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1 **EFFECT OF HIGH HYDROSTATIC PRESSURE ON *SALMONELLA SPP***
2 **INACTIVATION AND MEAT-QUALITY OF FROZEN CHICKEN BREAST**

3
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11
12 **Abstract**

13 The aim of this study was to evaluate the effect of the pressure level and holding time on
14 the *Salmonella spp* inactivation during HHP processing in frozen chicken breast fillets.
15 Once identified the most effective process, meat quality (color and texture) was evaluated.
16 Results showed that the treatments at 500 MPa for 1 min and 400 MPa for 5 min were
17 enough to guarantee *Salmonella spp* inactivation in frozen chicken breast fillets. With
18 respect to quality parameters, an extension of shelf life is expected with both treatments, as
19 counts of indigenous microbiota were below the detectable level (< 2 logs CFU/g).
20 However, chromatic parameters and texture profile of the fillets treated with HHP suffered
21 significant changes. Even so, the treatment of 500 MPa for 1 min was more effective at
22 preserving chromatic parameters than treatment of 400 MPa for 5 min. The texture profile
23 between fillets treated was not significantly different

24 **Keywords:** *Salmonella spp*, frozen chicken breast, High Hydrostatic Pressure, Quality
25 properties

26

27 **1. Introduction**

28 Salmonellosis is a foodborne disease caused by non-typhoidal *Salmonella* enterica
29 serotypes (serotypes other than *S. Typhi* and *S. Paratyphi*) and is typically characterized by
30 a self-limiting gastroenteritis syndrome manifested as diarrhea, fever and abdominal pain
31 (Crump, Sjölund-Karlsson, Gordon & Parry, 2015; Parry & Threlfall, 2008). In healthy
32 humans, the infectious dose is generally of 10^6 to 10^8 organisms, but lower bacterial counts
33 can cause disease in older adults, especially immunocompromised patients and infants
34 (Chen, Wang, Su & Chiu, 2013). Although *Salmonella* is ubiquitous, its primary reservoir
35 is the intestinal tract of animals. Poultry populations, in particular, chicken and turkey, are
36 frequently colonized with *Salmonella* without detectable symptoms (sub-clinical
37 infections/healthy carriers) by horizontal and vertical transmission at the primary
38 production level (Barrow, Jones, Smith & Wigley, 2012; Cosby et al. 2015). The presence
39 of *Salmonella* in healthy poultry animals is considered as the main risk factor for foodborne
40 diseases, since allowing bacteria to easily transmit through eggs and poultry meat to
41 humans.

42 Preventive interventions at the farm and at the abattoir can decrease *Salmonella*
43 contamination in poultry products. Nevertheless, some extent of contamination in processed
44 meat products is practically unavoidable (Masana, Barrio, Palladino, Sancho & Vaudagna,
45 2015). As food industry needs to guarantee food safety in relation to pathogenic bacteria
46 such as *Salmonella spp*, different processing technologies have been widely investigated
47 (Morales-de la Peña, Welti-Chanes & Martín-Belloso., 2019) in different food matrices
48 such as dairy beverage (Cordeiro et al, 2019), cheeses (Cunha-Neto et al., 2019), leafy
49 greens (Erickson & Liao, 2019, Rossi et al., 2019), fruit as melons (Paudel, Bhargava &

50 Kotturi, 2019) and chicken meat (Cui, Bai, Changzhu, Liu & Lin, 2018; Duc, Son, Honioh
51 & Miyamoto, 2018.)

52 **High hydrostatic pressure (HHP), has been recognized as an alternative to high**
53 **temperatures processing to inactivate pathogens and spoilage bacteria (Lee &**
54 **Kaletunç, 2010; Wang, Hsu, Huang & Yang, 2013) and improving the shelf life of the**
55 **processed products. At the same time, HP technology no affecting the natural**
56 **characteristics of food, preserving the freshness of foods since no affects single**
57 **molecules responsible for color, aroma, flavor. Also, it allows to hold back of bioactive**
58 **compounds, so the nutritional value remains unaffected and preserving sensory**
59 **attributes of foods (Mújica-Paz, Valdez-Fragoso, Samson, Welti-Chanes, & Torres,**
60 **2011; Kaushik, Pal Kaur, Rao & Mishra, 2014; Misra, et al., 2017)**

