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29 **Abstract**

30 The combined effect of sage (0.3 and 0.6%) and high pressure processing (HPP) [300 MPa (10  
31 min, 9.2 °C) and 600 MPa (10 min, 15 °C)] on the antimicrobial and antioxidant characteristics  
32 of beef burgers during prolonged chilled storage (60 days) was analysed. Sage powder showed  
33 antioxidant and antimicrobial activities, but the addition of sage powder to burgers had no  
34 apparent effect on antimicrobial activity; however, antioxidant activity was detected as  
35 measured by TBARS, hexanal and photochemiluminescence (PCL). In general, lipid oxidation  
36 increased in all samples during storage. HPP at 600 MPa had no effect on lipid oxidation but  
37 caused mesophilic and psychrotrophic counts to remain close to the detection limit for at least  
38 6 days. Significant correlations were found between lipid oxidation measured by TBARS and  
39 PCL and between TBARS with hexanal over the storage period. Sage had no detrimental effects  
40 on sensory attributes of burgers.

41 **Industrial relevance**

42 Sage is an aromatic plant with excellent antimicrobial and antioxidant properties. High  
43 pressure processing HPP is an efficient non-thermal preservation technology. As far as the  
44 authors are aware, very few studies have holistically addressed the question of stability  
45 (microbial spoilage and oxidation of lipids) of traditionally-prepared burgers as affected by HPP  
46 and addition of a natural plant. This paper examines the possible application of both  
47 treatments so as to obtain beef burgers with suitable oxidative and microbiological stability  
48 over prolonged chilling storage without this affecting sensory attributes.

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52

53 *Highlights*

54

55 - Sage powder was an effective antioxidant in burgers over prolonged chilling storage.

56 - HPP of beef burgers did not induce lipid oxidation during prolonged chilling storage.

57 - Sensory attributes were unaffected by added sage powder.

58 - Burgers exposed to 600 MPa showed acceptable microbial quality after 60 days.

59

60 *Key words*

61

62

63 *Dried sage, High Pressure Processing, beef burger, antioxidant, antimicrobial, chilled storage*

64 **1. INTRODUCTION**

65 Burgers are among the most popular processed meat products in the world. They are highly accepted  
66 and consumed by large segments of the population, mainly due to convenience and low price.  
67 However, they have a very limited stability, mainly because of microbial spoilage and lipid oxidation,  
68 both with possible repercussions on safety and health. High initial counts of viable psychrotrophic  
69 and/or mesophilic microorganisms have been found during meat processing (Karpinska-Tymoszczyk,  
70 2010; Mohamed, Mansour and Farag, 2011), and these can be higher if burgers are prepared in a  
71 traditional way. Various methods have been studied to delay or avoid these effects, among the more  
72 interesting of which are ones that are more label-friendly (since no chemical additives are required)  
73 (Burt, 2004; Tajkarimi, Ibrahim, and Cliver, 2010).

74 High pressure processing (HPP) is the most successful non thermal food preservation  
75 technology developed so far and is becoming increasingly important in the production of minimally-  
76 processed foods and additive-free meat products. The application of HPP to food processing has  
77 been undertaken for a variety of reasons, among others, to reduce microbial load so as to improve  
78 food safety and prolong shelf life (Bajovic, Bolumar, and Heinz, 2012; Garriga, Grebol, Aymerich,  
79 Monfort, and Hugas, 2004; López-Caballero, Carballo and Jiménez-Colmenero, 2002). However, high-  
80 pressure treatment may also induce lipid oxidation in meat depending on processing time and  
81 especially on the pressure level applied and the origin of the meat. HPP-induced lipid oxidation in  
82 meat has been related to increased accessibility of iron from haemoproteins, membrane disruption  
83 and radical formation under high pressure (Bolumar, LaPena, Skibsted, and Orlie, 2016). The use of  
84 plant natural antioxidants (e.g. rosemary and garlic extracts, tomato products) in meat products has  
85 been shown to minimize pressure-induced lipid oxidation in various meat products (Alves,  
86 Bragagnolo, Silva, Skibsted, and Orlie, 2012; Bolumar, et al., 2016; Mariutti, Orlie, Bragagnolo and  
87 Skibsted, 2008).

88 The genus *Salvia* (sage) is one of the largest and the most important aromatic and medicinal  
89 genera of the Lamiaceae family, which contains 900 different species widespread throughout

90 Mediterranean region, South-East Asia and Central America. *Salvia officinalis* is a rich source of  
91 phytochemicals including phenolic acids, polyphenols, flavonoid glycosides, anthocyanins,  
92 sesquiterpenoids, diterpenoids, sesterterpenes and triterpenes (Sepahvand et al., 2014). It has been  
93 well documented that sage presents excellent antimicrobial activity (Burt, 2004; Gutierrez, Barry-  
94 Ryan and Bourke, 2008; Hayouni et al., 2008; Tajkarimi, Ibrahim and Cliver, 2010). However, the  
95 antimicrobial effect of sage (which has been generally evaluated as an essential oil) on meat matrices  
96 has produced conflicting results. While this has been shown to be effective against *Salmonella*  
97 inoculated in minced beef (Hayouni et al., 2008), in other cases it was ineffective, as its effect is  
98 dependent on the fat content (Burt, 2004). On the other hand, sage has been clearly identified as an  
99 effective antioxidant in different foods, including muscle-based food. Some researchers have  
100 reported that sage, or sage extracts, can effectively retard lipid oxidation in different meat products  
101 (Fasseas, Mountzouris, Tarantilis, Polissiou and Zervas, 2008; Mariutti, Nogueira and Bragagnolo,  
102 2011; McCarthy, Kerry, Kerry, Lynch and Buckley, 2001). In this regard sage has been successfully  
103 used to protect HHP-processed minced chicken breast against lipid oxidation (Mariutti et al., 2008).

