization of OHWT

Once the OHWT conditions were obtained, a characterization of bioactive compounds and antioxidant capacity was carried out; this to check the OHWT effectiveness. Ten fruits were selected, five of them received the OHWT and the remaining were considered as control. Once the treatment was applied to the fruits, the samples were stored at − 20 °C (Whirpool freezer, WC150140) immediately after the application of OHWT for evaluation.

Phenolic compounds

Methanol extracts (ME) of eggplant were obtained by maceration. Two grams of lyophilized tissue from peel and pulp of eggplant fruit were taken, to which 40 mL of distilled methanol were added. The container with the tissue and methanol was shaken, then sonicated for 20 min. The supernatant was collected in two 15 mL tubes and centrifuged for 10 min, then the supernatant was filtered through Whatman No.1 paper and the filtered sample was collected. The procedure was repeated 2 more times with washes of 20 mL of distilled methanol. The solvent was eliminated under vacuum (40 °C) with a rotary evaporator (BÜCHI Labortechnick AG, Switzerland), followed by removal of any residual solvent in a vacuum oven at 40 °C. The ME obtained were stored at − 20 °C in the dark until use.

Total phenolics were quantified by Folin–Ciocalteu (FC) method with slight modifications [17]. The determination was carried out in a 96-well microplate. Ten microliters of the ME (10 mg/mL), gallic acid (standard) or distilled water (negative control) were mixed with 100 µL of the FC reagent diluted in water (1:10, v/v). The microplate was gently shaken and allowed to stand for 2 min, 90 µL of a 10% sodium carbonate solution (Na_2CO_3) was added, the mixture was shaken and incubated at 40 °C for 30 min in the dark. Finally, absorbances were measured in a microplate reader (Multiskan Bichromatic, Fisher Scientific, USA) at 765 nm. Quantification was carried out using a gallic acid calibration curve (0–250 µg/mL) and the results were expressed as milligrams Gallic Acid Equivalents (GAE) per 100 g, dry base (mg GAE/100 g DB).

To evaluate the content of chlorogenic acid (CA), 50 mg of ME were dissolved in 5 mL of H_2O:methanol (9:1, v/v) and partitioned with ethyl acetate (5 mL/3 times); this phase was brought to dryness, the residue was dissolved in methanol (10 mg/mL) and filtered through a polyvinylidene difluoride membrane (17 mm × 0.45 µm). The mobile phase consisted of 1% formic acid (A) and acetonitrile (B), with an initial gradient of 0.5 of B, linear increase of 30% of B at 10 min, remaining so for 10 min, and finally linear increase until 60% B at 30 min. Total running time was 35 min. Flow rate was 0.4 mL/min and injection volume of 15 µL. Detection was performed at 320 nm. Quantification was performed using a calibration curve with a commercial CA standard and the results were expressed as micrograms of CA per milligrams of ME (µg CA/mg ME) [18].

The anthocyanin content was determined as previously reported in the "Optimization" section.

Antioxidant capacity

2,2-Diphenyl-1-picrylhydrazyl (DPPH) analysis was performed with some modifications [19]. In a 96-well microplate, an aliquot of 20 µL of the diluted methanolic extract (1:2 v/v) was mixed with 180 µL of the 150 mM DPPH radical (methanol). The mixtures were incubated for 30 min in complete darkness (27 °C) and were read at an absorbance of 525 nm in a microplate reader (SynergyTM HT Multi-Detection, Biotek, Inc., Winooski, VT). Trolox was prepared as a calibration curve (0–200 µg/mL) and the antioxidant capacity was reported as Trolox equivalent (µmol TE/100 g DB).

In the 2,2′-azinobis-3-ethylbenzotiazoline-6-sulfonic acid (ABTS) method, the reagent is oxidized to its corresponding radical $ABTS^{\cdot+}$, which shows an intense coloration in solution. The antioxidant substances transfer electrons or hydrogen atoms to the radical $ABTS^{\cdot+}$ species being inactivated and measured as the loss of color. This method was evaluated according to López-Angulo [19] with some modifications. The $ABTS^{\cdot+}$ was prepared by mixing ABTS (7 mM) reagent and potassium persulfate (2.45 mM) in a 1:1 ratio, and the mixture was allowed to stand for 12 h at 25 °C

in darkness. The $ABTS^{\cdot+}$ radical was diluted with phosphate buffer solution (7 mM, pH 7.4) to get an absorbance of 0.7 ± 0.02 at 734 nm. In assay tubes, 100 µL of samples were diluted in methanol (1:20) and mixed with 1900 µL of $ABTS^{\cdot+}$ diluted radical; the mixtures were allowed to stand for 30 min at 27 °C in darkness and their absorbances were measured at 734 nm. A Trolox calibration curve was prepared in the range of 0–225 µg/mL and the antioxidant activity expressed as Trolox Equivalent (µmol TE/100 g DB).

Statistical analysis

For the characterization technique, all experiments and analysis were completely randomized with five replicates. The statistical analysis of variance was performed using Statgraphics Centurion XVI software (Statpoint Technologies, Inc., Warrenton, VA, USA). The means specific differences were determined by Fisher's least significant difference (LSD) method, which was applied following the analysis of variance (ANOVA), significance of differences was defined at $P \leq 0.05$.

Results and discussion

Optimization process

Fitted model checking

Response surface analysis was applied to the experimental data. Table 1 shows the different combinations of temperature and time factors used to obtain an OHWT. ANOVA and least squares regression were performed for each of the response variables.