61 Several studies documented the effectiveness of HHP treatments to inactive
62 *Salmonella* in fresh poultry products. Argyri, Papadopoulou, Nisiotou, Tasso, &
63 Chorianopoulos, (2018) reported that HHP processing (500 MPa/10 min/ 20°C) in chicken
64 fillets inoculated with *Salmonella Enteritidis* (3, 5 or 7 log CFU/g) stored at 4 °C and 12 °C
65 enhanced the safety of chicken meat and increased the shelf life of it. Tananuwong,
66 Chitsakun, & Tattiyakul, (2012) reported that *Salmonella Typhimurium* counts in chicken
67 breast fillets were reasonably reduced (~2log reduction) with HHP treatments at 300 MPa,
68 35 °C, 1 min and 400 MPa, 30 °C, 1 min. Kruk et al. (2011) reported that HHP treatments
69 at 450 and 600 MPa at 15 °C achieved an inactivation below detectable level of *Salmonella*
70 *Typhimurium*, *Escherichia coli* and *Listeria monocytogenes* and improved the shelf life of
71 chicken meat. Patterson, McKay, Connolly, & Linton (2010) studied vacuum-packaged
72 cooked poultry meat treated at 18 °C in a range of pressure levels (400-600 MPa) and hold
73 times (1, 2 and 10 min) and reported that as the pressure level and holding time increased,

74 the number of surviving microorganisms decreased significantly. However, to the best of
75 our knowledge, there are no studies of the effectiveness of HHP treatments to inactive
76 *Salmonella spp* in frozen chicken breast fillets. It has been reported that the freezing
77 procedure decreases both, the undesirable discoloration of meat products and the bacterial
78 resistance, in HHP treatments (Masana et al., 2015; Szerman et al., 2011; Vaudagna et al.,
79 2012).

80 One of the main objectives in food processing is to combine appropriate conditions to
81 reduce the intensity of treatments and improve food safety and shelf-life while maintaining
82 meat-quality. Therefore, the aim of this study was to evaluate the effect of pressure level
83 and holding time on the *Salmonella spp* inactivation during HHP processing in frozen
84 chicken breast fillets, to select the best combination of pressure level and holding time, and
85 to evaluate the effect on meat quality.

86

87 **2. Materials and methods**

88 *2.1 Bacterial strains and inoculum preparation*

89 *Salmonella* strains were provided by Dr. Pablo Chacana from Pathobiology Institute,
90 INTA Castelar, Argentina. The strains were originally isolated at different stages of the
91 food chain and were identified as *S. Enteritidis*, *S. Typhimurium*, *S. Thompson*, *S.*
92 *Heidelberg*, and *S. Schwarzengrund*. The strains were kept in the frozen culture at -80°C
93 and activated separately in Xylose-Lysine-Desoxycholate agar (XLD, Oxoid, UK). One
94 colony of each strain was subcultured in Tryptic Soy Broth (TSB, Oxoid, UK). Cells were
95 harvested by centrifugation at 10000 rpm for 5 min and the pellets were washed twice with
96 phosphate-buffered saline (PBS, pH 7.2, Oxoid). The inoculum was prepared by mixing
97 equal volumes of each strain in PBS.

98 2.2 *Sample preparation*

99 Bags of chicken breasts were purchased from a local supermarket. Chicken breasts
100 were sliced with a punch in order to obtain samples of 25cm² and 25g. For the inoculation
101 procedure, 50µl of a *Salmonella* pool was applied onto the sample surface and evenly
102 spread with a drigalski spatula. The final concentration was approximately 6-7 log CFU/g.
103 The inoculated slices were allowed to dry for 15 min at room temperature in a biological
104 safety cabinet. All samples were individually vacuum-packed (Cryovac BB2000CB, Sealed
105 Air Co., Argentina) and kept at -20 °C for 24 h prior to HHP treatments.

106 2.3 *Experimental design*

107 The experimental design was carried out using different pressure levels and holding
108 times in order to identify the best combination of process factors with the greatest effect on
109 the lethality of *Salmonella spp.*

110 The design was divided into three phases. For the first phase, inoculated chicken
111 samples were exposed to 100, 200, 300, 400, 500 and 600 MPa for 1 min. For the second
112 phase, the highest-pressure level with positive counts was selected to study the effect of the
113 holding time. The inoculated chicken samples were exposed for 1, 3, 5, 7 and 9 min. For
114 the third phase, the pressure levels and holding times selected in the previous phases were
115 used to study the effect on indigenous microbiota, chromatic parameters and texture profile
116 in non-inoculated chicken breast fillets. Pressurized samples were compared with control
117 samples (unpressurized) and all experiments were carried out three times in duplicate.