104 Meat products are complex matrices with different physical properties and chemical  
105 composition that influence the lethality of the microorganisms during HPP. The combination of  
106 natural antimicrobials (e.g. plant bioactive compounds) and antioxidants (plant phenolic compounds)  
107 as additional hurdles through different mechanisms during HPP, can definitely be an effective and  
108 innovative means of improving the stability of processed meat products (Hygreeva and Pandey,  
109 2016). Therefore, combined protection against both deteriorative actions, could help to extend the  
110 shelf life of additive-free meat products which it involves expand logistic opportunities by allowing  
111 long-distance distribution in the global market, (Bolumar et al., 2016). Taking into account the above  
112 the aim of the present work was to study the combined antimicrobial effect associated with the  
113 application of high pressure processing [300 MPa (10 min, 9.2 °C) and 600 MPa (10 min, 15 °C)] and  
114 the antioxidant protection conferred by the incorporation of sage as natural ingredient (0.3 and 0.6%  
115 in powder form), on prolonged chilling stability of beef burger prepared in a traditional way.

116

## 117 2. MATERIAL AND METHODS

118

### 119 2.1. Sage preparation

120 *Salvia officinalis* (Lamiaceae) was collected in the area of El-kseur, Béjaia, Algeria, and authenticated  
121 by the Botany Department, Faculty of Science, University of Béjaia. After cleaning and drying (15–18  
122 days), the leaves were ground in an analytic mill (IKA A11 basic; IKA Werke GmbH & Co. KG, Staufen,  
123 Germany) and sieved (Tap sieve shaker AS 200; Retsch GmbH, Haan, Germany) through a 500 µm  
124 screen. This ground powder was used to formulate the meat products.

125

#### 126 2.1.1. Preparation of extracts and measurement of antimicrobial activity

127 6.25 g of sage powder was used in 50 mL of three different solvents with different polarities: 80%  
128 methanol (Pharma grade), 80% ethanol (Pharma grade) and distilled water. Extractions were carried  
129 out in a water bath shaker at 60 °C for 30 min, followed by centrifugation (Beckman J2-MC USA) at  
130 12000 x g, 5 °C. The antimicrobial activity of the sage extracts was evaluated by the disk diffusion  
131 method in agar as described in Arancibia, Giménez, López-Caballero, Gómez-Guillen and Montero  
132 (2014), against 10 strains of microorganisms selected for their impact on human health (either lactic  
133 acid bacteria or pathogens) or for being responsible for food spoilage. These were obtained from the  
134 Spanish Type Culture Collection (CECT): *Aeromonashydrophila* CECT 839T, *Bifidumbacteriumbifidum*  
135 DSMZ 20215, *Lactobacillus acidophilus* CETC 903, *Photobacteriumphosphoreum* CECT 4192,  
136 *Staphylococcus aureus* CECT 240, *Escherichia coli* CECT 515, *Pseudomonas fluorescens* CECT 4898,  
137 *Listeria monocytogenes* CECT 4032, *Vibrio parahaemolyticus* CECT 511T, *Shewanella putrefaciens*  
138 CECT 5346T and *Yersinia enterocolitica* CECT 4315. Sterile filter paper discs (6 mm diameter,  
139 Whatman® antibiotic assay; Sigma-Aldrich, Saint Louis, Missouri, USA) were soaked with 40 µL of the  
140 extracts. The disks were then placed on Brain Heart Infusion Agar (Oxoid, Basingstoke, UK) petri  
141 dishes previously seeded with 100 µL of different microorganisms ( $10^5$ – $10^6$  cfu/mL). Paper disks with

142 40 µL of each solvent were used for control purposes. Quantitative antimicrobial activity was  
143 measured from the inhibition diameter around the film disk (considered as antimicrobial activity)  
144 using Corel Photo-Paint X3 software. Results were expressed as diameter of growth inhibition (mm).  
145 Each determination was performed in duplicate.

146

## 147 **2.2. Burger preparation**

148 Beef top rounds (15 kg) were selected and trimmed of visible fat and connective tissue, cut into small  
149 pieces, and finally minced through a 4.5 mm diam. hole mincer plate (Vam.Dall. Srl. Modelo FTSIII,  
150 Treviglio, Italy). Lots of approximately 1.2 kg were vacuum-packed, frozen and stored ( $-18\text{ }^{\circ}\text{C}$ ) until  
151 use. For the preparation of burgers, meat packages were thawed (approx. 18 hr  $3 \pm 2\text{ }^{\circ}\text{C}$ , reaching  
152 between  $-3$  and  $-5\text{ }^{\circ}\text{C}$ ) and minced again through a grinder with a 6 cm diam. plate. Three different  
153 batches of burgers were prepared with 93.5 % of beef meat (8.31 % of fat and 20.54 % of protein  
154 content and pH of 5.93) and containing 0% (control sample), 0.3% and 0.6% of added power sage  
155 (proportions selected based on previous sensory essays), 1.2% NaCl and 5% added water. The  
156 burgers were prepared as follows. Meat was mixed for 1 min in a mixer (Mainca, Granollers, Spain);  
157 half of the salt, sage and water was added and the whole mixed again for 1 min; the rest of the salt,  
158 sage and water was added and mixed again for 2 min. The final temperature of the meat masses was  
159 between 5 and  $7\text{ }^{\circ}\text{C}$ . Burgers (90 g) were then prepared using a manual burger former and vacuum-  
160 packed in plastic bags (Cryovac<sup>®</sup> BB3050). Each type of formulation was randomly separated into  
161 three groups for further treatments.

162

## 163 **2.3. High pressure processing (HPP) of burger**

164 After preparation, burgers were immediately exposed to the different HPP treatments using a Pilot  
165 Food Processor, Model FGP7100:9/2C (Stansted Fluid Power LTD, Essex, UK) with a cylinder 10 cm in  
166 inner diameter and 22 cm in height. The pressure-transmitting fluid was water/propylene glycol (2:1,  
167 v/v). A non-pressurized control and the following HPP conditions were assayed: a) Treatment at 300



168 MPa: 45.5 s at 9.9 °C to reach pressurization, 10 min at 9.2 °C and 300 MPa and 18 s at 6.1 °C to  
169 depressurization process; and b) Treatment at 600 MPa: 90 s at 10.2 °C to reach pressurization, 10  
170 min at 15 °C and 600 MPa and 46 s at 2 °C to depressurization process.

171 Nine different samples were obtained in this way. Control burger without sage: non-  
172 pressurized (0S) and pressurized at 300 and 600 MPa (300/0S and 600/0S respectively). Burger  
173 containing 0.3% sage: non-pressurized (0.3S) and pressurized at 300 and 600 MPa (300/0.3S and  
174 600/0.3S respectively). Burger containing 0.6% sage: non-pressurized (0.6S) and pressurized at 300  
175 and 600 MPa (300/0.6S and 600/0.6S respectively).