The predictive models in terms of coded factors were Eq. (6) for PME, Eq. (7) for ΔE, Eq. (8) for firmness and Eq. (9) for anthocyanins content:

$$PME = 1.62 + 0.53(T) + 0.25(t) + 0.027(T)(t) + 0.27(T)^2 + 0.12(t)^2 \quad (6)$$

(p-value of model < 0.0001; adjusted $R^2 = 98.82$)

$$\Delta E = 1.10 + 0.033(T) + 0.017(t) + 3.47810^{-3}(T)(t) + 2.85810^{-3}(T)^2 + 4.39810^{-3}(t)^2 \quad (7)$$

(p-value of model < 0.0001; adjusted $R^2 = 92.58$)

$$\text{Firmness} = 31.32 - 2.40(T) - 0.70(t) - 0.31(T)(t) - 1.04(T)^2 - 0.64(t)^2 \quad (8)$$

(p-value of model < 0.0001; adjusted $R^2 = 93.28$)

$$Anthocyanins = 4.74 - 1.12(T) - 0.36(t) - 0.10(T)(t) - 0.48(T)^2 - 0.32(t)^2 \quad (9)$$

(p-value of model < 0.0001; adjusted $R^2 = 94.92$)

The ANOVA showed that the models were highly significant for all responses ($p < 0.0001$). The F values for the four responses were significant. The predictive capability of the model is usually explained by the adjusted coefficient of determination, which for all responses were $R^2_{adj} > 0.8$, indicating that a high proportion of variability was explained by the data. The lack of fit did not result in a significant p value, indicating that these models should be used for predicting those responses. As a rule, the coefficients of variation (CV) should not be greater than 10%. In this study in particular, the CVs were less than 10% for all responses. This indicates that the models were adequate for describing the behavior of the response variables.

Effect of process variables on PME activity

Eggplants subjected to HWT showed PME activities ranging from 1.333 to 2.922 (U/g FW) (Table 1). The analysis of variance of the prediction model for PME activity in eggplant showed the relationship between this response variable and the HWT factors (T and t). Contour and response surface graphs were constructed from the mathematical prediction model of PME activity in which the effect of the HWT factors on PME activity is observed (Fig. 1a). It is important to mention that applying the treatment with the higher temperature (54.14 °C) (Fig. 1a), did not inactivate the PME enzyme and on the contrary, an irreversible activation was carried out for all treatments, as desired. PME activity has a key role in the degradation process of the cell wall of eggplants [6]. In this study, as the factors (temperature and time) of the treatments increased, PME had greater activity (Fig. 1a). These results could be due to the participation of the PME enzyme in the fruit softening process, this enzyme catalyzes pectin demethylation, which allows PG to further degrade the cell wall, this process would be accelerated when the fruit is subjected to high temperatures, since the application of a moderate stress causes the activation of PME enzyme [6]. Likewise, in previous studies it has been mentioned that this difference in the activity