118 2.4 *HHP treatments*

119 For HHP treatments, frozen samples were placed into a Stansted Fluid Power HHP
120 System (model FPG 9400:922, vessel capacity: 2 L and maximum working pressure: 900
121 MPa; Stansted, United Kingdom). The compression fluid was a mix of propylene glycol

122 and distilled water (30:70 v/v). The initial temperature of the compression fluid was 5 °C.
123 The compression rate applied was 300 MPa.min⁻¹. After treatment, samples were held at
124 4°C.

125 2.5 *Microbiological analysis*

126 Samples were transferred into sterile stomacher bags with 225 ml of 0.1% peptone
127 water (PW, Oxoid, UK). Immediately after, samples were homogenized with a stomacher
128 (easy Mix, AES, France) for 60 s and serial dilutions were prepared. For inoculated
129 samples, *Salmonella* counts were performed in Tryptic Soy agar (TSA, Oxoid, UK), as
130 non-selective media, and XLD as selective media. The difference in microbial counts
131 between TSA and XLD represented the injured population. All plates (in duplicate) were
132 incubated overnight at 37 °C. For non-inoculated samples, the Plate Count Agar (PCA)
133 medium (Merck, Germany) was used for counting total aerobic mesophilic and
134 psychotropic cells after incubation at 37 °C for 48 h and at 5 °C for 11 days, respectively.
135 Enterobacteriaceae counts were performed on Violet Red Bile Dextrose agar (VRBD,
136 Oxoid, UK) incubated at 37 °C for 24 h and Lactic acid bacteria (LAB) in Man Rogosa
137 Sharpe Agar (MRS, Oxoid, UK) incubated at 30 °C for 72 h.

138 2.6 *Chromatic parameters analysis*

139 Chromatic parameters of the control and pressurized samples were determined using
140 a Konica Minolta colorimeter (model CR-400, Konica Minolta Sensing, Osaka, Japan) at
141 25 °C, with illuminant D₆₅, 2° observer angle and calibrated using a standard white tile.
142 Measurements were done in five points of each piece. The parameters measured were L*
143 (lightness), a* (redness) and b* (yellowness). The hue angle (h), Chroma (C*) and Color
144 difference (ΔE) were calculated by the software of the colorimeter. All measurements were
145 carried out three times per treatment.

146 2.7 *Texture profile analysis*

147 Texture profile analysis was carried out with a Texture Analyzer Stable
148 MicroSystems (model TA-XT2i, Surrey, U.K.) and using a load cell of 50kg. The test
149 Warner–Bratzler blade was employed to cut a cooked sample stick. The parameters used
150 were a constant test speed of 1 mm/s, a cutting distance of 30 mm and a trigger force of 5 g.
151 Firmness (maximum cutting force, g) and work area (area under the force–deformation
152 curve, g.s.) were determined using the Texture Expert software. Before texture analysis,
153 samples (25 g piece) were cooked at 80 °C until reached 70 °C at the core (internal
154 temperature was monitored with T thermocouples) in an electric convection oven (Oster,
155 CKSTPA488, China). Then, samples were cut into sticks of 1.5 cm in diameter and 1.5 cm
156 in thickness and cut parallel to the muscle fiber orientation. Three sticks were obtained
157 from each sample. Measurements were carried out at room temperature in triplicate for
158 each treatment.

159 2.8 *Statistical analysis*

160 An analysis of One factor-ANOVA was carried out using the SPSS software package,
161 version 21 (SPSS Inc., Chicago, Ill., U.S.A.). Tamane's test was applied to compare the
162 mean values when ANOVA showed significant differences ($p < 0.05$).

