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Comparison of the quality attributes of carrot juice pasteurized by ohmic heating and conventional heat treatment

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ABSTRACT

This study aimed to evaluate the effect of pasteurization of carrot juice by heat treatment (80 °C–7 min) and ohmic heating (OH) on its physicochemical and chromatic parameters, enzymatic and microbiological inactivation, volatile compounds, antioxidant capacity, carotene content, and sensory characteristics, compared to control juice (CJ). Both treatments allowed obtaining microbiologically stable and safe carrot juice and achieved 99% peroxidase inactivation levels. Significant differences were obtained in L* parameter, polyphenoloxidase activity and total antioxidant capacity, compared to HT-treated and CJ. The OH-treated juice presented greater color preference (p < 0.05) by semi-trained panelists and a high level of acceptance among consumers (corresponding to "like extremely"). The results allow concluding that OH could be an alternative to conventional HT for the pasteurization of carrot juice, providing benefits in its sensory perception.

1. Introduction

One of the main problems in horticulture and its derived industries is the generation of significant amounts of waste and by-products, usually discarded or underutilized. In some cases, these by-products can account for up to 75% of the harvested material (Llorach et al., 2003). However, in recent years, progress has been made in developing food and technologies to reevaluate horticultural by-products (Aimaretti et al., 2016; Fernandez et al., 2018; O'Shea et al., 2012). In this context, the discards from carrot (*Daucus carota L.*), one of the most popular vegetables with a worldwide production (Bhat et al., 2017; Riganakos et al., 2017), can be used to produce carrot juice. Carrot juice is currently widely marketed thanks to its high nutritional value (Picouet et al., 2015) due to its high content of carotenoids, such as α -carotene, β -carotene, lutein, and lycopene (Riganakos et al., 2017), some of which may exhibit provitamin A activity (Cakmakci et al., 2014). It is also a good source of vitamin C and phenolic compounds, which substantially contribute to its antioxidant potential (Zhang et al., 2005). The importance of antioxidant components in the maintenance of health and protection against coronary heart disease and cancer continues to be a relevant aspect of food as the trend of the future moves towards functional foods with specific health effects (Sharma et al., 2012; Wootton-Beard et al., 2011).

Carrot juice should be consumed within 1–2 days from its production (Picouet et al., 2015). The action of endogenous enzymes, mainly polyphenol oxidase (PPO), peroxidase (POD), and pectin methylesterase (PME), is conducive to juice deterioration (Gouma et al., 2020; Mannozzi et al., 2019). PPO and POD are mostly responsible for enzymatic browning due to the oxidation of phenolic compounds, while PME

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causes cloud destabilization and hence clarifies carrot juice due to pectin degradation. The inactivation of these enzymes and the inactivation and/or inhibition of the growth of microorganisms are critical for extending the shelf-life of carrot juice (Gouma et al., 2020). Conventional heat treatment (HT) is usually applied (Dima et al., 2015) to achieve that purpose. However, the high temperatures used can affect the sensory and nutritional quality of the juice (Quitão-Teixeira et al., 2009; Riganakos et al., 2017). Thus, an ongoing challenge for the food industry is developing technologies that minimize the adverse effects of HT on quality attributes, decrease the processing times, and ensure the safety of the products.

In this context, ohmic heating (OH), an electrical resistance heating method in which an alternating current (50-100 kHz) passes through the food (Sensoy, 2012), maybe an alternative. Since the amount of heat generated is directly related to the current induced by the voltage gradient in the field, the most important parameter in the applicability of ohmic heating is the electrical conductivity of the material (Kumar, 2018). The advantage of OH over conventional HT methods is the volumetric nature of the heating effect. Instead of being transferred by conduction or convection, heat is generated very fast inside the food due to the electric current, achieving shorter processing times. This high heat transfer rate allows achieving microbial safety without causing product degradation (Sensoy, 2012) and improving the quality of some processed foods, especially those containing large particles (Zareifard et al., 2015). Despite the mentioned advantages of OH, the main drawbacks are possible electrochemical reactions at the electrodes, which lead to their erosion and the electrolysis of the product (Jaeger et al., 2016). OH has been applied in several fruits, juices and vegetable purees, at pasteurization temperatures, and the deterioration of some compounds of interest (such as carotenoids, antioxidants and vitamin C) was lower than that observed after the application of HT (Dima et al., 2015; Funcia et al., 2020; Kumar, 2018; Müller et al., 2020). Also, several works have studied the inactivation of microorganisms through the application of OH compared to HT, and the main effect has been attributed primarily to heat (Jaeger et al., 2016). Despite this, a non-thermal microorganism inactivation effect is still under discussion (Kubo et al., 2020).

Despite the encouraging results mentioned above, the effect of the OH on carrot juice is not yet well studied; thus, the present study aimed to evaluate the effect of the pasteurization of carrot juice by OH and HT (80 $^{\circ}$ C–7 min) on its physicochemical and chromatic parameters, enzymatic and microbiological inactivation, volatile compounds, antioxidant capacity, carotene content and sensory characteristics, compare to control juice. Also, microbial growth was evaluated for 60 days under refrigerated storage conditions (4 $^{\circ}$ C).