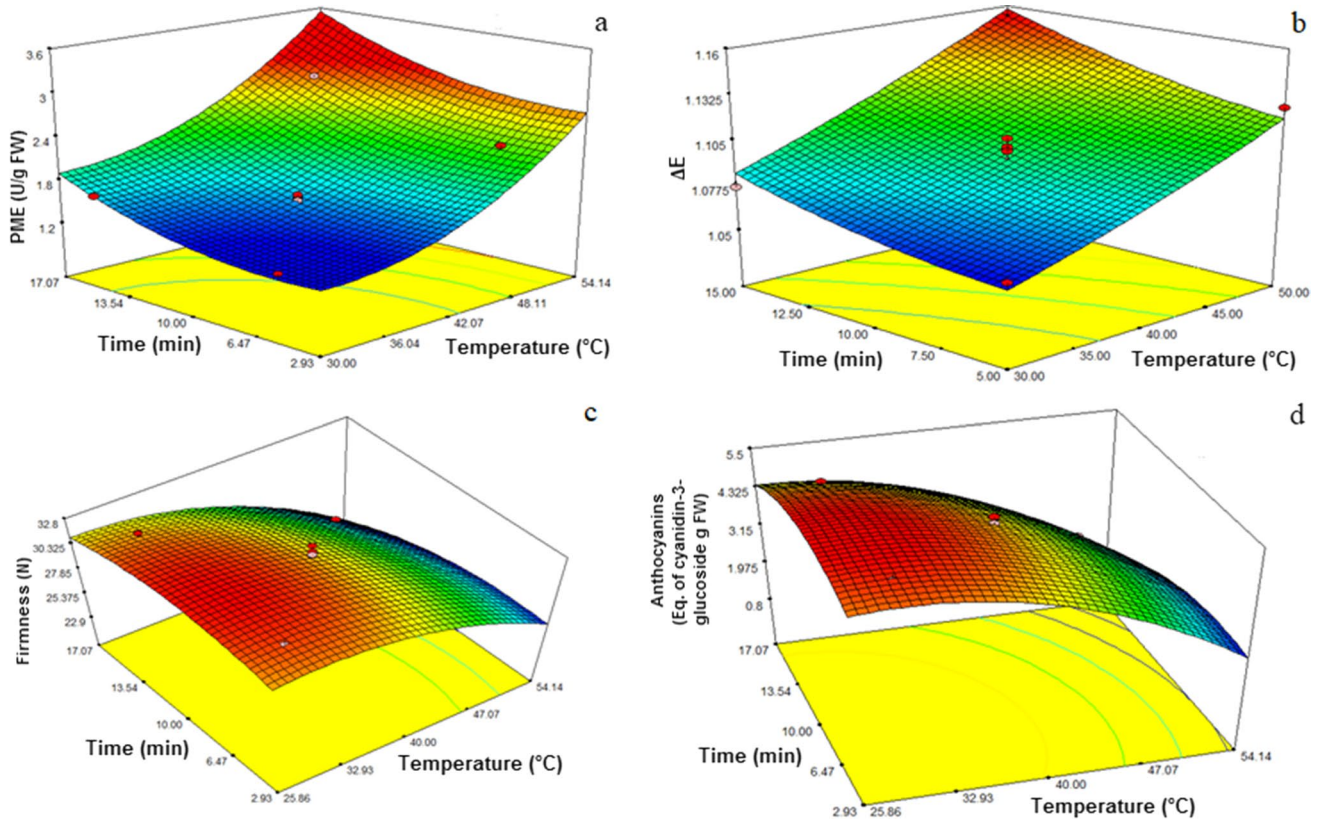

Fig. 1 Response surface showing the effect of HWT with different temperature and time on the PME enzyme activity (**a**), total color difference ΔE (**b**), firmness (**c**) and anthocyanin content (**d**) in eggplant fruits

of the enzyme under different temperatures could be due to a low thermostability of the enzyme, since it is the increase in temperature that influences the activity of PME [20]. Similar results were previously reported in kiwi fruits [21], when temperature (25–45 °C) and time (from 0 to 25 min) were increased, a higher PME activity was obtained, which tripled when the fruit was subjected to 45 °C (9.00 µmol COO^{-} g^{-1} min^{-1}) compared to the application of 25 °C (3.19 µmol COO^{-} g^{-1} min^{-1}). Similarly, when a HWT at 55 °C for 60 s in eggplant was applied, it was obtained a higher PME activity compared to the control after 15 days of storage [6]. These results could be attributed to the fact that the application of HWT at high temperatures caused a higher solubilization of pectin as a result of the catalytic action of cell wall enzymes [6]. A HWT was applied at 60 °C for 1 min on melon fruits, with higher PME activity noted in the treated fruits compared to the control [22]. In the same way, in another study, greater PME activity was obtained in lettuce samples subjected for 1 min to a HWT at 50 °C compared to those subjected to 25 °C [23].

Effect of process variables on color

From the mathematical prediction model of the ΔE variable, response surface graphs were created (Fig. 1b) in which the effect of the HWT factors (T and t) on the ΔE was observed. Color is a particularly important sensory attribute in the eggplant fruit, since its decrease is an indicator of loss of quality. In this study, it was observed that the higher the temperature and time of the HWT in eggplant fruit, the greater changes occurred in their external color. This behavior obtained for color change could be attributed to the exposure of the eggplant to a moderate stress factor (HWT) that could be causing a greater degradation of temperature-sensitive phenolic compounds such as anthocyanins [24]. In accordance with this work, in a research carried out in cucumber [14], four different hydrothermal treatments were applied, it was found that when applying two intermittent HWT (40 °C for 30 and 50 min) and two continuous (40 °C for 30 and 50 min) these had positive effects in terms of reducing the development of yellow zones in the peel of the fruit; this was also reported in spinach leaves (heat shock treatment) [25],

while the continuous treatment at a temperature of 40 °C for 50 min caused severe stress, triggering an evident etiolation during storage. Another research conducted on fresh-cut eggplant [5] concluded that the behavior of browning when applying a HWT (50 °C × 60 s) was similar to that of the general appearance, since temperatures below 40 °C and above 60 °C produced a significant decrease in the scores of this parameter.

Effect of process variables on firmness

Figure 1c shows that the shorter the application temperature and time (25.86 °C × 10 min) of HWT, the higher the firmness (32.75 N), i.e., firmness is closer to that of fresh product due to temperature is low. This parameter has great importance because it determines the commercial value of eggplants. The results obtained in this study agrees with the evaluation of the activity of the PME enzyme, where it was reported that at a longer time and temperature, a higher activity of this enzyme was obtained, which is the cause of an accelerated loss of firmness. This process occurs in various fruits and vegetables products, due to the degradation of polyuronides, which leads to the degradation of cell wall components, such as cellulose, hemicellulose, and pectin [6, 26]. Which would explain the results found in this study, given that when the PME activity was higher, a greater loss of firmness was obtained in the fruits. It has been reported in minimally processed kiwi fruit that their immersion in hot water (45 °C for 25 min) maintained firmness for a period of 10 days, and then, after a storage period of 3 days the control samples suffered a rapid loss (64%) of firmness compared to the first 10 days of storage [21]. These samples showed significantly different firmness in relation to those treated with a HWT. Similarly, other authors found greater firmness retention in melon immersed in hot water (70 °C) for 3 min, compared to melon immersed in cold water (10 °C) for 20 min [27]. These authors mentioned that firmness retention was related to the activity of cell wall degrading enzymes (PG, PME, glucanase, and galactosidase); which coincides with the results obtained in this research, since when PME activity was lower, firmness variable was higher.

