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1 **Evaluation of quality parameters of orange juice stabilized by two thermal treatments (helical**
2 **heat exchanger and ohmic heating) and non-thermal (high-pressure processing)**

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20 **Abstract**

21 **This work aims to compare the impact of three thermal (helical coil heat exchanger HCHE,**
22 **ohmic heating OH, and mild pasteurization MP) and one non-thermal (high-pressure**
23 **processing HPP) treatments on orange juice by using industrial plants. Nutritional (total**
24 **phenolic content (TPC), ascorbic acid (AA) and total antioxidant capacity (TAC)), physical**
25 **(viscosity, colour, browning index (BI) and suspended pulp (SP)), sensory (Triangle test and**
26 **QDA) as well as chemical (¹H NMR spectroscopy) aspects were analysed. Results revealed a**
27 **significant (p<0.05) increase in viscosity for HPP (+ 20%) compared to untreated samples while**
28 **the opposite effect was observed for all thermal treatments (-22%). The lowest a* values were**
29 **observed in HPP and HCHE samples. Total phenolic content decreased significantly only in**
30 **HCHE (-14%), while the highest ascorbic acid content was observed in HPP samples and it**
31 **resulted not significantly different from untreated. Regarding the chemical profile, treated**
32 **samples (except for MP) led to a significant (p<0.05) decrease of all selected marker peaks,**
33 **mainly including sugars (alpha- and beta-glucose, beta-fructose, and sucrose) and amino acids**
34 **compared to untreated ones. HPP samples showed a similar sensory profile if compared with**
35 **the untreated sample, showing only a significant difference (p<0.05) in terms of orange aroma;**
36 **on the contrary, OH, HCHE, MP rated the lowest acceptances due to, among all considered**
37 **descriptors, orange aroma, cooked aroma, sweetness, cooked taste.**

38

39 **Keywords:** Thermal processing; colour; phenols; viscosity; sensory evaluation

40

41 Introduction

42 The global consumption of fruit-based beverages reached 95.7 billion litres in 2018 of which
43 soft drinks accounted for 37.2 billion. This was followed by nectars (10.9 billion), 100% juice
44 (16.6 billion), and powdered and concentrated juices (30.4 billion). Citrus juices including
45 orange juice (OJ), made from the endocarp of the *Citrus sinensis* fruit, accounts for 60% of all
46 juices and juice-based beverages in Western Europe (AIJN, 2014). Orange juice represented
47 43.8% of the juice market, as well as in the juice drinks segment, with 26.2% of the market
48 (Neves et al., 2020). Orange juice is an essential source of bioactive and antioxidant compounds
49 such as vitamin C (Davey et al., 2000), carotenoids (Rodriguez-Amaya et al., 1997), provitamin
50 A and flavanones (Gil-Izquierdo et al., 2001). In the last decades, the demand for more natural,
51 functional, quality, and minimally processed foods has increased exponentially (Bansal et al.,
52 2015).

53 While pre-pasteurized and minimal processed orange juices have favourable sensory and
54 nutritional characteristics, they are synonymous with having a short shelf life due to the
55 presence of spoilage causing bacteria, yeasts, and endogenous enzymes (Lee & Coates, 1999).

56 Thermal pasteurization (TP) is often used to inactivate bacteria and endogenous enzymes and
57 therefore extend the shelf life of OJ. However, TP may cause irreversible sensorial, nutritional,
58 and physical-chemical changes in the OJ (Braddock, 1999). For example, OJ has been shown
59 to lose representative aroma and taste components, and vitamin C during thermal treatments
60 (94°C/30s) and subsequent storage (4°C/15 days) (Yeom et al., 2000). Moreover, regarding the
61 energy consumption of the process, Pardo and Zufía (2012) studied the environmental impacts
62 of traditional and novel preservation technologies through LCA methodology: Authors
63 reported that thermal technologies showed a high environmental load, related directly or
64 indirectly to the fossil fuel combustion processes involved in the thermal energy generation