2. Materials and methods

2.1. Preparation of carrot juice

Fresh carrots (Daucus carota L.) were supplied by a producer in Santa Rosa de Calchines (31°25'S, 60°20'W), Santa Fe province, Argentina. After harvesting, carrots classified as discarded due to their shape or size were immediately transported to our lab. Carrots were packed in polyethylene bags with perforations (49 \times 80 cm, 24 holes, 70 μm thickness) and kept under refrigeration at 4 °C (relative humidity: 95%) until their use. Before processing, carrots were washed and disinfected by dipping in chlorinated water (200 ppm) for 5 min and dried. Then, carrots were manually peeled and chopped into small pieces (20 \times 20 \times 20 mm). Carrot juice was extracted using a juice extractor (JG1700, Yelmo, China). The average yield of the juice was 50.91 \pm 0.51 g per 100 g of carrot. The juice was placed into polyethylene terephthalate (PET) bottles (1000 mL) and immediately stored at 4 \pm 1 $^\circ\text{C}.$ A total of 9000 mL of juice was prepared, 3000 mL for each treatment (OH and HT) and 3000 mL for the control (preserved without treatment). The OH and HT treatments were applied after 12 h of refrigeration. The whole procedure was repeated three times, so nine batches of carrot juice were obtained

on three different days.

2.2. Ohmic heating

2.2.1. Ohmic heating apparatus

The laboratory-scale OH system was designed and manufactured at the Laboratorio de Control de Accionamientos, Tracción y Potencia (Departamento de Electrónica, Facultad de Ingeniería, Universidad de Buenos Aires, Argentina). It consists of an ohmic heating unit (Fig. 1), which heats the liquid to be pasteurized by passing an alternating current with a frequency of 60 kHz through it. It should be noticed that the high frequency used by the unit is high enough to avoid electrolysis phenomena (Diaz Ramos & Tacca, 2013). The OH unit includes two stainless steel electrodes (height: 10.1 cm; width: 9.8 cm) located in a heating cell (length: 33.5 cm; width: 9.8 cm) constructed with a 10-mm-thick acrylic and a workload of 2.5 L. Temperature during processing was measured by a stainless steel-coated PT100 resistor located at the midpoint of the heating cell. The temperature, voltage, current and power applied were monitored by a microprocessor board, which transmitted the information to the computer at constant intervals (0.1 s).

Two nested closed-loops controlled the temperature of the sample. The external loop compares the measured temperature with the reference value, which could be either a constant value or a rising ramp. The temperature control loop supplied a current reference to the inner loop, which set the rms voltage by varying the duty cycle of the output voltage waveform. The temperature of each sample was assumed to be uniform in the cell since the maximum difference among the temperatures measured at different locations was approximately 1 $^{\circ}$ C.

2.2.2. Electrical conductivity measurement

The OH system measures the output voltage (V) and current (I) passing through the load in real-time. Then, the microprocessor calculates the rms values to calculate the power applied to the load and performs the quotient between the I and the V to estimate the conductance of the food sample at each instant. The conductivity (σ) is calculated according to Equation (1) (Diaz Ramos & Tacca, 2013)

$$\sigma = \frac{IL^2}{VVol} \tag{1}$$

where I is the rms current, V is the rms output voltage, L is the distance between electrodes (cm) and Vol is the sample volume (mL). The values of both the power and conductivity are sent through a Bluetooth interface to a host PC that presents the values on the screen in a graph as a function of time.

2.2.3. Ohmic pasteurization

The ohmic heating system described in 2.2.1 was used to pasteurize the carrot juice. The heating cell was filled with 2500 mL carrot juice at 4 °C and the temperature sensor was inserted and fixed into the geometric center of the cell to monitor the sample temperature continuously. The samples were sandwiched between the two electrodes in the heating cell and heated ohmically with an alternating current with 60 kHz frequency and voltage adjustable by a power relay (the electric field at the maximum voltage applied was 120 V/cm). The sample was ohmically heated from 15 to 80 °C, and then the temperature was kept constant at 80 \pm 1 °C for 7 min (Fig. S1). The pasteurized juice was packaged in bottles in aseptic conditions, which were immersed in an ice-water bath and then stored in refrigeration (4 °C). The experiments were replicated three times.

2.3. Conventional heat treatment

The carrot juice assigned to the HT treatment was divided into five cook-in bags (Cryovac CN640, Sealed Air) containing 500 mL each. All bags were then placed in a water bath (Lindberg/Blue M, mod.

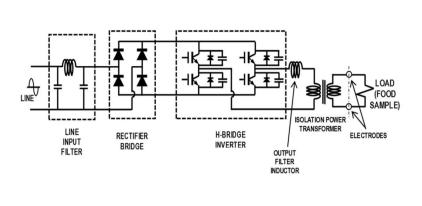




Fig. 1. Block diagram of the ohmic heater and picture of the ohmic heating cell, temperature sensor and voltage signal generator.