176 Analyses were performed using two patties per day at 1, 3, 6, 10, 24, 34, 44 and 60 of chilling  
177 storage ( $2 \pm 2$  °C).

178

#### 179 **2.4. Proximate analysis**

180 Moisture and ash contents were determined by the AOAC methods (2005) and fat content according  
181 to Bligh and Dyer (1959). Protein content was measured with a LECO FP-2000 Nitrogen Determinator  
182 (Leco Corporation, St Joseph, MI, USA). All analyses were done in triplicate in samples without HPP  
183 treatment since this treatment does not affect composition of burgers.

184

#### 185 **2.5. Sensory evaluation**

186 A semi-trained 48-member sensory panel, recruited among staff of the ICTAN-CSIC with previous  
187 experience in descriptive analysis, was specifically instructed to evaluate the burgers in two sessions  
188 at the beginning of storage. Given the number of samples and that in previous studies it was  
189 observed that the application of high pressure produced no significant changes in sensory attributes  
190 (Hygreeva and Pandey, 2016), the panellists only tested the non-pressurized samples with and  
191 without sage. Burgers were cooked for 2.5 min on a grill until the centre of the product reached 70  
192 °C. A quarter portion of each burger was presented to the assessors in random order. The assessors  
193 evaluated acceptability of flavour, acceptability of odour and overall acceptability of the burgers

194 using a 10-point hedonic scale from “dislike extremely” to “like extremely”. The assessors were  
195 provided with mineral water and bread to rinse their mouths between samples.

196

## 197 **2.6. pH determination**

198 The pH was determined for all samples (in triplicate) on 10 g homogenates in 100 ml of distilled  
199 water using a pH meter (827pH Lab Methrom, Herisau, Switzerland).

200

## 201 **2.7. Microbiological analysis**

202 Samples were prepared in a vertical laminar-flow cabinet (model AV 30/70, Telstar, Madrid, Spain).

203 Ten grams of each sample (from 2 pieces per sample) were taken and placed in a sterile plastic bag

204 with 90 ml of peptone water (0.1%) (Panreac Química, S.A. Madrid, Spain). After 2 min. in a

205 stomacher blender (Stomacher Colworth 400, Seward, UK), appropriate decimal dilutions were pour-

206 plated (1 mL) on the following media: Plate Count Agar (PCA) for the total mesophile count (TMC)

207 (30°C for 72 h) and for Psychrotrophic bacteria (4 °C for 7-10 days); and Violet Red Bile Glucose Agar

208 (VRBG) for *Enterobacteriaceae* (37 °C for 24 h). All microbial counts were converted to logarithms of

209 colony-forming units per gram (Log cfu/g).

210

## 211 **2.8. Lipid stability evaluation**

### 212 **2.8.1. TBARs assay**

213 Lipid oxidation was evaluated by changes in TBARs (thiobarbituric acid-reactive substances) in fresh

214 burgers, pressurized and non-pressurized, during storage as described by Serrano, Cofrades and

215 Jiménez-Colmenero (2006) with slight modifications. Briefly, 5 g of each sample was homogenized in

216 35 ml of 7.5% trichloroacetic acid (Panreac) for 1 min at high speed in an Omnimixer blender (ES

217 Homogenizer, OMNI International Inc., Gainesville, VA, USA). The blended sample was centrifuged

218 (3000g, 2 min) and 5 mL of the supernatant was mixed with 5 mL of 20 mM thiobarbituric acid;

219 finally, the solution was mixed and then incubated in the water bath at 90 °C for 15 min. Colour was

220 measured spectrophotometrically (Lambda 15UV/VIS spectrophotometer, Perkin-Elmer, USA) at 532  
221 nm. A calibration curve was plotted with 1,1,3,3-tetraethoxypropane (Sigma Chemical Co., St. Louis,  
222 MO, USA) to obtain the malonaldehyde (MDA) concentration and results were expressed as mg  
223 malonaldehyde/kg of sample. TBARs determinations for each sample were performed in duplicate.

224

### 225 **2.8.2. Hexanal assay**

226 Lipid oxidation was also analysed by changes in hexanal content. Minced samples (3 g) and 7 mL of a  
227 0.2% EDTA water solution were dispensed in glass vials and thoroughly mixed for 3 min. The vials  
228 were then sealed with Teflon-face silicone septums and aluminium caps. The vials were frozen at -80  
229 °C until use, when they were thawed overnight (12h) at 4 °C, and resuspended by stirring for 30 s.  
230 Prior to injection into the Gas chromatography-mass spectrometer (CG-MS), sample was heated to  
231 80 °C for 15 min following preconcentration for 2 cycles in an active carbon cap (carbopack),  
232 desorbing at 300 °C. Samples were injected into a CG-MS using TurboMatrix HS 40 Trap Automated  
233 headspace sampler (Perkin Elmer, Massachusetts, USA). CG-MS analysis of sample headspace was  
234 carried out using an Agilent system (Waldbronn, Germany) consisting of a 6890N gas chromatograph  
235 coupled to a (EI) 5973N quadrupole mass spectrometer and a HP computer. The interface and the  
236 source temperature were 240°C and 230°C respectively. Electron impact mass spectra were recorded  
237 in SIM mode at an ionization energy of 70 eV. Separation was performed on a fused-silica bonded  
238 phase capillary column HP5MS (J&W Scientific, Folsom, CA, USA) (30m x0.25mm x0.25µm) at  
239 constant pressure (12 psi) provided by a HS-40 Autosampler. The temperature was programmed  
240 isothermally at 50°C for 7 min, then raised to 150°C at 20°C min<sup>-1</sup> and to 240°C at 50°C min<sup>-1</sup>; this  
241 temperature was held for 5 min. Blank analyses were carried out with the same trapping material  
242 and following the same procedure, starting from distilled water as the sample.