Effect of process variables on anthocyanin content

In Fig. 1d it can be observed the effect that the temperature and time factors had on the anthocyanin content, since when these factors were higher (54.14 °C × 10 min), the anthocyanin content was lower (2.417 Eq. of cyanidin-3-glucoside/g FW). Which may be indicators of condensation with other compounds, greater degradation of anthocyanins and non-enzymatic browning reactions, that have been reported to produce the brown and polymeric color pigments that are believed to be one of the main causes of quality loss during postharvest handling and processing of fruit due to cleavage of covalent bonds, oxidation or intensification of oxidation reactions [28]. In a study carried out on eggplant [28], it was mentioned that changes in its coloration and anthocyanins degradation were affected to a greater extent by an increase in temperature and exposure time, due to a loss in the stability and concentration of anthocyanins, being delphinidin and nasunin the most abundant anthocyanins [29]. Where the accumulation of other than nasunin and delphinidin 3-rutinoside (tulipanin) in eggplant is extremely rare [4]. Furthermore, they reported that the brown color occurred along with the reduction of anthocyanin content, which is consistent with this and previous works [30]. It was reported that monomeric anthocyanin degradation was faster than the formation of nonenzymatic browning products in reconstituted blackberry juice at high temperatures [30]. Similar results were reported when applying a blanching treatment in yams, where a loss of anthocyanins of up to 14.7% was observed [31]. It is important to mention that the degradation of anthocyanins causes a darkening phenomenon in the peel when exposed to high temperatures and changes the antioxidant capacity that depends largely on the food system and the method used to apply heat [2], which matches with the results observed for color change in this study.

Optimization

The RSM was used through the application of a CCD, where different combinations of temperature and immersion times of the HWT were established to optimize the process. Figure 2 was obtained from the superposition of the previously described contour graphs (Fig. 1). The central point of the optimization region in Fig. 2 corresponds to the optimal combination of process variables to obtain adequate values of the HWT, which were: temperature of 40 °C and a time of 15 min. The predicted values of PME activity, ΔE, firmness, and anthocyanin content, using the prediction models of each of the response variables and the optimal conditions of the HWT were 1.90 U/g FW, 1.12, 29.99 N, and 4.05 Eq. of cyanidin-3-glucoside/g FW, respectively (Fig. 2). To validate the predictions the HWT was fulfilled using the optimal conditions of temperature and time. The experimental values of PME activity, ΔE, firmness, and anthocyanin content obtained with optimal conditions (1.62 U/g FW, 1.09, 31.32 N, and 4.74 Eq. of cyanidin-3-glucoside/g FW, respectively) were similar to the previously mentioned predicted values, indicating that the optimal conditions of the HWT process were suitable and reproducible.

Chemical characterization of OHWT fruits

As shown in Fig. 3a, results indicated 10.5 mg GAE/100 g for control and 17.76 mg GAE/100 g for OHWT for phenolic

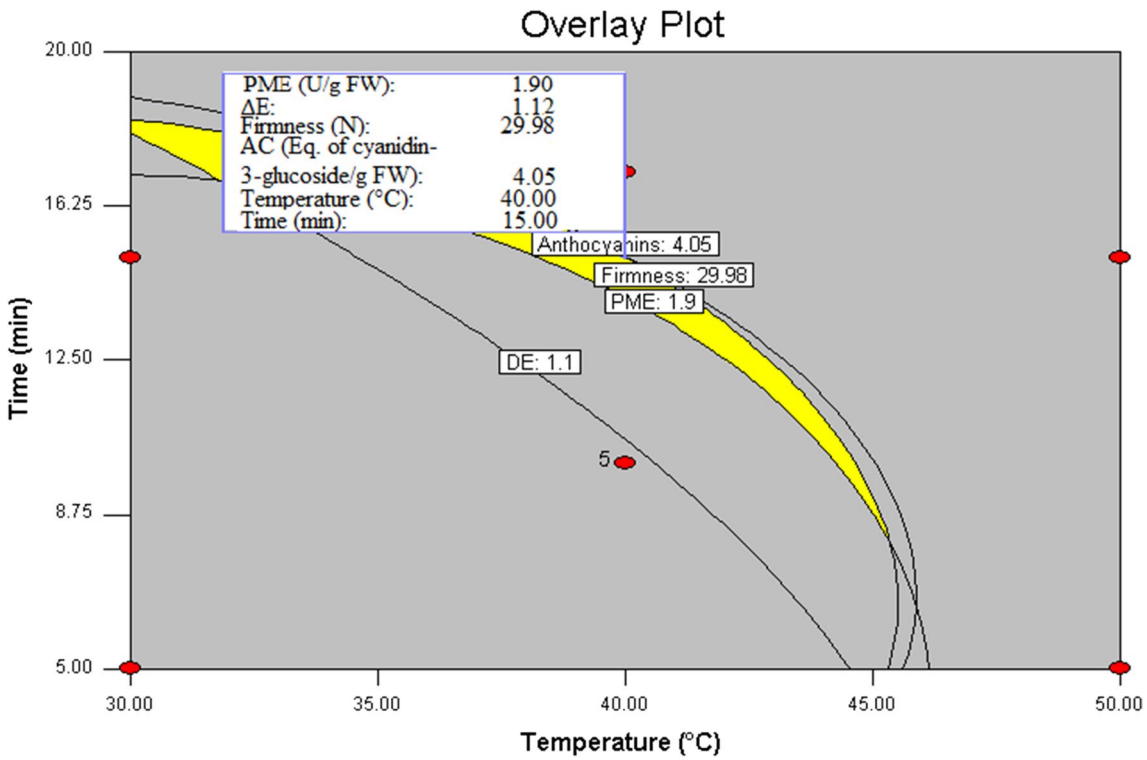

Fig. 2 Overlay plot showing the region with the best combination of the process variables with which a HWT was optimized for eggplant fruits