RWB3220CY-1, NC USA) at 82 °C. A combination of 80 °C and 7 min was applied as the lowest heating point (Picouet et al., 2015). Temperatures were measured using flexible T-type thermocouples, placed in the bags by stuffing box devices (Ecklund Harrison Tech, USA). The data were recorded with a digital multimeter (Fluke Hydra 2625A, USA). The temperature readings were taken at 10-s intervals, and the measurement precision was ± 0.1 °C (Fig. S2). Immediately after HT, bags were immersed in an ice-water bath and stored under refrigeration (4 °C).

2.4. Determination of quality attributes

2.4.1. pH and total soluble solids (TSS)

The pH in carrot juices was measured at room temperature (20 ± 1 °C) by using a digital pH meter (Thermo Orion, 710A+) with a pH electrode (8102BN, ROSS, Thermo Orion) and ATC probe (Thermo Orion). TSS were determined as °Brix at 20 ± 1 °C using a refractometer (Atago Master T, Tokyo, Japan). The measurements were carried out in duplicate for each sample.

2.4.2. Chromatic parameters

The chromatic parameters of carrot juices were measured using a Minolta CR-400 chroma meter provided with a sample holder CR A505 and a 20-mm glass cell (CM-A99 to measure liquids, Konica Minolta, Japan) with an illuminant D65 and 2° observer angle. Results are expressed as L* (lightness), a* (redness/greenness) and b* (yellowness/ blueness) in the CIE Lab system. These values were used to calculate the Hue degree and Chroma (Equations (2) and (3)) (Pathare et al., 2013).

$$H^{\circ} = \operatorname{arctangent}[b^{*}a^{*-1}] \tag{2}$$

$$Ch = (a^{*2} + b^{*2})^{1/2}$$
(3)

Also, the total color difference between the sample and the control (ΔE) was calculated by Equation (4) (Mannozzi et al., 2019).

$$\Delta E = \left[\left(L^* - L_0^* \right)^2 + \left(a^* - a_0^* \right)^2 + \left(b^* - b_0^* \right)^2 \right]^{1/2} \tag{4}$$

where L*, a* and b* are the chromatic parameters of the sample and L_0^* , a_0^* and b_0^* are the chromatic parameters of the control juice. Differences in perceptible color can be classified as not detectable (0–0.5), slightly detectable (0.5–1.5), detectable (1.5–3.0), well detectable (3.0–6.0) and highly detectable (6.0–12.0) (Cserhalmi et al., 2006). The browning index (BI) was calculated by Equation (5) (Mannozzi et al., 2019).

$$BI = [(100(x - 0.31)]/0.172$$
(5)

where $x = (a^* + 1.75L^*)/(5.645L^* + a^* - 3.012b^*)$.

The measurements of chromatic parameters were performed in triplicate for each sample and the results were averaged.

2.4.3. Enzyme activities

The enzyme activities for peroxidase (POD), polyphenol oxidase (PPO), and pectin methylesterase (PME) were determined as described by Fernández et al. (2018). More details of the methodology can be found in the Supplementary material (sections S.1.a and S.1.b). One unit of enzyme activity was defined as the change of 0.001 of absorbance at the corresponding wavelength. The measurements were carried out in duplicate for each sample. Results are expressed as $\Delta A/\min/L$, and the percentage of relative enzymatic activity relative to the control carrot juice was calculated.

2.4.4. Microbial analysis

All samples were serially diluted for microbial analysis with a sterile 0.1 g/100 mL peptone solution, and 1.0 mL of each dilution was plated into duplicate plates of the appropriate agar. Plate Count Agar medium (Merck, Germany) was used to determine the total aerobic mesophilic counts (TPC) after incubation at 37 °C for 48 h. Yeast Extract Dextrose Chloramphenicol Agar medium (Merck, Germany) was used to count yeast and mold cells (Y&M) after incubation at 25 °C for 7 days. Lauryl Sulfate Broth medium (Merck, Germany) was used to count total coliforms (TCC) after incubation at 37 °C for 48 h. Finally, in-depth seeding on Sulfite Polymyxin Sulfadiazine Agar medium (Merck, Germany) was used to count clostridium sulfite-reducers (CSR) after incubation at 37 °C for 48 h. The results are expressed as decimal logarithms of the number of colony-forming units per mL (CFU/mL) of juice. The plates were considered below the quantification limit of 1.0 Log CFU/ mL when up to five colonies were counted. Microbiological analyses were carried out in control, OH-treated and HT-treated carrot juice after 0, 15, 30, and 60 days of refrigerated storage (4 $^{\circ}$ C).

2.4.5. Volatile compounds (VOCs)

The carrot juice VOCs were extracted by headspace solid-phase microextraction, as described by Kebede et al. (2014) with some modifications. Briefly, 5 mL of juice was equilibrated (40 °C - 20 min) and VOCs were extracted (40 °C - 10 min) by 2 μ m–50/30 μ m DVB/Carboxen/PDMS Extractive Fiber (Supelco, Bellefonte, USA). Then, the VOCs extracted were determined by gas chromatography-mass

spectroscopy (GC–MS). More details of the methodology can be found in the Supplementary material (section S.2). Results are expressed as arbitrary area units (AAU). VOCs were determined in duplicate.