243

### 244 **2.8.3. Antioxidative activity by photochemiluminescence (PCL)**

245 Antioxidant activity was determined for the sage and for the burgers in triplicate using an automated  
246 photochemiluminescent system (Photochem, Analytik Jena Model AG; Analytik Jena USA, The  
247 Woodlands, TX, USA) which measures the capacity to quench free radicals (Popov and Lewin, 1996).  
248 This method is based on controlled photochemical generation of radicals, part of which is quenched  
249 by the antioxidant, and the remaining radicals are quantified by a sensitive chemiluminescence-  
250 detection reaction. Briefly, 1 g of sample was homogenized for 30 s in an Omnimixer blender (ES  
251 Homogenizer, OMNI International Inc., Gainsville, VA, USA) with 50 mL of methanol (PANREAC,  
252 UHPLC Supergradient). After mixing for 30 s, sample was filtered through Whatman No. 1 paper. 20  
253  $\mu$ l of filtrate was added to reagent kits supplied by the manufacturer and the automated PCL system  
254 measured the total antioxidant capacity. Trolox (Sigma–Aldrich, Inc., St. Louis, MO, USA) was used as  
255 a standard, and results were expressed in Trolox equivalents (mmol TE/g sample).

256

### 257 **2.9. Statistical analysis**

258 The entire experiment was fully replicated on two different days. One-way analyses of variance  
259 (ANOVA) were carried out to evaluate the statistical significance ( $P < 0.05$ ) of the formulation, and  
260 two-way ANOVA as a function of formulation and storage time and their interaction using the  
261 general linear model (GLM) procedure of SPSS Statistics (v.20, IBM SPSS Inc., Chicago, IL).  
262 Formulation and storage time and their interaction were assigned as fixed effects and replicate as a  
263 random effect. Least squares differences were used for comparison of mean values between  
264 treatments and Tukey's HSD test to identify significant differences ( $P < 0.05$ ) between formulations  
265 and storage time. The SPSS correlation procedure was used to determine Pearson's correlation  
266 coefficients and significant levels among lipid oxidation (TBARs and hexanal) and antioxidant activity  
267 (PCL).

268

## 269 **3. RESULT AND DISCUSSION**

270

271 **3.1. Antimicrobial activity of the sage extracts**

272 Sage, which is rich in phenolic acids (e.g. rosmarinic, syringic acid), monoterpenes (e.g. 1-8-cineole,  $\beta$ -  
273 thujone,  $\alpha$ -thujone) and diterpenes (e.g. carnosol and carnosic acid) (Hayat, Cherian, Pasha, Khattak  
274 and Jabbar, 2008; Mekinic et al., 2012), showed antimicrobial activity. *S. aureus* was found to be one  
275 of the most sensitive microorganisms (data not shown). This is very important given the high  
276 incidence of *S. aureus* in foods during handling (Jay, 2002). Spice antimicrobial compounds have a  
277 greater effect on Gram-positive microorganisms than Gram-negatives due to the latter's cell wall  
278 (Gómez-Estaca, López de Lacey, López-Caballero, Gómez-Guillen and Montero, 2010; Mekinic et al.,  
279 2014), which hinders access to the plasmatic membrane. However, in the present work, individual  
280 variability between strains also appeared to determine antimicrobial activity since the extracts  
281 showed no activity against Gram-positive *L. monocytogenes* or against Gram-negative *E. coli*.

282

283 **3.2. Proximate composition**

284 As expected, formulation had little effect on proximate composition (Table 1). All samples had similar  
285 ( $P > 0.05$ ) protein, moisture and ash contents irrespective of formulation. Only the fat content  
286 increased slightly, that could be explained with sage fat binding properties related with its fibre  
287 proportion (Jiménez-Colmenero and Delgado-Pando, 2013).

288

289 **3.3. Sensory evaluation**

290 Overall, the sensory evaluation of beef burgers was unaffected by formulation (Table 2). Panellists  
291 were unable to distinguish a difference ( $P > 0.05$ ), in terms of flavour and odour acceptability and  
292 general acceptability, between burgers containing sage, irrespective of the concentration (Table 2).  
293 As also reported by Zhang, Lin, Leng, Huang and Zhou (2013), these results indicate that sage could  
294 be incorporated into beef burgers without any detrimental effects on sensory attributes. However, a  
295 spicy odour and flavour was observed in precooked turkey thigh when sage decoction (amount  
296 obtained from 35 kg of sage in 30 L of water boiled (100 °C) at atmospheric pressure) was used

297 (Mielnik, Sem, Egelanddal and Skrede, 2008). Similarly, Hayouni et al. (2008) reported that minced  
298 beef containing 1.5% of essential oil of *S. officinalis* was acceptable, but at higher concentrations it  
299 was unacceptable to the panellists, probably because sage essential oil has a strong, warm, spicy,  
300 herbaceous, and camphoraceous scent. This negative smell–taste effect is inherent in the use of  
301 essential oils (or their components) but is not evident when powdered leaves are used, even at 0.6%  
302 (Table 2).

303

#### 304 **3.4. pH**

305 The addition of sage to burgers did not affect ( $P > 0.05$ ) pH levels neither initially nor during storage  
306 (Table 3). During storage of pork patties at 4 ° C (9 days), the pH of patties containing sage was found  
307 to be quite variable (McCarthy et al., 2001). However, the same authors reported that the pH of  
308 those with ginseng and rosemary increased and those with fenugreek and mustard decreased. In  
309 cooked turkey meatballs, the addition of sage resulted in a decrease in pH (Karpinska-Tymoszczyk,  
310 2007). Moreover, in the present case a slight increase in pH was observed after high-pressure  
311 treatment in all batches (Table 3). This behaviour was observed in dry fermented meat products after  
312 HPP (300 MPa) or raw sausages pressurized above 200 MPa as a consequence of protein  
313 denaturation and the formation of new linkages (Mandava, Fernández, and Juillerat, 1994; Marcos,  
314 Aymerich, and Garriga, 2005). Moreover, Suzuki, Watanabe, Iwamura, Ikeuchi, & Saito (1990)  
315 attributes this effect particularly to conformational changes of histidine. Macfarlane, McKenzie,  
316 Turner, and Jones (1981) observed an increase in the pH of beef muscle caused by pressure  
317 treatment attributed to a loss of free protons as a result of a redistribution of ions as consequence by  
318 the increased ionisation that occurs at elevated pressures. Microbial metabolism did not appear to  
319 influence the pH of hamburgers during storage (Tables 3-4). Thus, the increase in the counts,  
320 especially in those lots without high pressure treatment, could result in a pH increase due to the  
321 accumulation of basic compounds. Nevertheless, with small fluctuations, no significant differences

322 were observed ( $p > 0.05$ ) either by effect of pressure nor by the sage, as conservation progresses  
323 (Table 3).