content evaluation, with significant difference. For the determination of anthocyanin content, it was obtained 0.3467 and 0.2819 Eq. of cyanidin-3-glucoside/g FW for control and OHWT, respectively (Fig. 3b). Regarding the content of chlorogenic acid, fruit of both treatments showed differences obtaining 4.74627 µg CA/mg ME for control and 5.9715 µg CA/mg ME for OHWT (Fig. 3c). Eggplant is a fruit with a high content of phenolic compounds, in both the peel and the pulp, being anthocyanins and chlorogenic acid the most abundant [29]. Previous studies have shown that the phenolic content can decrease or increase in fruit and vegetables depending on storage conditions, such as cold storage (chilling injury temperatures), and the application of physical and chemical treatments [32]. In this study, total phenolic content increased in response to the OHWT (Fig. 3a), which could be the result of the generation of a moderate stress in the fruit due to the application of the OHWT causing the activation of the non-enzymatic antioxidant system and the synthesis of these compounds [33]. HWT also produces lower oxidative stress, which changes the amount of antioxidants and promotes tolerance to subsequent severe stress in horticultural products [8]. Similar results were previously reported in heat-treated (boiling, steaming, and microwaving) eggplant where an increase in phenolic compounds, and a reduction in the activity of the PPO enzyme (the enzyme responsible for the oxidation of phenolic compounds) was observed compared to untreated eggplant [34]. Secondary metabolites present in plant tissues, such as phenolic substances, are believed to have antioxidant properties, so the phenolic substances they produce are beneficial in counteracting oxidative stress [28, 35]. During the present experiment, it was observed that the presence of total anthocyanins was affected by the application of an OHWT (Fig. 3b). This may be because temperature is shown to be a crucial factor in the degradation of anthocyanins and its degradation increases in proportion to the increase in temperature. Hot water treatments by immersion cause even greater loss of anthocyanin content than in other thermal processes, as more water is involved, a greater dissolution of anthocyanins occurs, degrading more easily, since they are compounds of a water-soluble nature [28]. The same behavior was reported previously in eggplant when evaluating anthocyanins and antioxidant capacity in the peel subjected to different heat treatments (steam or boiled) [28]. In addition, eggplant stands out for its antioxidant activity, which is related to its content of phenolic compounds, with chlorogenic acid (5-*O*-caffeoylquinic acid) being the most abundant in the pulp [36]. On this study, it was observed a higher content of chlorogenic acid in the OHWT (Fig. 3c), which may be due to an increase in the isomerization and hydrolysis reactions, which leads to the distribution of phenolic acid concentration, due to the massive trans-etherification occurring during the application of the heat treatment [36]. These results are similar to those previously reported in heat treated eggplant,

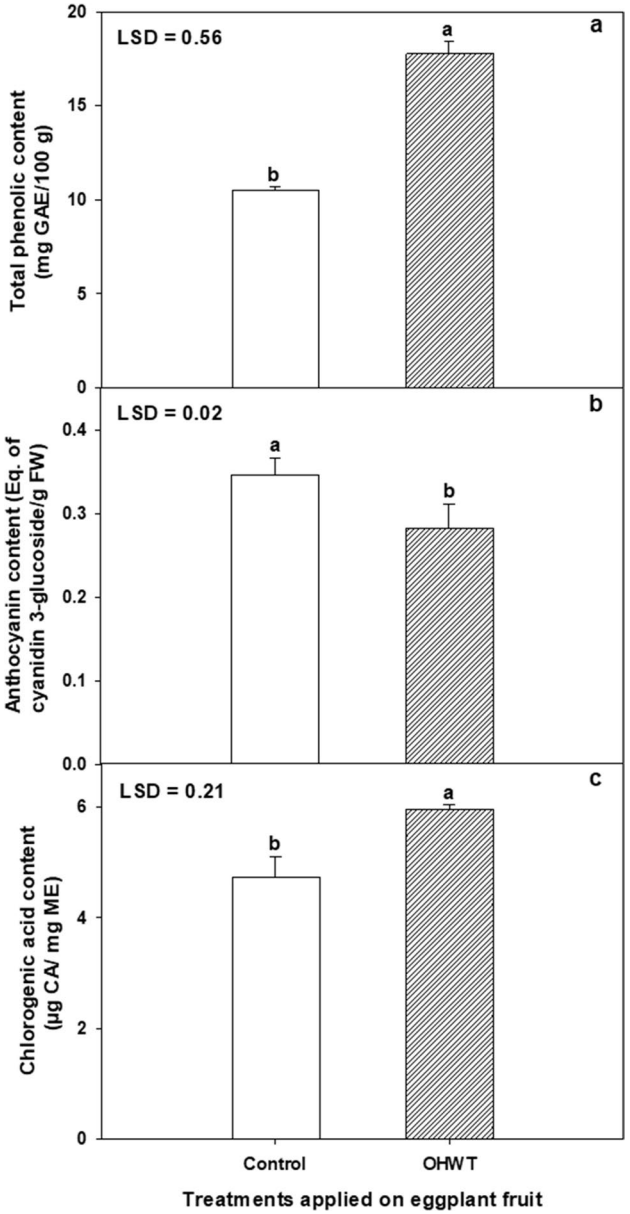

Fig. 3 Effect of the application of an optimum hot water treatment (OHWT) (40 °C, 15 min) on total phenolic (**a**), anthocyanin (**b**) and chlorogenic acid contents (**c**) in eggplant fruits. Data represents the mean + standard deviation ($n = 5$). Different letters in each graph indicate a significant difference (Fisher $p < 0.05$)