2.4.6. Total antioxidant capacity

Total antioxidant capacity (TAC) was determined using the Ferric reducing antioxidant power (FRAP) methodology, according to Chen et al. (2015) with some modifications, and calculated by using 6-hy-droxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, Sigma-Aldrich, St. Louis, USA) as standard and expressed as μ M of Trolox equivalent antioxidant capacity. The details of the technique can be found in the Supplementary material (section S.3).

2.4.7. Carotenoids

The carotenoid extraction procedure was adapted from Rossetti et al. (2010). Briefly, 5 g of juice was homogenized with 25 mL of the buffer; 1 mL of homogenate was mixed with 2 mL of pyrogallol and incubated (70 °C - 2 min). Saponification was carried out with 300 μ L KOH (70 °C - 30 min). Carotenoids were extracted with hexane and analyzed by high-performance liquid chromatography (HPLC). More details of the methodology can be found in the Supplementary material (section S.4). The measurements were carried out in duplicate for each sample.

2.4.8. Sensory evaluation

Semi-trained panelists (16) carried out a preference ranking test to assess color and flavor differences between the control, OH-treated and HT-treated carrot juices. The preference ranking test to assess color was carried out in a cabinet with standardized light (Verivide, CAC 120, UK) D65 illuminant and the visual angle was kept constant during all tests. Samples were placed in transparent glass bottles, simulating a commercial presentation. The preference ranking test to assess flavor was carried out in panel booths. Panelists received 25 mL of each sample, served in plastic glasses at 4 \pm 1 °C. A balanced-block design was used for order presentation.

The pasteurized carrot juice with the highest score in the preference ranking test was selected to evaluate the consumers' acceptance. A ninepoint hedonic scale (Meilgaard et al., 2007) was used (1: dislike extremely; 9: like extremely). The test was carried out with 117 casual consumers recruited in the organic products market "*Sabe la Tierra*" located in San Fernando (Buenos Aires, Argentina). Consumers (54 males, 62 females) were between 20 and 79 years old. Samples were delivered at 4 °C and served in plastic glasses.

2.5. Statistical analysis

A completely randomized block design with three fixed treatments and three randomized blocks was applied. Each processing batch corresponded to a block. The results were statistically evaluated by using the Statistical Package SPSS 12.0 (Chicago, USA). For all the parameters evaluated in the present study, the block effect was non-significant (p >0.05). One-way analysis of variance (ANOVA) was used for independent samples (when equal variances were not assumed) and linear regression was used to analyze the effect of the treatment on the quality attributes of the carrot juice samples. Bonferroni post-hoc test was used to compare means. The areas of VOCs were analyzed by principal component analysis (PCA). The scores obtained from each PCA were analyzed by ANOVA to test for significant differences between treatments. The Friedman test analyzed preference ranking data.

3. Results and discussion

3.1. Electrical conductivity

The electrical conductivity (σ) of carrot juice increased linearly with the increase in temperature (Fig. 2). The data were adjusted to a linear regression model, according to Equation (6).

(6)

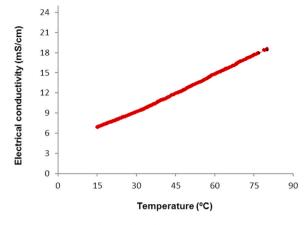


Fig. 2. Electrical conductivity of carrot juice.

 $\sigma = 4.11 + 0.18 \times T R^2 = 0.998$

where σ is the electrical conductivity (mS/cm), and T is the temperature (°C) of the carrot juice during the OH treatment. These results are consistent with those reported by Quitão-Teixeira et al. (2008), who obtained σ values between 5 and 20 mS/cm (from 5 to 65 °C) when treating carrot juice with pulsed electric fields (PEFs). Kumar (2018) also reported linear increases in the σ of various fruits with the increase in temperature during OH, with a constant voltage gradient.

The increase in σ with the temperature may be explained by the fact that, in a liquid, the electric current is transported by the movement of ions (Kong et al., 2008), and therefore, when the temperature increases, the resistance to the movement of ions is lower (Jittanit et al., 2017).

3.2. pH and TSS

The pH and TSS of the three carrot juices evaluated (control, OHtreated and HT-treated) are shown in Table 1. The pH values of the OH- and HT-treated juices were significantly (p < 0.05) higher than those of the control juice (Table 1). Luo et al. (2008) obtained similar results, observing a slight increase in the carrot juice pH treated by PEFs at 25 kV/cm for 200 μ s at 1 Hz. The increase of pH values in the OH- and HT-treated juices could be a consequence of the diffusion of materials or salts from the cell to the medium. On the one hand, the application of electric fields enhances this diffusion phenomenon (Rinaldi et al., 2020; Varghese et al., 2014); and on the other, the HT may cause cell membrane disruption (Patras et al., 2009). Regarding TSS, we observed no significant differences (p > 0.05) between the treatments. Furthermore, Jiménez-Sánchez et al. (2017) reported that the electrical treatments of fruit juices would not affect TSS content.