324

### 325 **3.5. Microbial stability: Considerations regarding the antimicrobial combined effect of HPP and** 326 **sage**

327 Table 4 shows the microbial counts of burgers produced by emulating artisanal processing  
328 conditions. The addition of sage scarcely modified the microbial counts ( $P > 0.05$ ). Similarly,  
329 Mohamed et al. (2011) reported that the addition of natural herbal extracts—0.04% v/w essential  
330 oils (sage among them)—to ground beef did not significantly change the psychrotrophic bacterial  
331 counts during chilled storage (5 °C). However, Karpinska-Tymoszczyk (2007) found that the addition  
332 of sage ethanol extracts (0.1%) to turkey meatballs reduced microorganism mesophiles by 1 log  
333 cycle. It is known that this discrepancy may be due to differences in the characteristics of the spices  
334 (geographic location, seasonality, phenophase, etc.), and to how the sage itself is incorporated (as a  
335 spice powder, extract of different nature, essential oil, etc.). These changes can produce qualitative  
336 and quantitative variations in total phenols that may lead to modifications in biological activity  
337 (Mekinic et al., 2012). Despite the microorganism levels produced by handling in the production of  
338 burgers and by the sage powder, in the present case counts increased by only 1 log cycle over 10 d  
339 (Table 4). In this connection, counts in ground beef with added sage essential oil (0.04% v/w) have  
340 been found to register 8 log cfu / g after 12 days of storage at 7 °C, appearing spoiled (changes in  
341 colour, odour and texture) (Mohamed et al., 2011).

342 Pressurization at 300 MPa/10 min, 9 °C, reduced counts of psychrotrophic and mesophilic  
343 bacteria ( $P < 0.05$ ) by at least two log cycles, and these differences were observed up to 10 days.  
344 Similar results have been reported by Jung, Nam, Ahn, Kim and Jo (2013) in ground beef pressurized  
345 at 300 MPa for 5 min at 15 °C. Sage showed no activity in burgers at any of the concentrations  
346 studied (0.3% and 0.6%). Application of higher pressures (600 MPa) caused mesophilic and  
347 psychrotrophic counts to remain below or close to the detection limit for at least 6 days. Kruk et al.

348 (2011) reported that chicken breast fillets under 600 MPa / 15 ° C / 5 min reduced counts of some  
349 pathogenic organisms previously inoculated (*Salmonella thyphimurium* KCTC 1925 and *E. coli*  
350 KCTC1682 by 6-8 log cfu / g for 7-14 days and *L. monocytogenes* KCTC 3569 above 14 days). These  
351 authors found that at pressures of 300 MPa the reduction in counts was generally sustained at 1-2  
352 log cycles. In our study, the psychrotrophic counts in burgers treated at 600 MPa were < 6 log cfu / g  
353 at 60 days, showing the stability of the product over prolonged chilled storage (Table 4).  
354 Enterobacteria were inhibited by pressure (300 MPa or 600 MPa), remaining below the limit of  
355 detection during the experimental period. This is very important for purposes of improving hygiene  
356 during preparation of burgers and extending their shelf life. In this connection, a combined  
357 treatment of 0.3% sage and modified atmospheres (20% CO<sub>2</sub> / 80% N<sub>2</sub>) in turkey meatballs has been  
358 found to prevent the appearance of coliforms (an effect not observed in batches under modified  
359 atmospheres only) (Karpinska-Tymoszczyk, 2010).

360

### 361 **3.6. Lipid stability**

362 TBARs values were affected ( $P < 0.05$ ) by formulation, HPP and storage (Table 5). Initially, samples  
363 containing sage had lower ( $P < 0.05$ ) TBARs values than OS burgers irrespective of sage concentration.  
364 Lipid oxidation increased during storage, but those differences generally persisted after HPP and  
365 throughout storage (10 days for non-pressurized samples). Comparison of TBARs values in samples  
366 with/without added sage showed that these were generally little affected by pressurization during  
367 storage (Table 5). Lipid oxidation increased ( $P < 0.05$ ) during storage in the pressurized control  
368 samples (300/OS and 600/OS), while the increase of TBARs values was proportionately smaller in  
369 burgers containing sage. The fact that the TBARS values of burgers with added sage were significantly  
370 lower over storage indicates a lower lipid oxidation rate. The decrease found after 34 days in long-  
371 term storage samples (burgers pressurized at 600 MPa) could be the result of further reactions  
372 between secondary lipid oxidation products (TBARs) and other meat macromolecules or compounds,  
373 such as proteins, as reported by Utrera, Morcuende and Estévez (2014).



374 Hexanal levels were generally higher ( $P < 0.05$ ) in control burgers than in the products  
375 containing sage, although the effect was similar irrespective of the concentration (Table 6). This  
376 behaviour is consistent with the TBARS results. Hexanal concentrations increased significantly in all  
377 samples during storage, although the timing of the increase varied with formulation (presence of  
378 sage) and processing (pressurization). After increasing, the hexanal content declined ( $P < 0.05$ ) in  
379 non-pressurized samples, and in samples pressurized at 600 MPa after 24 days of storage,  
380 irrespective of formulation (Table 6). As reported by Utrera et al. (2014), hexanal is formed in the  
381 early stages of oxidation, and like TBARS undergoes further reactions which may be responsible for  
382 the decrease in hexanal content. Strong interactions between proteins and lipid oxidation products  
383 to form Schiff bases via condensation have been reported (Utrera and Estévez, 2013).

384 The sage extract showed an antioxidant activity measured of  $87.87 \pm 5.08$  mg eq trolox /mg  
385 sample much greater than activity shown by burgers which was affected ( $P < 0.05$ ) by formulation,  
386 HPP and storage (Table 7). Martins et al. (2014) reported antioxidant activity in various sage extracts  
387 (aqueous, methanol/water) obtained by decoction or infusion. Also, Grzegorzczuk, Matkowski and  
388 Wysokinska (2007) reported antioxidant potential in methanol and acetone extracts prepared from  
389 organs (shoots and hairy roots) and undifferentiated elements (cell and callus) in in-vitro cultures of  
390 *S. officinalis*. In the present case antioxidative activity was greater ( $P < 0.05$ ) in burger samples  
391 containing sage than in the control (0S); this behaviour correlated directly with sage concentration,  
392 irrespective of pressurization and storage. Significant differences were noted in some cases, but  
393 pressurization level and storage generally had a relatively small effect on the antioxidative activity of  
394 the burgers, with no clear trend (Table 7).