65 **phase.** Improving the heat exchange efficiency through design optimization using e.g.,
66 Computational Fluid Dynamics (CFD) techniques (Rinaldi et al., 2019) could be a valuable way to
67 reduce energy consumption in traditional thermal processes. Among heat exchangers **available to**
68 **date, helical coil heat exchangers are widely used in industrial applications due to the compact**
69 **structure, high heat transfer coefficient, and therefore represent a promising choice for energy**
70 **saving in the food industry (Jayakumar et al., 2008; Jayakumar et al., 2010).** Many researchers
71 have identified that a complex flow pattern exists inside a helical pipe due to which the enhancement
72 in heat transfer is obtained thanks to the development of secondary flow. The curvature of the coil
73 governs the centrifugal force while the pitch (or helix angle) influences the torsion to which the fluid
74 is subjected.

75 **High-pressure processing (HPP) has received a lot of attention in the last years because of the**
76 **widespread implementation of the clean-label approach and its minimal impact on sensory and**
77 **nutritional qualities (Roobab et al., 2021). HPP has been demonstrated to be effective in reducing**
78 **microbial flora in fruit beverages while retaining the sensory quality of the product without affecting**
79 **the structure of small molecules such as bioactive substances (Bull et al., 2004; Rastogi et al., 2007;**
80 **Oey et al., 2008; Barba et al., 2010).** This process applies high hydrostatic pressure to inactivate
81 several enzymes and pathogenic bacteria that cause food product quality degradation: in particular,
82 sufficiently high pressures and long processing times (700 MPa/1 min) in HPP treatments of OJ were
83 more effective at preserving the turbidity of OJ, that can be lost during storage **because of enzymatic**
84 **de-esterification and degradation by pectin-esterase (Goodner et al., 1999).** Electrical resistance
85 heating, joule heating, and electro-heating are all terms **applied to:** ohmic heating (OH). **OH, may be**
86 **a favourable food processing technology because it provides rapid and uniform heating**
87 **resulting in reduced thermal damage to the product and fouling phenomena in the heating**
88 **section when compared to traditional thermal pasteurisation. Few studies have evaluated the**
89 application of this technology to process orange juice and compared simultaneously with other

90 treatments (Leizeron and Shimoni, 2005a and 2005b; **Tumpanuvatr and Jittanit 2012;**
91 **Demirdöven and Baysal, 2014; Hashemi et al., 2019).**

92 By considering the quality parameters of OJ, several thermal and non-thermal technologies have been
93 tested and evaluated to process and stabilize OJ, such as conventional thermal methods, atmospheric
94 cold plasma, ozone treatments, microwave-assisted pasteurization, HPP, pulsed electric field, OH
95 (Goodner et al., 1999; Ayhan et al., 2002; Gil-Izquierdo et al., 2002; Sanchez-Moreno et al., 2005;
96 Leizeron et al., 2005ab; Baxter et al., 2005; Patil et al., 2009; Velazquez-Estrada et al., 2013; Alves
97 Filho et al., 2016; Brugos et al., 2018; Atuonwu et al., 2020).

98 Atuonwu et al. (2020) studied microwave, OH, HPP, and conventional thermal methods for energy
99 consumption per litre of orange juice, as well as associated greenhouse gas emissions. **Despite**
100 **innovative technologies (especially OH) showing significantly higher efficiency, the high costs**
101 **of grid electricity results in poorer processing economics when compared to conventional gas-**
102 **fired technologies. However, the authors stated that with shifting trend towards renewable**
103 **sources of energy, the ohmic technologies will eventually become more economically feasible.**
104 **Atuonwu et al. (2020) did not observe significant** differences among the different thermal and non-
105 thermal methods for some specific OJ quality parameters (flavour compounds, vitamin C), **this may**
106 **be due to the application** of mild thermal treatment conditions and short processing times employed.
107 Baxter et al., (2005), despite demonstrating that the HPP (600MPa / the 60s)-treated orange juice was
108 acceptable for odour and flavour profile up to 12 weeks of storage at 4°C, did not report significant
109 differences related to the sensory profile and shelf life compared to thermally pasteurized OJ.
110 **Conversely, other studies reported superior product quality characteristics for OJ processed**
111 **using non-thermal technologies: particularly preservation of bioactive and thermolabile**
112 **compounds were reported to be favourable in HPP than in pasteurization (Cortés et al., 2008;**
113 **Vieira et al., 2018).** other researchers reported that also OH was more gentle than standard

114 pasteurization in retaining some specific flavour compounds (Leizeron and Shimoni, 2005a and
115 2005b).