3.3. Chromatic parameters, ΔE and BI

The values of L*, a*, b*, Ch, H°, ΔE and BI of the control, OH-treated and HT-treated carrot juices are shown in Table 1. The L* values of the OH-treated juice were significantly lower (p < 0.05) than those of the control and HT-treated juices. Also, the a* values of OH- and HT-treated juices were significantly higher (p < 0.05) than those of the control juice, whereas the H° values were significantly lower. Parameters b*, Ch and BI of OH- and HT-treated juices showed no significant changes (p > 0.05) in comparison to those of the control juice.

The application of HT in carrot juice can cause variations in color, with a decrease in a* parameter causing a change from red to yelloworange (Chen et al., 1995). Also, browning reactions may explain the less red and yellower colorations of carrot juices (Szczepańska et al., 2020). However, in this study, the HT applied increased the "redness" of the juice and decreased parameter H°, tending towards reddish tones,

Table 1

Effects of OH and HT on the pH, total soluble solids and	d chromatic parameters of carrot juice.
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Treatment	pH	TSS (°Brix)	Chromatic parameters						
			L*	a*	b*	Ch	\mathbf{H}°	ΔE	BI
Control	6.52 ± 0.02^{b}	11.15 ± 0.08^{a}	43.04 ± 0.19^a	16.80 ± 0.41^{b}	31.51 ± 0.53^a	35.71 ± 0.66^{a}	61.93 ± 0.23^{a}	_	144.7 ± 3.4^{a}
HT	6.59 ± 0.02^{a}	$11.17\pm0.08^{\rm a}$	$\textbf{42.25}\pm\textbf{0.20}^{a}$	$19.41\pm0.43^{\rm a}$	$30.44\pm0.56^{\rm a}$	36.10 ± 0.69^a	$\textbf{57.48} \pm \textbf{0.25}^{\text{b}}$	$\textbf{3.49}\pm\textbf{0.43}^{a}$	$146.2\pm3.5^{\rm a}$
OH	6.66 ± 0.02^{a}	11.12 ± 0.09^a	41.73 ± 0.21^{b}	18.96 ± 0.46^a	$\textbf{30.44} \pm \textbf{0.59}^{a}$	$\textbf{35.87} \pm \textbf{0.73}^{a}$	$58.14 \pm \mathbf{0.26^b}$	$\textbf{4.60} \pm \textbf{1.57}^{a}$	148.6 ± 3.8^{a}

Data are expressed as means \pm standard error (n = 9). Values with different letters (a, b, c) in the same column were significantly different (p < 0.05) from each other. L*: lightness, a*: redness, b*: yellowness, Ch: Chroma, H°: Hue degree, Δ E: total color difference, BI: browning index, TSS: total soluble solids.

without affecting b* parameter. Furthermore, Patras et al. (2009) and Bhale (2004) observed an increase in a* values after applying HT (70 °C) to carrot juice and OH to carrot cubes, respectively. Bhale (2004) also reported a decrease in L* and H° values of OH-treated carrot cubes. HT could improve the carotenoid extraction by disrupting cell membranes and carotenoid-protein complex (Patras et al., 2009). This effect may explain the higher a* and lower H° values in HT-treated juices than the control one. In OH-treated juices, the lowest L* and higher a* values could be associated with a higher diffusion of dyes and salts through cell membranes than in HT, probably explained by the phenomenon of electroosmosis (Loypimai et al., 2015).

The values of ΔE (Table 1) for the OH- and HT-treated juices showed no significant differences (p < 0.05) and would be in the "well visible" range (Cserhalmi et al., 2006). These results indicate that both OH and HT generate changes that could be perceived even by inexperienced observers. Other authors have reported that the values of ΔE obtained for carrot juices treated by thermal (Gong et al., 2015; Gouma et al., 2020) and non-thermal (Szczepańska et al., 2020) technologies were similar.

3.4. Enzyme activities

The values obtained from the residual activities of POD, PPO and PME of the control, OH-treated and HT-treated carrot juices are shown in Table 2.

POD activity in the control juice was 177.5 \pm 2.8 $\Delta A/min/L$. Both OH and HT treatments led to inactivation levels greater than 99%. Similarly, Mannozzi et al. (2019) achieved 80% of POD inhibition in carrot pulp by applying a pretreatment with OH at 40–80 °C (114 V/cm).

PPO activity in the control juice was $2.34 \pm 0.11 \Delta A/min/L$. The OH treatment led to an inhibition of 80%, whereas HT led to an inhibition of 68%. In apple juice pretreated with HT (80 °C) and OH (20–80 °C, 114 V/cm), Mannozzi et al. (2019) achieved 75% of PPO inhibition.

Both PPO and POD are involved in the enzyme browning mechanism, which can generate off-flavor and result in the loss of nutritional and

Table 2

Effects of OH and HT on residual enzymatic activity, antioxidant capacity (FRAP) and carotenoids of carrot juice.