163

164 **3. Results and discussion**

165 3.1 *Effect of the HHP processing in the inactivation of the Salmonella spp.*

166 The effects of the pressure level and holding time are shown in Tables 1 and 2.
167 *Salmonella spp* counts in TSA after HHP-treatment were different from control samples
168 and among the pressure levels evaluated. *Salmonella spp* counts in TSA at 100, 200 and
169 300 MPa were 6.70, 6.19 and 5.61 log CFU/g with log reductions of 0.64, 1.15 and 1.73 log

170 CFU/g, respectively. At 400 MPa, *Salmonella spp* count was 4.55 log CFU/g with a log
171 reduction of 2.79 log CFU/g. At 500 and 600 MPa counts were undetectable therefore log
172 reductions were of at least 5 log CFU/g (detection limit 2 log CFU/g). In control samples,
173 *Salmonella spp* count in TSA was 7.34 log CFU/g and in XLD was 6.80 log CFU/g. The
174 difference between TSA and XLD (0.54 log CFU/g) reflects the amount of injured bacterial
175 before HHP treatment probably due to the freezing procedure. *Salmonella spp* counts in
176 XLD at 100, 200, 300 and 400 MPa were 6.39, 5.89, 5.39 and 3.43 log CFU/g,
177 respectively. The number of injured bacteria was ≤ 0.3 log CFU/g for all pressure levels
178 analyzed except for 400 MPa were injured bacteria raised up to 1.12 log CFU/g. The
179 increase in pressure level caused more *Salmonella spp* lethality rather than injury. An
180 effective treatment should at least cause 3 log CFU/g reductions given that the amount of
181 natural contaminated *Salmonella spp* in raw chicken meat is described to be lower than the
182 total viable count (2 logs CFU/g) (Tananuwong et al., 2012). Considering this, for
183 treatments with 1 min of holding time, a pressure level of 500 MPa onwards should be
184 enough. Some authors reported higher bacterial resistance while others reported lower
185 bacterial resistance (Morales, Calzada, Rodríguez, De Paz, & Nuñez 2009; Tananuwong et
186 al., 2012). Morales et al., (2009) worked with a pool of *S. Enteritidis* exposed at 300 and
187 400 MPa for 1 min at 12 °C and obtained log reductions of 0.82 log CFU/g at 300 MPa and
188 1.36 log CFU/g at 400 MPa. Tananuwong et al. (2012) studied the effect of HHP treatment
189 on *Salmonella Typhimurium* inactivation in fresh chicken breast when the compression
190 fluid temperature was 25 °C and reported a log reduction of approximately 2 log CFU/g
191 after 300 MPa for 1 min and a log reduction of approximately 4 log CFU/g after 400 MPa
192 for 1 min. The differences could be due to different strain resistance to HHP treatment
193 and/or treatment conditions.

194 For the second phase, in order to study the effect of holding time, a pressure level of
195 400 MPa was selected. *Salmonella spp* counts in TSA of control samples was 6.84 log
196 CFU/g. After treatments for 1, 3, 5, 7 and 9 min *Salmonella spp* counts in TSA were 4.68,
197 4.31, 3.09, 3.13 and 2.19 with log reductions of 2.16, 2.53, 3.75, 3.71 and 4.65,
198 respectively. Statistical differences were found between control and pressurized samples, as
199 well as, between treatments for 1 and 3 min compared to treatments for 5, 7 and 9 min. No
200 significant differences were found between treatments for 1 and 3 min and among
201 treatments for 5, 7 and 9 min (Table 2). When TSA counts were plotted against the holding
202 time, a concave curve upward shape was described. Similar results were reported by
203 Tananuwong et al. (2012) and Morales et al. (2009), who observed the same behavior when
204 applied HHP treatments at 400 MPa to different holding times in chicken breast fillets. This
205 behavior was probably due to the fact that sensitive cells were quickly destroyed, while the
206 remaining cells were able to adapt to the applied stress, implying higher resistance (Buzrul,
207 Alpas, Largeteau & Demazeau, 2008; Van Boekel, 2002). As to *Salmonella spp* counts in
208 XLD, in control samples was 6.04 log CFU/g while after treatments for 1 and 3 min were
209 3.32 and 3.01 log CFU/g. These results were different from control results but equal
210 between themselves ($p < 0.05$). The number of injured bacteria in control samples was 0.8
211 log CFU/g, whereas for treatments for 1 and 3 min was 1.36 and 1.30 log CFU/g,
212 respectively. After 5 min, onwards, *Salmonella spp* counts in XLD were undetectable. The
213 quantification of injured cells is highly important, as injured cells could recover viability,
214 given a situation of temperature abuse. For this reason, lethality should always be estimated
215 by bacterial counts in non-selective media.

216 Based on our results, the treatments at 500 MPa for 1 min and 400 MPa for 5 min were
217 enough to guarantee *Salmonella spp* inactivation in frozen chicken breast fillets when the
218 compression fluid temperature was 5 °C.