116 Understanding the effects of various technologies on juice quality features is critical for designing
117 and optimizing technological parameters to generate high-quality foods. Thus, the present work aims
118 to investigate the effects of mild pasteurization, and helical coil heat exchangers compared to ohmic
119 and high-pressure processing for the pasteurization treatment of orange juice. In particular, colour,
120 viscosity, total phenol content, ascorbic acid content, and sensorial quality were investigated.

121

122 **Materials and Methods**

123 *Samples, preparation, and storage*

124 **Fresh orange juice (untreated UNT) was prepared from *Citrus sinensis*. A mix of Valencia and**
125 **Navel varieties were purchased from MACE S.R.L (Ferrara, Italy).** The OJ was subjected to
126 different treatments: mild pasteurization (MP), a helical coil heat exchanger (HCHE), ohmic heating
127 (OH) and high hydrostatic pressure (HPP). Thermal pasteurization represents the **widest** treatments
128 for obtaining a shelf-stable product while HPP was tested as the less impacting treatment for a
129 refrigerated product. All the samples were stored at a refrigerated temperature ($+4 \pm 1^\circ\text{C}$) until the
130 analyses were performed.

131

132 *1. Mild pasteurization (MP)*

133 **Mild pasteurization treatments** were carried out in a pilot plant at the University of Parma
134 laboratories as reported in Rinaldi et al. (2018). The treatment was performed at a flow rate of 20 L/h
135 and a residence time of 20 s at $72 \pm 1^\circ\text{C}$ as **reported by** Vervoort et al. (2011) with a pasteurizing
136 effect after $F_{90}^{6.7} = 7 * 10^{-4}$. After the **MP** treatment, OJ was rapidly cooled using a continuous
137 cooling section to a final temperature $20 \pm 1^\circ\text{C}$, then packed in PET bottles under aseptic condition
138 and stored at refrigerated temperature ($+4^\circ\text{C}$) for further analysis. **Treatment was repeated three**
139 **times and 5 litres of OJ were treated each time.**

140

141 *2. Helical coil heat exchanger (HCHE)*

142 A helical coil heat exchanger (HCHE) (Sterideal® HX Coil, John Bean Technologies, Chicago,
143 Illinois, United States) **was studied which consisted of** a helical coil stainless steel 304 tube with an
144 internal diameter of 30 mm and a total length of 8.5 m. During the treatment, the flow rate was set to
145 2000 L/h, fastest particle holding time of 7.72 s at 97°C . Thermal treatment was designed with a

146 pasteurizing effect equal to $F_{90}^{6.7} = 1.5$ for as suggested by Leizeron & Shimoni (2005a) by
147 achieving a complete reduction of relevant pathogens and spoilage bacteria as well as a residual
148 activity of thermostable PME of about 5% (Velázquez-Estrada et al., 2012). During tests,
149 temperatures at the beginning and the end of the holding section were measured using a resistance
150 temperature detector Pt100 with a diameter of 3 mm (Endress+Hauser AG Reinach BL, Switzerland).
151 **Recorded temperature profile during tests confirmed the target F-value with a value equal to**
152 **$F_{90}^{6.7} = 1.54$.** After thermal treatment, the samples were rapidly cooled in the aseptic portion of the
153 plant, for about 5 min to reach 25 °C and aseptically packed in 10 L bags (Goglio Spa, Milano, Italia
154 - PE/MET/PE, thickness 77 µm using of commercial plant (AF200 Classic Aseptic Filler, John Bean
155 Technologies, Chicago, Illinois, United States). **Trials were performed on an industrial plant in**
156 **duplicate and for each trial approximately 400 kg of the product was used.**