395 TBARS, hexanal and PCL are all methods that provide information about the oxidative status  
396 of the system and the progress of lipid oxidation in meat products such as burgers, and so it is  
397 possible to establish a level of correlation among them. When all the experimental data (irrespective  
398 of formulation and storage time) were collated, significant correlations were found for TBARS/PCL (-  
399 0.502,  $P < 0.01$ ) and TBARS/hexanal (0.661,  $P < 0.01$ ), but for PCL/hexanal the correlation was not

400 significant ( $-0.209, P > 0.01$ ). This means that there is an inverse relationship between the progress of  
401 lipid oxidation and the radical quenching capacity of the system. Also, there is a direct relationship  
402 between the parameters used to evaluate the formation of secondary compounds from lipid  
403 oxidation in beef burgers with different formulations and processing. Rey, Hopia, Kivikari and  
404 Kahkonen (2005) also found a direct relationship between TBARs and hexanal content in cooked  
405 burgers after 3 days of refrigerated storage at 4 °C and with different plant extracts as natural  
406 antioxidants. Cofrades et al. (2011) found a significant correlation for TBARs/PCL in frankfurters  
407 enriched with n-3 fatty acids and containing antioxidants such as butylhydroxytoluene (BHT) and  
408 hydroxytyrosol (Hyt). However, other authors have reported no significant correlation between lipid  
409 oxidation and antioxidant capacity in fresh meat (Descalzo et al., 2008) and fish muscle (Medina,  
410 Gallardo, González, Lois and Hedges, 2007).

411         These results invite two main considerations: a) the antioxidant activity of sage, and b) the  
412 absence of prooxidant activity of HPP under the studied conditions. The antioxidative effect of sage  
413 demonstrated in this experiment is consistent with the results reported by various authors, although  
414 they used sage in different forms and on different matrices. In this regard, sage has been used in  
415 different forms, including essential oils (Fasseas et al., 2008; Mohamed et al., 2011; Unal, Babaoglu  
416 and Karakaya 2014), extracts (McCarthy et al., 2001) and dried powders (Mariutti et al., 2011;  
417 Mariutti et al., 2008) to study the oxidative stability of minced meat from different species (beef,  
418 pork, chicken) and as affected by cooking and/or under chilled and frozen storage. For example, the  
419 addition of 3% sage essential oil inhibited lipid oxidation in raw pork and in cooked bovine meat  
420 (Fasseas, et al., 2008). Addition of 0.1 % dried sage to minced chicken meat effectively minimized and  
421 delayed the oxidation of lipids and cholesterol during thermal processing and storage at  $-18\text{ °C}$   
422 (Mariutti et al., 2011). There are no reports in the literature associating the demonstrated natural  
423 antioxidant activity of sage with conditions of use in minced meat, but it seems that the presence of  
424 phenolic compounds (rosmarinic acid and carnosic acid, among others) contributes to its antioxidant  
425 activity through reductive, free radical-scavenging and lipid oxidation-inhibiting activities (Zhang et

426 al., 2013). In this connection, the authors observed an increase in the system's ability to scavenge  
427 free radicals, associated with the presence of sage (Table 7).

428 It has been reported that high-pressure treatment of meat favours oxidation of  
429 polyunsaturated fatty acids and promotes radical formation in fresh meat, although this effect  
430 depends on factors associated with HPP conditions (pressure level/time/temperature) (Guyon,  
431 Meynier and de Lamballerie, 2016). In this regard, several studies have concluded that treatment at  
432 pressures above 300-400 MPa is essential to induce a prooxidant effect (Guyon et al., 2016; H. Ma  
433 and Ledward, 2013; Mariutti et al., 2008) which is consistent with the results observed in the samples  
434 treated at 300 MPa (Tables 5-6). Alves et al. (2012) reported a decline in the concentration of radicals  
435 during storage of chicken meat pressurized at 300 MPa, suggesting that the radicals formed during  
436 pressure treatment are scavenged and hence cannot further enhance lipid oxidation. The absence of  
437 pressure-induced lipid oxidation at 600 MPa should be considered in light of the fact that the effect  
438 of HPP on lipid oxidation, is strongly dependent on the type of meat matrix (Guyon et al., 2016). For  
439 instance, it has been reported that beef was more resistant to pressure than chicken, so that the  
440 critical pressures for chicken breast and beef sirloin were established at 400 MPa and 600 MPa  
441 respectively (Schindler, Krings, Berger and Orlien, 2010). The lipid oxidation of raw ground beef was  
442 not significantly influenced by HPP treatment up to 600 MPa during storage (10 days) (Jung et al.,  
443 2013). However, Ma, Ledward, Zamri, Frazier and Zhou (2007) found that pressure treatment  $\geq 400$   
444 MPa considerably increased lipid oxidation in beef, and that it was more prone to lipid oxidation than  
445 chicken meat. On the other hand, Beltran, Pla, Yuste and Mor-Mur (2003) observed no effect on the  
446 oxidative stability of minced chicken breast subjected to 500 MPa. These conflicting results have  
447 been put down to differences in meat matrix conditions and characteristics. In this regard Schindler  
448 et al. (2010) posited that post-slaughter history and small variations in the quality of the raw material  
449 may have different effects on the development of lipid oxidation at pressures in the vicinity of the  
450 critical pressure. As in this experiment, various studies have demonstrated that after treatment at

451 pressures between 300 and 800 MPa for chicken and between 200 and 600 MPa for beef, the TBARS  
452 content generally increases during chilling storage (Guyon et al., 2016; Mariutti et al., 2008).

453 Mariutti et al. (2008) reported TBARS values directly indicating that sage protected the lipids  
454 against pressure-induced oxidation of chicken meat during chilling storage for two weeks. No such  
455 effect was observed in the present experiment since, although sage effectively inhibited lipid  
456 oxidation in beef burgers over storage, this does not seem to have been related to pressurization  
457 (Table 5).