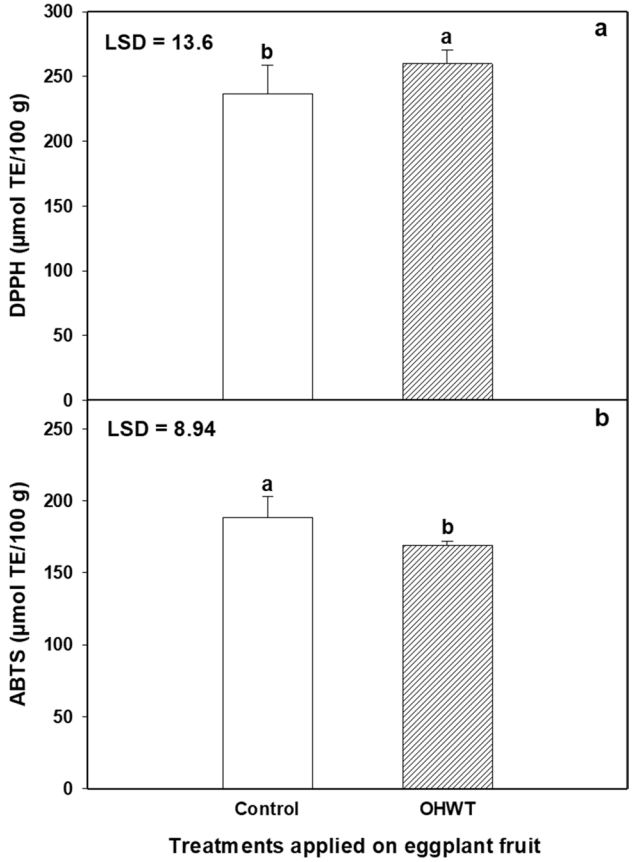

Fig. 4 Effect of the application of an optimum hot water treatment (OHWT) (40 °C, 15 min) on DPPH (**a**), and ABTS (**b**) antioxidant capacities in eggplant fruits. Data represents the mean + standard deviation ($n = 5$). Different letters in each graph indicate a significant difference (Fisher $p < 0.05$)

where it was observed an increase of chlorogenic acid in samples treated during four weeks of storage [36].

Thermal treatments generally affect antioxidant content [5]. Furthermore, during postharvest storage, the free radicals produced may be responsible for the overall decrease in total antioxidants in fresh fruits [35]. For DPPH analysis, it was obtained 236.60 and 259.10.91 µmol TE/100 g for control and OHWT, respectively (Fig. 4a). Meanwhile, for the total antioxidant capacity using ABTS methodology it was obtained as results 188.18 and 169.17 µmol TE/100 g for control and OHWT respectively as shown in Fig. 4b. Treatments evaluated by both methods showed significant differences ($p < 0.05$). Antioxidative systems respond to temperature stress, so if horticultural products are subjected to moderate and reversible stress (heat treatment) in the fruit tissue with different levels of severity, which will depend on the temperature and exposure time, species and variety; it is possible to lower oxidative stress, which modulates antioxidant levels and induces a reversible suspension of ripening [8]. Studies reported that some small molecules with antioxidant capacity could be distilled away with the application of HWT [28]. Similar to the results presented in this study, it has been reported that total antioxidant capacity levels in mume fruit were higher in HWT fruits than in control fruits during complete storage [37]. At the beginning of the experiment, OHWT showed a significant increase in antioxidant capacity compared to the control (Fig. 4a). These results coincide with those obtained in radish slices treated with HWT (50 °C, 1.5 min) and an immersion in a 2% ascorbic acid solution, where it was determined that

the antioxidant capacity was significantly higher than the untreated samples [38]. This may be due to heat treatments also employ a beneficial effect on the antioxidant capacity of eggplant fruit as a result of the disruption of the cell wall and a consequent release of antioxidant compounds, which leads to an increase in antioxidant capacity. It has been issued that the antioxidant capacity was higher in steamed and boiled eggplant fruit than in control fruit [34]. It is possible that high temperatures cause a rupture in the cell wall and release phenolic compounds causing an increase in antioxidant capacity, just as it is well known that these compounds exert antioxidant capacities and that the content of these compounds is affected and could decrease or increase depending on exposure to thermal processes [34]. Temperature and storage could also be determining factors in the antioxidant capacity of the fruit since in this study it was observed that the control fruit had a higher antioxidant capacity (ABTS) as shown in Fig. 4b, possibly related to a high content of phenols in eggplants, and also that possibly other substances of a nature other than polyphenols contribute to the antioxidant capacity, such as anthocyanins, beta carotenes, and others [5]. This coincides with a previous report [39], where it was evaluated the changes in the content of phenolic compounds and antioxidant capacity during storage and it was observed an increase in the antioxidant capacity of eggplant fruit stored at 10 °C, which was attributed to the accumulation of the phenolic compounds in the fruit during the storage at low temperatures (0 °C). This is consistent with a previous report [5], where changes in the content of phenolic compounds, antioxidant capacity and activities of oxidative enzymes were evaluated when applying a HWT (50 °C × 60 s).

Conclusions

The optimal hot water treatment conditions for eggplant were 40 °C for 15 min; being the first experiment where the thermal variables are optimized. This could serve as a basis for other studies on eggplant and may be applied for extending its shelf life for exportation with a positive effect. These results prove that OHWT had a benefit in numerous quality attributes and maintained the antioxidant capacity thanks to the retention of bioactive compounds.

Acknowledgements The authors are grateful to the program for the promotion and support of research projects (PROFAPI 2022, project PRO_A7_006) of the Universidad Autónoma de Sinaloa.

Author contributions Data curation, investigation, validation, writing—original draft: DADC; Conceptualization, methodology, resources, writing—review & editing: MELL and LEA-R; Validation, writing—review & editing, supervision: JC-C and RG-D; Validation, Resources: PJB-B; Concept, planning methodology, supervision, writing of the manuscript, final manuscript review and corrections, submission: MOV-G.

Data availability Data is available from corresponding author upon request.

Declarations

Conflict of interest The authors declared that there is no conflict of interest.

Ethical approval This study does not present any ethical concerns.