157

158 **3. High-pressure processing (HPP)**

159 High hydrostatic pressure treatments were conducted in a 300 L high-pressure plant (Avure
160 Technologies Inc., Erlanger, Kentucky, United States). Samples were packed in PET bottles with an
161 internal volume of 250 ml. An indirect direct method for generation of high isostatic using means of
162 cold water (4 °C) was used, and the temperature increase due to compression was not higher than 2-
163 3 °C/100 MPa. **HPP** treatments were conducted at 550 MPa for 90 s, chosen based on the goal of
164 inactivation of pectin methyl esterase (PME) (Nienaber & Shellhammer, 2001) and to be
165 **microbiologically safe (Linton et al., 1999)**. The treated samples were then stored at a refrigerated
166 temperature (+4 °C). **HPP treatments were conducted in triplicate and for each repetition 15**
167 **bottles were treated.**

168

169 **4. Ohmic heating (OH)**

170 Ohmic heating (OHM, Sterideal DT ®, John Bean Technologies, Chicago, Illinois, United States):
171 temperature increases up to 97 °C was obtained using an ohmic heater at a flow rate of 2,000 L/h

172 obtaining the same sterilizing effect reported above for HCHE. The industrial plant used the same
173 holding section already described for HCHE and experimental F-value was equal to $F_{90}^{6.7} = 1.60$.
174 **The electrical power required was precalculated using plant software at the set temperature**
175 **based on samples electrical conductivity measured by using a digital conductivity meter (0.413**
176 **Sm⁻¹ at 25 °C and 0.832 Sm⁻¹ at 60 °C) and by reported values (Leizeron & Shimoni, 2005a).**
177 **The final precise electrical power adjustment was then obtained using a feedback control on**
178 **product temperature measured at the outlet of the ohmic section and the product used for this**
179 **setting was discarded.** After thermal treatment, the samples were cooled and aseptically packed in
180 10 L bags (Goglio Spa, Milano, Italia - PE/MET/PE, thickness 77 μm). **Trials were performed on**
181 **an industrial plant in duplicate and for each trial, approximately 400 kg of the product was**
182 **used.**

183 The degree of thermal damage in thermal treatments was expressed in terms of cook value $C_{T_{ref}}^z$ and
184 obtained from the integration of the heat penetration curve:

185
$$C_{T_{ref}}^z = \int_0^t 10^{(T-T_{ref})/z} dt$$

186 where:

187 **t = time at T**

188 **T = temperature of the product at different times**

189 **T_{ref} = reference temperature; set equal to 100 °C**

190 **z = temperature increase that induces a 10-fold increase of the reaction rate of the chemical reaction**
191 **taken as a reference; z was set at 33°C.**

192

193 ***Physical and chemical characterization of orange juice***

194 ***Total soluble solids, suspended pulp, and pH***

195 The total soluble solids of orange juice (°Brix) were determined in triplicate at 25°C by refractometer
196 (Model 2WAJ, Optika, Italy). The pH was measured in triplicate using a pH meter (Model 3150,
197 Jenway, UK). Suspended pulp was measured using **the** IFU Method No 60 (2005).

198

199

200 *Viscosity*

201 The apparent viscosity profile of the orange juice samples was measured with concentric cylinder
202 geometry (Couette cell) mounted on Ares strain-controlled (ARES) rheometer (TA Instruments, New
203 Castle, DE, USA) and connected to a thermostatic bath (NESLAB RTE 111, Thermo Fisher
204 Scientific, Massachusetts, USA) for temperature control during the analysis. The dimensions of the
205 geometry were 34 mm cup diameter, 32 mm bob diameter, and height of 33 mm. 15 mL of the sample
206 were transferred using a graduated cylinder to the rheometer cup set at 25°C and sample temperature
207 left to equilibrate for 2 min before starting the test. The tests were performed from 10 to 200 s⁻¹ shear
208 rates and viscosity values at 100 s⁻¹ were compared.