219 3.2 Effect of the HHP processing in the meat-quality parameters

220 From the results described above, the effect of the treatments at 400 MPa for 5 min and
221 500 MPa for 1 min on meat-quality parameters were assessed. As to the effect on
222 microbial counts of indigenous microbiota, results are shown in Table 3.
223 Enterobacteriaceae was not detected as part of the indigenous microbiota of chicken breast
224 fillets. The microbial counts were, for all determinations, different between control and
225 pressurized samples ($p < 0.05$). Counts for psychotropic and lactic acid bacteria of the
226 pressurized samples were below the detectable level ($< 1 \log \text{CFU/g}$). Significant
227 differences were not found on counts of mesophilic bacteria ($< 1.5 \log \text{CFU/g}$) between
228 treatments at 400 MPa for 5 min and 500 MPa for 1 min. As microorganisms are the
229 primary agents responsible for fresh meat spoilage, an extension in shelf-life should be
230 expected (ICMFS, 2006). Argyri et al. (2018) not only reported that after HHP-treatment
231 microbial counts were under the limit of detection but also remained below or near the limit
232 of detection during 18 days of storage at 4 °C.

233 Results regarding the effect of HHP on chromatic parameters of chicken breast fillets
234 are shown in Figure 1. Pressurized samples exhibited a paler color ($> L^*$) with greater
235 intensity ($> C^*$ values) and yellowness ($> b^*$) compared to control samples ($p < 0.05$), but
236 with the same pink tone ($= h$ and a^* values) characteristic of the raw chicken breast fillets.
237 Comparing HHP treatments, b^* and C^* parameters were equal while the L^* parameter was
238 significantly higher at 400 MPa for 5 min than at 500 MPa for 1 min ($p < 0.05$). The
239 paleness ($> L^*$) of meat after HHP processing was also observed by other authors, who

240 reported that even at lower pressure levels (< 300 MPa), a “whitening” effect is produced
241 (Carlez, Rosec, Richard & Cheftel, 1993; Kruk et al., 2011). In this work, the color
242 difference was 15.25 at 400 MPa for 5 min and 11.06 at 500 MPa for 1 min. Jung, Ghoul
243 & De Lamballerie-Anton, (2003) and Tananuwong et al. (2012) reported that a ΔE value
244 greater than 10, is considered a significant difference in meat color. In our work, the
245 treatment at 500 MPa **for 1 min** resulted to be the most appropriate to preserve the
246 appearance of the chicken breast fillets, in terms of color parameters. Similar results were
247 reported by Jung et al. (2003) and Olmo, Del Morales, Ávila, Calzada & Nuñez (2010). The
248 authors observed that meat discoloration was significantly influenced by the holding time
249 and reported that the discoloration was produced when the holding time of the HHP
250 processing was longer than 1 min.

251 Texture profile results of chicken breast fillets after HHP treatment are shown in
252 Figure 2. Compared to control, HHP treatments significantly increased the firmness and
253 work area of the cooked chicken breast fillets ($p < 0.05$). Between HHP treatments, no
254 significant differences ($p > 0.05$) were found. Changes in texture profile of meat **depend on**
255 the meat protein system, the rigor state of meat, the working temperature, the pressure level
256 and the holding time (Sun & Holley 2010; Rodríguez-Calleja et al., 2012; Vaudagna et al.,
257 2012). HHP treatments at a pressure above 200-400 MPa (at temperature > 0 °C) could
258 influence in the meat protein conformation and induce protein denaturation, aggregation or
259 gelation, which can result in the meat becoming either tenderized or toughened (Vaudagna
260 et al., 2012). Gonzalez et al., (2009) observed, by CryoSEM analysis, that HP processing at
261 400 MPa for 1 min provokes a decrease in the size of muscle cells and the tissues were
262 more compact but without changes in shape. However, when the level pressure increased at
263 600MPa for 1 min, this caused the flattening and deformation of the cells. This effect

264 became more evident when the holding time increased to 5 min, where elongation of the
265 cellular tissue was observed. Tananuwong et al. (2012) reported that the shear force and
266 area under the curve of the pressurized-then-cooked chicken meat samples were
267 significantly higher than the control samples ($P \leq 0.05$), and reported that the structural
268 changes could be due to the denaturation of the protein in myofibrils and connective tissues
269 induced by pressure.