458

#### 459 **4. CONCLUSIONS**

460 It was concluded that sage powder was effective as an antioxidant, retarding lipid oxidation in HPP  
461 treated beef burgers over 60 days of chilling storage. Beef burgers did not undergo lipid oxidation  
462 during prolonged chilling storage as a result of pressurization at 300 and 600 MPa, and their  
463 microbial quality was judged acceptable after 60 days refrigerated storage when pressurized at 600  
464 MPa with and without sage. Natural dried sage powder, even at high concentrations, displayed  
465 potential in maintaining sensory eating quality in cooked beef burgers.

466

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Table 1. Proximate analysis (%) of burgers

<b>Sample</b>	<b>Moisture</b>	<b>Fat</b>	<b>Protein</b>	<b>Ash</b>
<b>0S</b>	71.96±0.18 <sup>a</sup>	6.20± 0.04 <sup>a</sup>	19.12±0.10 <sup>a</sup>	1.94±0.04 <sup>a</sup>
<b>0.3S</b>	72.20±0.33 <sup>a</sup>	6.89±0.19 <sup>ab</sup>	19.34±0.51 <sup>a</sup>	1.95±0.08 <sup>a</sup>
<b>0.6S</b>	72.14±0.33 <sup>a</sup>	7.30±0.56 <sup>b</sup>	19.15±0.10 <sup>a</sup>	2.04±0.03 <sup>a</sup>

0S: Control burger; 0.3S: Burgers containing 0.3% of sage; 0.6S: Burgers containing 0.6% of sage.  
Different letter indicated significant differences ( $P < 0.05$ ).

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Table 2. Sensory evaluation of burgers

<b>Sample</b>	<b>Flavor acceptability</b>	<b>Odor acceptability</b>	<b>General acceptability</b>
<b>0S</b>	5.57±2.59 <sup>a</sup>	5.45±2.58 <sup>a</sup>	5.82±2.69 <sup>a</sup>
<b>0.3S</b>	6.07±2.28 <sup>a</sup>	6.50±2.09 <sup>a</sup>	6.36±2.21 <sup>a</sup>
<b>0.6S</b>	6.09±2.13 <sup>a</sup>	6.70±1.86 <sup>a</sup>	6.35±2.38 <sup>a</sup>

0S: Control burger; 0.3S: Burgers containing 0.3% of sage; 0.6S: Burgers containing 0.6% of sage.  
Means ± standard deviation. Different letter indicated significant differences ( $P < 0.05$ ).

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684 Table 3. pH of burgers over the storage time

Samples	Storage (days at 2 °C)							
	1	3	6	10	24	34	44	60
<b>0S</b>	5.87±0.01 <sup>a2</sup>	5.57±0.25 <sup>a12</sup>	5.45±0.12 <sup>a1</sup>	5.70±0.52 <sup>a12</sup>				
<b>0.3S</b>	5.89±0.01 <sup>a2</sup>	5.56±0.26 <sup>a12</sup>	5.4±0.05 <sup>a1</sup>	5.68±0.56 <sup>a12</sup>				
<b>0.6S</b>	5.90±0.02 <sup>a1</sup>	5.55±0.27 <sup>a12</sup>	5.40±0.06 <sup>a1</sup>	5.73±0.49 <sup>a12</sup>				
<b>300/0S</b>	6.02±0.04 <sup>b2</sup>	5.83±0.23 <sup>a12</sup>	5.99±0.02 <sup>b2</sup>	5.93±0.16 <sup>a12</sup>	5.77±0.02 <sup>a1</sup>			
<b>300/0.3S</b>	6.03±0.00 <sup>b2</sup>	5.82±0.20 <sup>a1</sup>	6.05±0.02 <sup>b2</sup>	5.93±0.14 <sup>a12</sup>	5.76±0.03 <sup>a1</sup>			
<b>300/0.6S</b>	6.05±0.02 <sup>b2</sup>	5.85±0.19 <sup>a12</sup>	6.05±0.01 <sup>b2</sup>	5.95±0.17 <sup>a12</sup>	5.78±0.08 <sup>a1</sup>			
<b>600/0S</b>	6.05±0.01 <sup>b2</sup>	5.85±0.17 <sup>a1</sup>	6.06±0.00 <sup>b2</sup>	5.97±0.18 <sup>a12</sup>	6.07±0.02 <sup>b2</sup>	6.05±0.02 <sup>a2</sup>	6.07±0.06 <sup>a2</sup>	5.99±0.06 <sup>a12</sup>
<b>600/0.3S</b>	6.05±0.00 <sup>b2</sup>	5.87±0.17 <sup>a1</sup>	6.06±0.01 <sup>b2</sup>	5.94±0.15 <sup>a12</sup>	6.07±0.02 <sup>b2</sup>	6.08±0.01 <sup>b2</sup>	6.07±0.05 <sup>a2</sup>	5.96±0.07 <sup>a12</sup>
<b>600/0.6S</b>	6.06±0.02 <sup>b2</sup>	5.86±0.20 <sup>a1</sup>	6.06±0.03 <sup>b2</sup>	5.93±0.17 <sup>a12</sup>	6.11±0.01 <sup>b2</sup>	6.07±0.01 <sup>b2</sup>	6.07±0.05 <sup>a2</sup>	5.97±0.10 <sup>a12</sup>

685 Control burger: non-pressurized (0S) and pressurized at 300 and 600 MPa (300/0S and 600/0S, respectively). Burger containing 0.3% of sage: non-  
686 pressurized (0.3S) and pressurized at 300 and 600 MPa (300/0.3S and 600/0.3S, respectively). Burger containing 0.6% of sage: non-pressurized (0.6S) and  
687 pressurized at 300 and 600 MPa (300/0.6S and 600/0.6S, respectively).

688 Means ± standard deviation. Different letters (a,b,c) within the same column or numbers (1-3) in the same row indicate significant differences ( $P < 0.05$ ).

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696 Table 4. Microbiological count (log cfu/g) in burgers over storage.