209

210 *Colourimetric analyses*

211 The colour of orange juice samples was measured using image analysis with a desktop flatbed
212 scanner (Hewlett Packard Scanjet 8200, Palo Alto, CA, USA) at 600 dpi of resolution, equipped with
213 **a cold cathode lamp for reflective scanning. During image acquisition, the scanner was held in**
214 **a black box, it excludes surrounding light and external reflections. Flatbed scanner colour**
215 **(RGB) was corrected as previously reported by N'Dri et al. (2010) and converted to CIE Lab**
216 **using of ImageJ software (National Institutes of Health (NIH), Maryland, USA). The**
217 colorimetric analysis was performed in triplicate.

218 The browning index (BI) was measured using the method of Tiwari et al. (2008) and absorbance was
219 obtained at 420 nm by using a Phillips PV 8700 spectrophotometer.

220

221 ***Total phenolic content (TPC) and ascorbic acid content***

222 The Folin-Ciocalteu colorimetric test (Papagiannopoulos et al., 2004) was used to calculate the total
223 phenolic content (TPC) of the samples. 1 mL of sample was mixed vigorously with 70 mL pure water
224 and 5 mL Folin-phenol Ciocalteu's reagent (Sigma-Aldrich, Switzerland). After 5 minutes, 10 mL of
225 a saturated sodium carbonate solution was added, and the sample was brought up to a final volume
226 of 100 mL with distilled water. A UV–V is spectrophotometer was used to assess absorbance after
227 60 minutes at 720 nm. Total phenols were calculated as mg of (+)-catechin equivalents per kilogram
228 of total phenols. Samples were homogenized and diluted with distilled water before being used in a
229 colorimetric experiment.

230 HPLC–DAD was used to determine the amount of ascorbic acid in the sample. Before HPLC analysis,
231 materials were diluted with a 6 percent aqueous metaphosphoric acid solution, uniformly mixed with
232 Ultra-Turrax (T25 basic IKA®, IKA-Werke, Staufen, Germany), and filtered using a paper filter and
233 a 0.45 µm syringe drive filter unit. Solutions of 0.5 to 100 mg/kg of L-ascorbic acid in 6%
234 metaphosphoric acid were made as calibration standards.

235

236 ***Chemical profile analysis***

237 **¹H NMR spectroscopy method was used for the analysis.** For ¹H NMR analysis, 300 µL of OJ
238 samples were added to 400 µL of D₂O (Sigma Aldrich, Saint Louis, MO, USA) and 100 µL of 3-
239 (trimethylsilyl)-propionate-d₄ sodium salt, 98% atom D (TSP) (Sigma Aldrich, Saint Louis, MO,
240 USA) at 1 mg mL⁻¹ as internal standard. After centrifugation (at 4 °C, 3900 g, for 30 min), 600 µL
241 of the supernatant were filtered and transferred into 5 mm NMR glass tubes. For each thermal
242 treatment, triplicates were prepared and analyzed NMR spectra were recorded on a Bruker Avance™
243 III 400 MHz NMR Spectrometer (Bruker BioSpin, Rheinstetten, Karlsruhe, Germany) operating at a
244 magnetic field-field strength 4 T. Spectra were acquired at 298 K, with 32 K complex points, using a
245 90° pulse length and 3 s of relaxation delay (d1). A total of 128 scans were acquired with a spectral

246 width of 9595.8 Hz and an acquisition time of 1.707 s. The relaxation delay and acquisition time
247 allow the complete relaxation of the protons, allowing their integrals for quantitative purposes.
248 Acquired ¹H NMR spectra were processed applying a Fourier transform, transferred to MestReNova
249 software (release 6.0.2, Mestrelab Research, Spain) and referenced to TSP (0 ppm). The assignment
250 of ¹H NMR signals was supported using data available in the literature (Pham et al., 2021) and the
251 metabolomics data repository for NMR Metabolomics (bmr.io). An integration pattern was defined
252 by choosing buckets manually in the range between 0 and 9 ppm was considered spectra in the
253 overlapped form. This procedure permitted the choice of buckets sufficiently large to compensate for
254 the small chemical shift fluctuations in each spectrum, corresponding to a defined signal or to a group
255 of signals, which simplifies the interpretation of statistical results. The defined pattern was used for
256 automatic integration of all spectra and referred to TSP area.