270 Similar results were observed by Jung, De Lamballerie-Anton & Ghoul (2000) and
271 reported that HHP treatment (130 or 520 MPa and 10 °C for 260 s) significantly increased
272 the mechanical resistance of cooked (65 °C, 1 h) post-rigor beef compared with the control
273 sample. They reported that the highest values of beef mechanical resistance were observed
274 at the highest-pressure level evaluated. Realini, Guàrdia, Garriga, Pérez-Juan &, Arnau
275 (2011) reported that cured pork loin treated with HHP at 400 or 600 MPa at -15 or -35 °C
276 for 6 min showed higher values of Warner Bratzler shear force than control samples and
277 that the increment of toughness was independent of the pressure level. Kruk et al. (2011)
278 reported as pressure level increased, so did hardness. Nonetheless, in the texture sensory
279 evaluation, significant differences were not found between treated chicken breasts at 300,
280 450 and 600 MPa. In this case, the authors indicated that this result was due to the cooking
281 of the samples and not to the effect of HHP processing. Finally, our study demonstrated
282 that HHP technology effectively improved food safety and extended shelf life of the
283 processed products as successfully inactivated foodborne pathogens such as *Salmonella spp*
284 and reduced indigenous microbiota counts, under the limit of detection. In the case of the
285 quality parameters, a sensory test using consumers and trained panel (Vidal et al, 2019;
286 Horita et al., 2017) is recommended to determine whether the cooked product, exposed to
287 the recommended treatments, is acceptable.

288

289 **4. Conclusion**

290 Treatments at 400 MPa for 5 min and 500 MPa for 1 min were enough to ensure
291 more than 3 log CFU/g reductions of *Salmonella spp.* Besides, an extension in shelf-life
292 should also be expected with both treatments, as endogenous microbiota counts were
293 significantly reduced. Treatment at 500 MPa for 1 min resulted to be more effective at
294 preserving color parameters of raw chicken breast fillets than treatment at 400 MPa for 5
295 min. Nonetheless, it is important to take into consideration that pressurized chicken breasts
296 suffered significant modifications in chromatic parameters and texture profile compared to
297 control samples. A sensorial test is recommended to determine whether the cooked product,
298 exposed to the recommend treatments, is acceptable for consumers.

299

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304

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455 **Table 1.** *Salmonella spp* counts (log CFU/g) in chicken breast fillets after the application
 456 of high hydrostatic pressure treatments at 0.1, 100, 200, 300, 400, 500 and 600 MPa for
 457 1 min.

Pressure level (MPa)	Counts in TSA (log CFU/g)	Log reductions (log CFU/g)	Counts in XLD (log CFU/g)	Injured cells (log CFU/g)
0	7.34 (0.49) ^a	0	6.80 (0.42) ^a	0.54
100	6.70 (0.34) ^b	0.64	6.39 (0.44) ^{ab}	0.31
200	6.19 (0.31) ^c	1.15	5.89 (0.50) ^{bc}	0.3
300	5.61 (0.32) ^d	1.73	5.39 (0.65) ^c	0.22
400	4.55 (0.22) ^e	2.79	3.43 (0.43) ^d	1.12
500	ND	>5	ND	-
600	ND	>5	ND	-

458 *TSA: tryptic soy agar and XLD: xylose-lysine-desoxycholate. Values expressed as means and standard deviation of three replicates.
 459 Different letters mean significant differences ($p < 0.05$) according to Tamane test. *ND: non-detected, counts below the limit of
 460 detection (< 2 CFU/g).
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470 **Table 2.** *Salmonella spp.* counts (log CFU/g) in chicken breast fillets after high hydrostatic
 471 pressure treatments at 400 MPa for 0.1, 1, 3, 5, 7 and 9 min.

Holding time (min)	Counts in TSA (log CFU/g)	Log reductions (log CFU/g)	Counts in XLD (log CFU/g)	Injured cells (log CFU/g)
0.1	6.84 (0.15) ^a	-	6.04 (0.68) ^a	0.80
1	4.68 (0.35) ^b	2.16	3.32 (0.56) ^b	1.36
3	4.31 (0.17) ^b	2.53	3.01 (0.61) ^b	1.30
5	3.09 (0.50) ^c	3.75	ND	-
7	3.13 (0.45) ^c	3.71	ND	-
9	2.19 (0.95) ^c	4.65	ND	-

472 TSA: tryptic soy agar and XLD: xylose-lysine-desoxycholate

473 Values expressed as means and standard deviation of three replicates. Different letters mean significant differences ($p < 0.05$) according
 474 to Tamane test. *ND: non-detected, counts below the limit of detection (< 2 CFU/g).