			-			
Treatment	POD RA (%)	PPO RA (%)	PME RA (%)	FRAP (µM TE)	α-carotene (mg/L)	β-carotene (mg/L)
Control	100	100	100	1734 ± 147 ^c	22.5 ± 0.9^a	$69.6\pm3.2^{\text{a}}$
HT	$0.06 \\ \pm \\ 0.00^{b}$	31.8 ± 3.45^{a}	15.0 ± 1.32^{a}	$\begin{array}{c} 2051 \\ \pm \ 155^b \end{array}$	21.4 ± 0.9^{a}	65.5 ± 3.3^{a}
ОН	$0.26 \\ \pm \\ 0.02^{a}$	19.9 ± 2.55^{b}	17.2 ± 1.19^{a}	$\begin{array}{c} 2709 \\ \pm \ 165^a \end{array}$	22.2 ± 1.0^{a}	68.9 ± 3.5^a

Data are expressed as means \pm standard error (n = 9). Values with different letters (a, b, c) in the same column are significantly different (p < 0.05). POD RA: residual activity of peroxidase, PPO RA: residual activity of polyphenoloxidase, PME RA: residual activity of pectinmethylesterase, FRAP: Ferric reducing antioxidant power.

economic value of juices (Terefe et al., 2014). According to the inactivation levels reached for both enzymes in OH- and HT-treated juices here evaluated, it is expected to have a favorable effect on their quality.

Regarding PME, control juice activity was $10.57 \pm 0.54 \Delta A/min/L$, and the OH and HT treatments inhibited this enzyme by 83 and 85%, respectively. Generally, PME is responsible for losing viscosity and turbidity in juices (Espachs-Barroso et al., 2006). Although no other studies have evaluated the effect of OH on PME activity in carrot juice, Luo et al. (2008) achieved 37.35% of PME inactivation by applying PEFs (40 °C), which resulted in turbidity similar to that of fresh juice during its 28-day shelf-life.

Therefore, the application of the OH was more efficient than the HT in the inactivation of PPO from the carrot juice; although, similar results were obtained in the inactivation of PME and POD. This result could be associated with a non-thermal effect that caused the inactivation of PPO. The activation and inactivation of enzymes due to OH may be associated with conformational changes and the influence of electrostatic forces on the tertiary structure (Samaranayake & Sastry, 2018). Also, the oscillating electric field and the dissipation of energy by friction would induce molecular movement that changes the structure, and consequently, the activity of the enzymes (Shao et al., 2019). Despite the numerous published studies, it is difficult to accurately distinguish the thermal and non-thermal effects of electric fields on enzyme activity (Kubo et al., 2020). The existence of non-thermal effects can be more or less evident or indistinguishable depending on several parameters and factors, such as electric field strength, frequency, power, temperature distribution, time temperature history, food medium/matrix, and strain or type of biological material to be evaluated (Kubo et al., 2020).

3.5. Microbiological analysis

The values obtained for TPC, Y&M, TCC and CSR counts in the control, OH-treated and HT-treated carrot juices during the 60 days of storage at 4 $^{\circ}$ C are shown in Table 3.

The counts in the control juice at time 0 (Table 3) were in the range usually measured for carrot juice (Gong et al., 2015; Riganakos et al., 2017). The application of OH and HT significantly reduced all microbiological counts, with values below the quantification and/or detection levels, which indicated a correct pasteurization.

After 60 days of storage, the TPC counts increased to 2.27 ± 1.07 and 2.65 ± 1.19 Log CFU/mL in HT and OH-treated juices, respectively. Besides, the TPC counts in the control juice exceeded the recommended limits for consumption (<5 Log CFU/mL) after 30 days of storage. All other counts remained at values below the quantification and/or detection levels until the end of storage. It has been described that, in OH treatments, microorganisms are inactivated through heat since the force of the electric field (10 kV/cm) necessary to cause their lysis is much higher than that usually applied (Jaeger et al., 2016). Also, no microorganisms or pathogenic strains with particular resistance to OH treatment were reported (USA-FDA, 2000).

3.6. Volatile compounds

In control, OH-treated and HT-treated carrot juices, we identified 36, 37 and 41 VOCs, respectively, comparing their MS with those in the

Table 3

Microbial parameters of carrot juice during 60 days of storage at 4 $^\circ\mathrm{C}$ after OH and HT.

Treatment	Storage time (days)	TPC Log CFU/mL	Y&M Log CFU/mL	TCC Log CFU/mL	CSR Log CFU/mL
Control	0	$3.09 \pm$	$1.76 \pm$	$1.50 \pm$	ND
		0.64	0.66	0.55	
	15	4.21 \pm	$2.16 \pm$	1.54 \pm	ND
		0.68	0.66	0.27	
HT	0	<1	ND	<1	ND
	15	<1	ND	<1	ND
	30	1.70 \pm	ND	< 1	ND
		0.78^{a}			
	60	$\textbf{2.27}~\pm$	ND	< 1	ND
		1.07^{b}			
OH	0	ND	ND	<1	ND
	15	<1	ND	<1	ND
	30	$2.05~\pm$	ND	<1	ND
		0.75 ^A			
	60	$2.65~\pm$	ND	< 1	ND
		1.19^{B}			

Data are expressed as means \pm standard error (n = 3). A,B and a,b represent different comparison groups (OH vs Control; HT vs Control), whereas different letters in the same column represent a significant difference within the same time (p < 0.05).