257

258 *Sensorial analysis*

259 All sensorial tests were carried out at Stazione Sperimentale per l'Industria delle Conserve Alimentari
260 (SSICA) in a laboratory compliant to UNI EN ISO 8589:2010. **Sensorial test were carried out on**
261 **all samples except for MP: mild pasteurization is a stabilization technique that is generally**
262 **coupled with refrigerated storage and no complete inactivation of microorganisms. Based on**
263 **the microbiological results (data not shown), MP samples were excluded from sensorial**
264 **analyses for safety reasons.** Initially, a consumer discriminant test was conducted with 23 untrained
265 subjects (13 males, 10 females: average age 25 ± 8 y). The participants were asked to refrain from
266 eating, smoking, drinking, or chewing gums for 1 h prior to testing. A triangle forced-choice
267 procedure was used to determine differences between samples from different treatments. Participants
268 were requested to determine which sample was the odd one. Each sample was identified by a 3-digit
269 code and the order of sample presentation was randomized. Moreover, a quantitative descriptive
270 analysis (QDA) was performed with 10 trained judges on the following attributes: colour orange

271 aroma, cooked aroma, off-flavours, sweetness, bitterness, acidity, cooked taste, off-taste, taints,
272 overall liking. The intensity of each attribute was rated using unstructured line scales (scaled 0–10).

273

274 *Statistical analysis*

275 SPSS (v. 27.0, SPSS Inc., Chicago, USA) was used to calculate means, standard deviations and to
276 perform one-way analysis of variance (ANOVA) with Tukey HSD post-hoc test to evaluate the
277 significant differences among treatments ($p < 0.05$).

278 **Results and discussion**

279 *Physico-chemical analyses*

280 The total soluble solids content and pH of the orange juices and ranged from 10.8 to 11.3° Brix and
281 from 3.6 to 3.8, respectively (results not shown), and presented not significant differences between
282 treatments. Our results are in accordance with Demirdoven et al. (2014), **who** did not observe
283 significant differences among OH, pasteurized and raw OJ samples related to water soluble matter
284 and pH values. Also, Timmermans et al., (2011) **reported no** statistical differences in terms of pH
285 and °Brix for OJ untreated and treated with HPP and mild heat pasteurization.

286

287 *Viscosity*

288 **The** viscosity data of orange juices at 100 s⁻¹ are **shown in Figure 1**; among the samples, two different
289 trends were **observed**: HPP samples showed significantly higher viscosity compared to UNT while,
290 all thermally treated OJ expressed significantly lower viscosity. This result agrees with previous
291 studies on OJ treated with HPP (Polydera et al., 2005; Xu et al., 2015). **Generally, larger, and**
292 **irregular particles are reported to contribute to a higher viscosity in fruit juices due to the**
293 **higher hindrance to the flow compared to finer and more regular particles (Espinosa-Muñoz et**
294 **al., 2013). Suspended pulp results (Table 1) partially confirmed this observation with the**
295 **highest value of SP % in HPP samples among the other treated samples. All thermal treatments**
296 **(HCHE, OH and MP) despite their very low cook values, probably caused the non-enzymatic**
297 **degradation and the base-catalysed splitting of pectin chains via the β-elimination reaction with**
298 **a consequent reduction of viscosity. Suspended pulp results are not significantly different**
299 **between UNT and HPP (Table 1) but probably, despite the same total weight of sedimented**
300 **pulp, volume of particles could be different due to gelatinization of pectin caused by high**
301 **pressure as previously reported (Agcam et al., 2021). Sanchez-Moreno et al., (2005) observed that**
302 **HPP and high pasteurization OJ showed significantly lower viscosity than freshly squeezed orange**
303 **juice.**

304

305 ***Colour analysis***

306 Colorimetric parameters L^* and b^* did not present significant differences among the samples (**Figure**
307 **2**) while, **about** a^* values, a shift to negative values of this latter parameter (indicating a less red
308 colour) was **observed for** all the treatments **except for** HPP. This result agrees with Sánchez-Moreno
309 et al. (2005) that reported the same trend after thermal and HPP treatments. In addition, total colour
310 difference (ΔE) was calculated and the highest value was obtained for MP (3.0) samples while the
311 lowest one for HPP (2.0), as expected (Oey et al., 2008).