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487 **Table 3.** Endogenous microbiota counts (log CFU/g) in chicken breast fillets after of the application of the high hydrostatic pressure

Pressure level (MPa)	Holding time (min)	Mesophilic bacteria (log CFU/g)	Psychrotrophic bacteria (log CFU/g)	Lactic acid bacteria (log CFU/g)	Enterobacteriaceae (log CFU/g)
0.1	0.1	3.09 (0.32) ^a	2.42 (0.14) ^a	2.14 (0.05) ^a	ND
400	5	1.54 (0.06) ^b	ND	ND	ND
500	1	1.10 (0.17) ^b	ND	ND	ND

488 treatments at 400 MPa for 5min, 500 MPa for 1 min and 0.1 MPa for 0.1 min.

489 Values expressed as means and standard deviation of three replicates. Different letters mean significant differences ($p < 0.05$) according to Tamane test. *ND: non-detected, counts below the limit of
490 detection (< 2 CFU/g)

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Figure captions

Fig 1. Chromatic parameters of the control samples and pressurized chicken breast fillets.

Different letters indicate significant statistical differences ($P < 0.05$)

Fig 2. Texture profile of the control samples and pressurized chicken breast fillets.

Different letters indicate significant statistical differences ($P < 0.05$)

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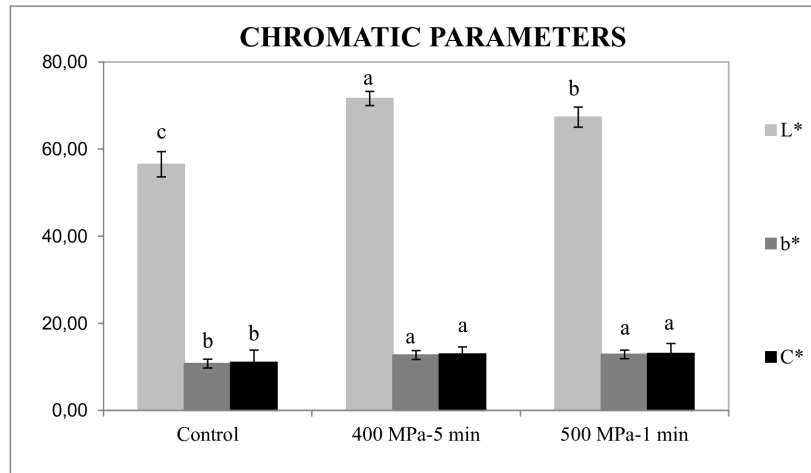


Figure 1

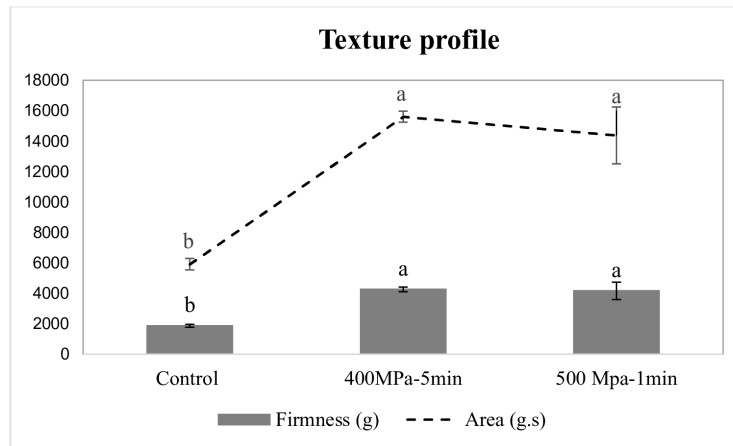


Figure 2

Highlights

- *Salmonella* inactivation was guaranteed at 400MPa/5min and 500MPa/1min
- Both HHP treatments successfully reduced the indigenous microbiota counts
- Both HHP treatments affected the quality properties of the chicken breast.
- Treatment at 500 MPa/1 min was more effective at preserving color parameters

Conflict of Interest and Authorship Confirmation Form

Please check the following as appropriate:

- All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version.
- This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue.
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