TPC: total aerobic mesophilic count, Y&M: yeast and mold cells count, TCT: total coliforms count, CSR: clostridium sulfite-reducers count. ND: non detected, <1: below the quantification limit.

mass spectral database NIST (Version 2.0, 2002; NIST USA). Terpenes were the chemical group present in the highest amounts in the three juices. The high proportion of monoterpenes among the total VOCs of the control juice (90.0 \pm 0.5%) was not altered (p > 0.05) by the application of HT or OH (88.8 \pm 0.5 and 90.0 \pm 0.2%, respectively). Also, the proportion of sesquiterpenes in HT- and OH-treated juices (9.6 \pm 0.6 and 9.0 \pm 0.2%) was similar (p > 0.05) to control juice one (9.6 \pm 0.5%). In agreement with previous studies (Umair et al., 2019), γ -terpinene, γ -pinene, sabinene, terpinolene, β -myrcene, p-cymene, α -thujene, limonene, α -terpinene, β -pinene, β -caryophyllene, and α -patchoulene represented 90% of the total VOCs of the three juices (Fig. 3). It should be noted that the HT-treated carrot juice presented the lowest value of the monoterpene terpinolene (Fig. 3). Previous studies have described that carrot monoterpenes contribute to the consumers' ratings "citrus-like" and "fruity", while sesquiterpenes contribute to the consumers' ratings "spicy" and "woody", related to the bitter and rough taste of carrots that often causes consumers' rejection (Bayindirli, 2010).

To investigate the existence of underlying dependencies between VOCs and the different treatments, we performed a PCA, for which we selected the VOCs that showed significant differences (p < 0.05)

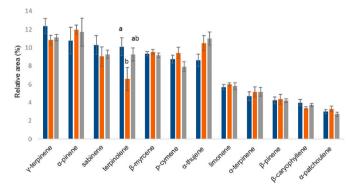


Fig. 3. Main volatile compounds in control, OH-treated and HT-treated carrot juice.

Data are expressed as means \pm standard error (n = 5). Values with different letters (a, b, c) in the same terpene are significantly different (p < 0.05) from each other (control HT-treated, OH-treated).

between treatments (five monoterpenes, four sesquiterpenes and two from other chemical groups). Fig. 4 shows a biplot of the carrot juices and VOCs. The first two components of the PCA explained 70.7% of the data variability (PC1 = 52.5%; PC2 = 18.2%).

PC1 was positively correlated with camphor, α -humulene, α -bisabolene and α -longipinene and negatively correlated with α -bergamotene, β -ocimene and sabinene. PC2 was only positively correlated with hexanal. The PCA showed that the HT-treated juice was characterized by the highest values of camphor, α -bisabolene, α -humulene and α -long-ipinene, and the lowest values of sabinene, α -bergamotene, and β -ocimene relative to the control and OH-treated juices. On the other hand, the control juice presented the highest values of sabinene, α -bergamotene, β -ocimene, and hexanal and the lowest ones of the sesquiterpenes α -bisabolene, α -humulene and α -longipinene.

The OH-treated juice seemed to have a profile similar to that of the control juice but with lower values of sabinene, α -bergamotene, β -ocimene and hexanal. These results could be explained by the fact that, during processing, the terpenoids in the samples can either be produced directly or be converted, from an initial product, into sesquiterpenes or monoterpenes by oxidation, dehydrogenation and other reactions (Korkmaz et al., 2017). Although it has been described that a reduction in carrot terpenes would affect the characteristic flavor of carrot juice (Kebede et al., 2014), it is to be hoped that changes in terpenes associated with heat treatments have no impact on juice flavor as they represent a minority fraction.

3.7. Total antioxidant capacity

The TAC of the control carrot juice (1734 \pm 147 μ M) (Table 2) was slightly higher than that previously reported by Wootton-Beard et al. (2011) (1369–1533 μ M). The TAC of carrot juice increased significantly (p < 0.05) after OH treatment (Table 2). Also, the antioxidant capacity of the OH-treated juice was significantly (p < 0.05) higher than that of the HT-treated juice. Similarly, Mannozzi et al. (2019) reported an increase in the TAC of carrot juice, as measured by the ABTS technique, when the pulp was pretreated (HT 80 °C and/or OH from 20 to 80 °C).

Most of the antioxidant activity of fruits and vegetables is generated by the presence of phenolic compounds (hydroxycinnamic acids, flavan-3-ols, anthocyanins, flavonols and dihydrochalcones) (Zhang & Hamauzu, 2004). In pumpkin juice, Bhat et al. (2017) obtained the highest values of total phenols after treating it with OH (80 °C - 4 min), intermediate values after treating it with HT (90 °C - 5 min), and the lowest values in the control juice.