312 On the contrary, no significant differences were observed in hydro-soluble colour at 420 nm (**Table**
313 **1**) that is generally used as an indicator of non-enzymatic browning in fruit juices (**Valdramidis et**
314 **al., 2010**). **This result was in** adherence with the non-significant differences in colour measured by
315 sensorial analysis (**Table 3**). Finally, HPP allowed the complete retention of suspended pulp
316 compared to UNT as previously reported by Parish (1998), due to the absence of shearing by pumps
317 in the thermal pasteurization systems.

318 ***Total phenolic content (TPC), total antioxidant capacity (TAC) and ascorbic acid (AA) content***

319 Total phenolic content (TPC) of untreated samples was 747 mg/L (**Table 1**) and HCHE was the only
320 treatment that caused a significant decrease of this parameter; no significant differences were
321 observed for other treated samples. This result agrees with calculated cook values for thermal
322 treatments: HCHE presented the highest value of C_0 (0.69 min) followed by OH (0.42 min) **due** to
323 the very high heating rate and by MP (0.15 min) thanks to the low treatment temperature. Similar
324 results were obtained for ascorbic acid content and total antioxidant capacity (**Table 1**). Moreover,
325 results agree with sensorial data with reference to bitterness: phenolic compounds are generally
326 recognised as responsible of bitterness in OJ and no differences were observed for this descriptor
327 (**Table 3**)

328 **TPC in OJ measured by Vieira et al., (2018), revealed no significant changes between the HPP**
329 **and thermally pasteurized samples before storage. However, after storage,** TPC decreased in the
330 thermally pasteurized **samples** than in HPP. Sanchez-Moreno et al., (2005) observed that HPP
331 treatment increased the content of naringenin (20.2%), and hesperidin (39.9%) and this change may
332 be due to the modification in the structure of vesicles in the orange juice and to a greater extraction
333 of flavanones and other bioactive compounds such as carotenoids.

334 HPP samples also showed the highest value in terms of AA **when** compared to the other treated
335 samples, but a similar value **when** compared to UNT (**Table 1**). This observation was also in
336 accordance with previous findings that reported a lower degradation of AA in beverages treated with
337 HPP when compared to thermally treated **samples** (Barba et al., 2010). Also concerning the TAC,
338 UNT together with HPP showed the highest values ($23.0 \pm 1.15 \mu\text{mol}_{\text{Trolox}}/\text{g}$ and 22.4 ± 1.18
339 $\mu\text{mol}_{\text{Trolox}}/\text{g}$, respectively) **when** compared to the thermally treated **samples** (**Table 2**).

340

341 *Chemical profile of orange juice samples ¹HNMR*

342 The spectra of the analyzed OJ samples (**Figure 3**) were dominated by resonances especially from
343 amino acids, sugars (glucose, fructose and saccharose), organic acids and other secondary compounds
344 as ethanol and ethyl acetate. Among these, 9 compounds (**Table 2**) clearly gave diagnostic signals in
345 the NMR spectrum without overlapping, which permitted an accurate determination of their
346 concentrations (g/L), so they were selected as marker peaks to explore composition changes
347 throughout different stabilization processes on OJ.

348 Compared to UNT sample, HPP and all thermal treatments (except for MP) led to a significant
349 ($p < 0.05$) decrease of all selected marker peaks, mainly including sugars (alpha- and beta-glucose,
350 beta-fructose, and sucrose) and amino acids, which are suggested to play a significant role in non-
351 enzymatic browning reactions of thermal treated OJ (Pham et al., 2021). Results agree with SP values
352 (**Table 1**) with significant lower values for all thermally treated samples. Lower pulp content means
353 that suspended pulp was damaged by the treatment and its content released in the serum. OH, and