References

1. D.K. Saini, P. Kaushik, Visiting eggplant from a biotechnological perspective: a review. Sci. Hortic. **253**, 327–340 (2019)
2. Y. Zhang, Z. Deng, H. Li, L. Zheng, R. Liu, B. Zhang, Degradation kinetics of anthocyanins from purple eggplant in fortified food model system during microwave and frying treatments. J. Agric. Food Chem. **68**(42), 11817–11828 (2020)
3. F. Mariano-Nasser, C. Vanz-Borges, J. Arruda-Ramos, M. Dominguez-Nasser, G. Alencar-Lundgren, K. Aparecida-Furlaneto, T. Regina-Kovalski, R. Lopes-Vieites, Bioactive compounds and enzymatic activity in minimally processed eggplant packed under active modified atmosphere. Semin. Cienc. Agrar. **40**(1), 139–148 (2019)
4. A. Ohyama, H. Yamaguchi, K. Miyatake, S. Negoro, T. Nunome, T. Saito, H. Fukuoka, Genetic mapping of simply inherited categorical traits, including anthocyanin accumulation profiles and fruit appearance, in eggplant (*Solanum melongena*). Mol. Biol. Rep. **49**, 9147–9157 (2022)
5. M.L. Lemos, D.R. Gutiérrez, M.J. Farias, S.C. Rodríguez, Application of antioxidants and hot water treatments to improve shelf life of fresh-cut eggplants (*Solanum melongena* L.) during storage. Rev. Fac. Nac. Agron. **76**(2), 10381–10392 (2023)
6. O.N. Alkan, D. Önder, S. Önder, Y. Karakurt, Cell wall composition and enzyme-related activities in eggplant as affected by hot water, 1-MCP and calcium chloride treatments. Food Sci. Technol. Int. **29**(6), 665–679 (2023)
7. D.A. Díaz-Corona, M.E. López-López, L.E. Ayón-Reyna, J.G. López-Velázquez, B.A. López-Zazueta, M.O. Vega-García, Impact of hot water-calcium on the activity of cell wall degrading and antioxidant system enzymes in mango stored at chilling temperature. J. Food Biochem. **44**, e13286 (2020)
8. D.G. Önder, S. Önder, A.T. Uysal, Y. Karakurt, Impact of postharvest hot water, 1-MCP and $CaCl_2$ treatments on antioxidant enzymes and related genes during cold storage in sweet cherry (*Prunus avium* L.). J. Food Meas. Charact. **15**, 5744–5758 (2021)
9. A. Wormit, B. Usadel, The multifaceted role of pectin methylesterase inhibitors (PMEIs). Int. J. Mol. Sci. **19**(10), 2878 (2018)
10. W. Zhang, M. Guo, W. Yang, Y. Liu, Y. Wang, G. Chen, The role of cell wall polysaccharides disassembly and enzyme activity changes in the softening process of Hami melon (*Cucumis melo* L.). Foods **11**, 841 (2022)
11. S. Ferarsa, W. Zhang, N. Moulai-Mostefa, L. Ding, M.Y. Jaffrin, N. Grimi, Recovery of anthocyanins and other phenolic compounds from purple eggplant peels and pulp using ultrasonic-assisted extraction. Food Bioprod. Process. **109**, 19–28 (2018)
12. A.M. Sinela, C. Mertz, N. Achir, N. Rawat, K. Vidot, H. Fulcrand, M. Dornier, Exploration of reaction mechanisms of anthocyanin degradation in a roselle extract through kinetic studies on formulated model media. Food Chem. **235**, 67–75 (2017)