According to the results obtained, the OH treatment greatly benefits the TAC of carrot juice. This could be mainly due to the release of antioxidant compounds induced by electropermeabilization during electrical treatment (Mannozzi et al., 2019), which is an additional effect to that of HT. Therefore, OH would have the potential to extract the greatest amount of phenolic compounds with minimal damage to the integrity of the product (Bhat et al., 2017).

3.8. Carotene content

The β -carotene content of the control carrot juice (69.6 \pm 3.2 mg/L) (Table 2) was agree with previously reported values 50–200 mg/L (Kun et al., 2008; Quitão-Teixeira et al., 2009). However, the α -carotene content was lower (22.5 \pm 0.9 mg/L) than that reported (90 mg/L) by Kun et al. (2008). The amount of carotenoids depends on numerous variables such as species of carrot, soil, climate, etc. (Kun et al., 2008).

The OH and HT treatments did not significantly change (p > 0.05) the contents of α -carotene or β -carotene of the carrot juice (Table 2). Marx et al. (2003) obtained similar results in HT-treated carrot juice (95 °C), whereas other authors found that the application of OH in carrot cubes (Lemmens et al., 2009), did not significantly change the β -carotene content.

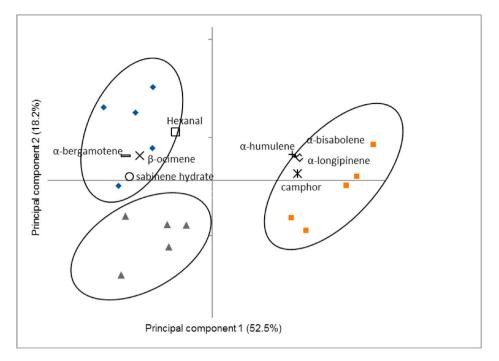


Fig. 4. PCA biplot based on volatile compounds of the control () OH-treated () and HT-treated () carrot juice.

3.9. Sensory analysis

The results of the sensory evaluation indicated that, regarding color, the OH-treated juice was preferred (p < 0.05) over the other samples. Regarding flavor, the results showed no significant differences between the treated juices and the control (p > 0.05). Therefore, in this study, the application of OH and HT treatments seems to have had no effect over flavor, preserving that of the control juice. It is important to highlight that previous studies in which carrot juice was treated with OH did not provide data on sensory assessments. Regarding other formulations, Dima et al. (2015) reported that the sensory evaluation of mixed juice (carrot, pumpkin, celery and citrus fruits) treated by OH (70 °C) achieved a higher score relative to the juice treated by HT (70 °C - 10 min), indicating that aroma and appearance were the most appreciated characteristics. On the other hand, Tumpanuvatr and Jittanit (2012) indicated that the color and flavor of orange juice and pineapple juice treated by OH (80 °C) did not differ from those treated by HT (60 °C).

Considering the results obtained in the color and flavor preference test, the carrot juice treated by OH was selected to carry out a hedonic test with consumers. The results indicated that 47.9% of consumers considered the OH-treated juice as "like extremely", thus receiving the highest score (9) of the hedonic scale used. At the same time, 99% of the consumers placed the OH-treated juice in the range between "like extremely" and "like slightly" (Fig. 5).

4. Conclusions

The OH and HT treatments allowed obtaining carrot juice that was microbiologically stable for 60 days under refrigerated storage at 4 °C. Both treatments also led to inhibition of POD, PME and PPO enzyme activities, with the OH treatment leading to more significant PPO inactivation than HT. The flavor of OH- and HT-treated juices was not modified compared to the control juice, which may be associated with the fact that the terpene profile was unchanged in treated and control juices. Also, the OH-treated carrot juice presented differences in its chromatic parameters regarding the control and HT-treated juices. The semi-trained panelists perceived these differences since most of them preferred the OH-treated juice due to its color. Besides, the OH-treated

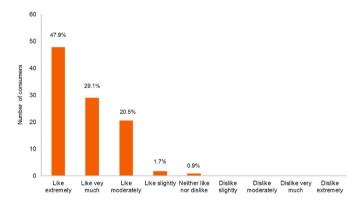


Fig. 5. Consumers' acceptance test of the OH-treated carrot juice carried out in the organic products market "*Sabe la Tierra*" (San Fernando, Buenos Aires, Argentina) (n = 117).

juice had a higher total antioxidant capacity than the control and HTtreated juices, the same carotene content as the control juice, and a high level of acceptance among consumers.

Thus, the application of OH would be an alternative to conventional HT in carrot juice production. This study provides technical information that could be used to take advantage of discarded carrots and constitute a promising basis to continue and carry out future studies on an industrial scale. Complementary studies should aim to assess the stability of quality attributes during storage and identify any non-thermal effect of OH on the quality parameters.

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Authors' contributions

All authors contributed equally to the manuscript.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2021.111255.

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