354 HCHE presented low SP value but probably reducing sugars were consumed by Maillard reaction
355 more than in MP due to the lower treatment temperature in the latter (**Rattanathanalerk et al., 2005**).
356 As previously discussed, HCHE presented the highest C₀ value and the lowest content of fructose
357 (**Table 2**) as it is recognized as the main precursor of reactive carbonyl species, indicating its
358 important role in the Maillard reaction (Paravisini and Peterson, 2019). Overall, HCHE and HPP are
359 the treatments that mostly caused the loss of nutrients in OJ, likely due to degradation reactions. On
360 the other hand, MP samples showed significant ($p < 0.05$) greater concentrations of all marker
361 compounds. This is probably because mild thermal treatment processes promoted the solubilization
362 of the water-soluble compounds without leading to their degradation due to the mild temperatures
363 and short time. **On the contrary, ethanol, ethyl acetate, D-alanine did not show significant**
364 **differences for UNT, HCHE and MP samples ($p > 0.05$); however, they significantly differed**
365 **from HPP and OHMIC (Table 2).** Vervoot et al., (2011) **reported** that no significant difference or
366 impact on sugars after HPP and pasteurization. So, also considering the previous observations from
367 color analysis, these results **generally suggest** that HCHE and HPP treatments are mainly responsible
368 to affect the visual and chemical/nutritional profile of the final product.

369

370 *Sensorial analyses*

371 Consumer discriminant test **demonstrated** that all treated samples were significantly recognized
372 ($p < 0.05$) from UNT, by 23/23 panellists, by 21/23 and by 17/23 for OH, HCHE and HPP,
373 respectively. To better understand the most important descriptors responsible for the differences,
374 QDA scores were analysed (**Table 3**): among all considered descriptors, orange aroma, cooked
375 aroma, sweetness, cooked taste of thermally treated OJ samples **were** significantly different (in almost
376 all the cases) from UNT ($p < 0.05$). HPP samples showed a similar sensory profile **when** compared
377 with UNT, showing only a **significant difference ($p < 0.05$) in terms of orange aroma. A previous**
378 **study (Leizeron and Shimoni, 2005a) reported results on OJ sensory profile showing a similar**

379 **flavour profile for fresh and ohmic-heated OJ and they were not distinguishable by panellists.**
380 **However, in this study $F_{90}^{6.7} = 0.83$ significantly lower compared to our study and probably for**
381 **this reason ohmic heating resulted favourable. Moreover, by considering overall liking results,**
382 **UNT resulted the most appreciated together with HPP, while on the contrary OH resulted the**
383 **less appreciate (Table 3). This result is in agreement with Parish (1998) which reported that**
384 **panellists generally perceived the flavours of high pressure treated juices to be significantly**
385 **closer to that of fresh one than flavours of thermally treated ones.** Three direct comparisons
386 between treated samples were also performed: OH vs. HPP (17/23), HCHE vs. HPP (23/23), and OH
387 vs. HCHE (15/23). In all assessments, untrained panellists were able to significantly ($p < 0.05$)
388 recognize samples confirming significant differences obtained in QDA (Table 3). **On the basis of**
389 **this result, HCHE technology seems not favourable with respect to sensorial profile of treated**
390 **OJ.**

391 **Conclusions**

392 This study aimed to evaluate the effect of traditional and emerging stabilization technologies on
393 orange juice (OJ) quality characteristics. **Fresh OJ** was compared with **treated OJ** by MP, HCHE,
394 OH, and HPP. The results highlighted significant differences, in terms of physical (viscosity, colour),
395 chemical (mainly total phenolic content and total antioxidant activity, sugar and ascorbic acid
396 content), and sensory parameters of treated OJ samples compared to fresh juices. Among the
397 evaluated treatments, HPP showed to be the best processing technology if compared to fresh OJ
398 quality characteristics. HPP juice showed a considerably higher sensory rating if compared to the
399 other treatments, and similar sensory characteristics to the untreated juice. Conversely OH did not
400 show any significant and strong advantage if compared to traditional treatments (HCHE, MP), in
401 terms of OJ quality parameters. MP is the treatment that better preserved the chemical profile if
402 compared to the untreated orange juice.

403

404 **Conflict of interest**

405 The Authors declare that there are no conflicts of interest

406

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