13. J. Yang, M.R. Fu, Y.Y. Zhao, L.C. Mao, Reduction of chilling injury and ultrastructural damage in cherry tomato fruits after hot water treatment. Agric. Sci. China **8**(3), 304–310 (2009)
14. N. Zhang, Z. Yang, A. Chen, S. Zhao, Effects of intermittent heat treatment on sensory quality and antioxidant enzymes of cucumber. Sci. Hortic. **170**, 39–44 (2014)
15. N.Y. Salazar-Salas, L. Valenzuela-Ponce, M.O. Vega-Garcia, K.P. Pineda-Hidalgo, M. Vega-Alvarez, J. Chavez-Ontiveros, F. Delgado-Vargas, J.A. Lopez-Valenzuela, Protein changes associated with chilling tolerance in tomato fruit with hot water pretreatment. Postharvest Biol. Technol. **134**, 22–30 (2017)
16. E.S.M. Abdel-Aal, P. Huel, A rapid method for quantifying total anthocyanins in blue aleurone and purple pericarp wheats. Cereal Chem. **76**(3), 350–354 (1999)
17. T. Hatami, S.A. Emami, S.S. Miraghaee, M. Mojarrab, Total phenolic contents and antioxidant activities of different extracts and fractions from the aerial parts of artemisia biennis willd. Iran. J. Pharm. Res. **13**(2), 551–559 (2014)
18. G. López-Angulo, J. Montes-Avila, S.P. Díaz-Camacho, R. Vega-Aviña, J.A. López-Valenzuela, F. Delgado-Vargas, Comparison of terpene and phenolic profiles of threes wild species of *Echeveria* (Crassulaceae). J. Appl. Bot. Food Qual. **91**, 145–154 (2018)
19. G. López-Angulo, J. Montes-Avila, S.P. Díaz-Camacho, R. Vega-Aviña, Y.P. Ahumada-Santos, F. Delgado-Vargas, Chemical composition and antioxidant, α-glucosidase inhibitory and antibacterial activities of three *Echeveria* DC. species from Mexico. Arab. J. Chem. **1**, 1–10 (2014)
20. A.F. Cerón, D.F. Mejía, O. Osorio, Cinética de inactivación térmica de la enzima pectinmetilesterasa en zumo de tomate de árbol (*Solanum betaceum* Cav). Información Tecnológica. **27**(2), 67–76 (2016)
21. S. Beirão da Costa, A. Cardoso, L. Louro-Martins, J. Empis, M. Moldão-Martins, The effect of calcium dips combined with mild heating of whole kiwifruit for fruit slices quality maintenance. Food Chem. **108**, 191–197 (2008)
22. A.C. Silveira, E. Aguayo, V.H. Escalona, F. Artés, Hot water treatment and peracetic acid to maintain fresh-cut Galia melon quality. Innov. Food Sci. Emerg. Technol. **12**, 569–576 (2011)
23. A.B. Martin-Diana, D. Rico, C. Barry-Ryan, J.M. Frias, J. Mulcahy, G.T.M. Henehan, Effect of calcium lactate concentration and temperature washing treatments on quality retention of salad-cut iceberg lettuce. Food Res. Int. **38**, 729–740 (2005)
24. A. Concellón, M.C. Añón, A.R. Chaves, Characterization and changas in polyphenol oxidase from eggplant frui (*Solanum melongena* L.) during storage at low temperature. Food Chem. **88**, 17–24 (2004)
25. F. Gómez, L. Fernández, G. Gustavo, J.J. Guiamett, A. Chaves, C.G. Bartoli, Heat shock increases mitochondrial H_2O_2 production and extends postharvest life of spinach leaves. Postharvest Biol. Technol. **49**, 229–234 (2008)
26. C. Zanotti-Gallon, T. Shiga, E. Purgatto, A.P. Jacomino, Occurrence of early softening in papaya due to specific cell wall-related changes. Sci. Hortic. **297**, 110915 (2022)
27. X. Fan, B. Anoas, J. Beaulieu, J. Sites, Effect of hot water surface pasteurization of whole fruit on shelf life and quality of fresh-cut cantaloupes. J. Food Sci. **73**, 91–98 (2008)
28. Y. Zhang, Y. Sun, H. Zhang, Q. Mai, B. Zhang, H. Li, Z. Deng, The degradation rules of anthocyanins from eggplant peel and antioxidant capacity in fortified model food system during the thermal treatments. Food Biosci. **38**, 1–12 (2020)
29. G. Niño-Medina, V. Urías-Orona, M.D. Muy-Rangel, J.B. Heredia, Structure and content of phenolics in eggplant (*Solanum melongena* L.)—a review. S. Afr. J. Bot. **111**, 161–169 (2017)
30. N. Jiménez, P. Bohuon, J. Lima, M. Dornier, F. Vaillant, A.M. Pérez, Kinetics of anthocyanin degradation and browning in reconstituted blackberry juice treated at high temperature (100–180°C). J. Agric. Food Chem. **58**, 2314–2322 (2010)
31. J. Tian, J. Chen, F. Lv, J. Chen, D. Liu, X. Ye, Domestic cooking methods affect the phytochemical composition and antioxidant activity of purple-fleshed potatoes. Food Chem. **197**, 1264–1270 (2016)
32. D.R. Utami, Sutrisno, Y.A. Purwanto, Effect of low temperature storage on quality and total phenolics of Thai eggplant (*Solanum melongena* Cv. Gelatik). Int. Food Res. J. **25**(6), 2385–2390 (2018)
33. M.E. López-López, J.A. López-Valenzuela, F. Delgado-Vargas, G. López-Angulo, A. Carrillo-López, L.E. Ayón-Reyna, M.O. Vega-García, A treatment combining hot water with calcium lactate improves the chilling injury tolerance of mango fruit. HortScience **53**(2), 217–223 (2018)
34. A. Chumyam, K. Whangchai, J. Jungklang, B. Faiyue, K. Saengnil, Effects of heat treatments on antioxidant capacity and total phenolic content of four cultivars of purple skin eggplants. ScienceAsia. **39**(3), 246–251 (2013)
35. M.W. Haider, M. Nafees, R. Iqbal, S. Ali, H. Asad, F. Azeem, M. Arslan, M.H. Rahman, A.R.Z. Gaafar, M.S. Elshikh, Combined application of hot water treatment and aucalyptus leaf extract postpones senescence in harvested green chilies by conserving their antioxidants: a sustainable approach. BMC Plant Biol. **23**, 576 (2023)
36. P. Šilarová, L. Boulekbache-Makhlouf, F. Pellati, L. Česlová, Monitoring of chlorogenic acid and antioxidant capacity of *Solanum melongena* L. (eggplant) under different heat and storage treatments. Antioxidants **8**, 234 (2019)
37. H. Endo, K. Ose, J. Bai, Y. Imahori, Effect of hot water treatment on chilling injury incidence and antioxidative responses of mature green mume (*Prunus mume*) fruit during low temperature storage. Sci. Hortic. **246**, 550–556 (2019)
38. R. Goyeneche, K. Di Sacala, M.G. Goñi, A. Vega-Galvez, I. Quispe, S.I. Roura, Application of ascorbic acid and mold heat shock to improve shelf life and ensure microbial safety of sliced radish (*Raphanus sativus* L). J. Food Process. Preserv. **39**, 3022–3031 (2015)
39. A. Concellón, M.J. Zaro, A.R. Chaves, A.R. Vicente, Changes in quality and phenolic antioxidants in dark purple American eggplant (*Solanum melongena* L. Cv. Lucía) as affected by storage at 0°C and 10°C. Postharvest Biol. Technol. **66**, 35–41 (2012)