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		Storage (days at 2 °C)							
Samples		1	3	6	10	24	34	44	60
	<b>0S</b>	7.48±0.00 <sup>b1</sup>	7.47±0.05 <sup>c1</sup>	8.07±0.03 <sup>d2</sup>	8.19±0.08 <sup>e2</sup>				
	<b>0.3S</b>	7.33±0.02 <sup>b1</sup>	7.83±0.04 <sup>c2</sup>	7.84±0.11 <sup>d2</sup>	7.30±0.03 <sup>d1</sup>				
	<b>0.6S</b>	7.31±0.04 <sup>b1</sup>	7.51±0.16 <sup>c12</sup>	7.8±0.13 <sup>d2</sup>	8.22±0.08 <sup>e3</sup>				
PSYCHROTROPHILIC	<b>300/0S</b>	5,46±0,06 <sup>a2</sup>	5.33±0.10 <sup>b2</sup>	4.00±0.00 <sup>a1</sup>	6.66±0.01 <sup>c3</sup>	8.13±0.03 <sup>b4</sup>			
	<b>300/0.3S</b>	5,22±0,13 <sup>a12</sup>	4.83±0.49 <sup>a1</sup>	5.28±0.28 <sup>b2</sup>	6.09±0.01 <sup>b3</sup>	7.95±0.02 <sup>b4</sup>			
	<b>300/0.6S</b>	5.30±0.06 <sup>a1</sup>	4.95±0.07 <sup>ab1</sup>	5.73±0.12 <sup>c2</sup>	6.29±0.06 <sup>bc3</sup>	8.30±0.02 <sup>b4</sup>			
	<b>600/0S</b>	-	-	-	-	5.18±0.04 <sup>a1</sup>	5.71±0.12 <sup>a2</sup>	5.24±0.34 <sup>a1</sup>	5.92±0.11 <sup>a2</sup>
	<b>600/0.3S</b>	-	-	-	-	5.28±0.01 <sup>a1</sup>	5.20±0.18 <sup>a1</sup>	5.15±0.21 <sup>a1</sup>	5.74±0.06 <sup>a2</sup>
	<b>600/0.6S</b>	-	-	-	-	5.09±0.09 <sup>a2</sup>	5.69±0.01 <sup>a3</sup>	5.24±0.34 <sup>a2</sup>	5.69±0.12 <sup>a3</sup>
	<b>0S</b>	7.23±0.01 <sup>b1</sup>	7.57±0.02 <sup>b12</sup>	7.85±0.05 <sup>e2</sup>	7.66±0.01 <sup>d2</sup>				
	<b>0.3S</b>	7.15±0.05 <sup>b1</sup>	7.37±0.07 <sup>b2</sup>	7.67±0.09 <sup>e2</sup>	7.58±0.05 <sup>d2</sup>				
	<b>0.6S</b>	7.14±0.02 <sup>b1</sup>	7.34±0.12 <sup>b1</sup>	7.71±0.08 <sup>e2</sup>	7.68±0.09 <sup>d2</sup>				
MESOPHILES	<b>300/0S</b>	5,58±0,02 <sup>a2</sup>	5.73±0.04 <sup>a2</sup>	4.83±0.49 <sup>c1</sup>	6.57±0.03 <sup>c3</sup>	8.17±0.04 <sup>c4</sup>			
	<b>300/0.3S</b>	5,50±0,00 <sup>a1</sup>	5.67±0.06 <sup>a1</sup>	5.76±0.00 <sup>d1</sup>	6.23±0.07 <sup>c2</sup>	8.00±0.05 <sup>c3</sup>			
	<b>300/0.6S</b>	5,54±0,01 <sup>a1</sup>	5.67±0.06 <sup>a1</sup>	5.79±0.05 <sup>d1</sup>	6.35±0.03 <sup>c2</sup>	8.19±0.09 <sup>c3</sup>			

<b>600/0S</b>	-	-	1.48±0.00 <sup>b1</sup>	2.50±0.00 <sup>b2</sup>	4.99±0.03 <sup>b2</sup>	5.48±0.00 <sup>a3</sup>	6.14±0.09 <sup>b4</sup>	5.96±0.17 <sup>a4</sup>
<b>600/0.3S</b>	-	-	1.00±0.00 <sup>a1</sup>	2.68±0.08 <sup>b2</sup>	5.23±0.01 <sup>b4</sup>	5.33±0.07 <sup>a4</sup>	4.80±0.28 <sup>a3</sup>	7.57±0.03 <sup>b5</sup>
<b>600/0.6S</b>	-	-	1.39±0.55 <sup>ab1</sup>	2.16±0.06 <sup>a2</sup>	3.43±0.04 <sup>a3</sup>	5.56±0.06 <sup>a4</sup>	5.80±0.28 <sup>b4</sup>	5.90±0.08 <sup>a4</sup>

	<b>Day1</b>	<b>Day3</b>	<b>Day 6</b>	<b>Day 10</b>	<b>Day24</b>	<b>Day34</b>	<b>Day44</b>	<b>Day 60</b>
<b>0S</b>	4.30±0.09 <sup>a2</sup>	3.66±0.64 <sup>a1</sup>	4.12±0.39 <sup>a12</sup>	4.48±0.01 <sup>b2</sup>				
<b>0.3S</b>	4.37±0.31 <sup>a1</sup>	4.33±0.17 <sup>b1</sup>	3.99±0.14 <sup>a1</sup>	3.82±0.01 <sup>a1</sup>				
<b>0.6S</b>	4.25±0.24 <sup>a1</sup>	4.45±0.04 <sup>b1</sup>	4.17±0.09 <sup>a1</sup>	4.09±0.01 <sup>ab1</sup>				

ENTEROBACTERIA	<b>300/0S</b>	-	-	-	-	-		
	<b>300/0.3S</b>	-	-	-	-	-		
	<b>300/0.6S</b>	-	-	-	-	-		

<b>600/0S</b>	-	-	-	-	-	-	-	-
<b>600/0.3S</b>	-	-	-	-	-	-	-	-
<b>600/0.6S</b>	-	-	-	-	-	-	-	-

698 Control burger: non-pressurized (0S) and pressurized at 300 and 600 MPa (300/0S and 600/0S, respectively). Burger containing 0.3% of sage: non-  
699 pressurized (0.3S) and pressurized at 300 and 600 MPa (300/0.3S and 600/0.3S, respectively). Burger containing 0.6% of sage: non-pressurized (0.6S) and  
700 pressurized at 300 and 600 MPa (300/0.6S and 600/0.6S, respectively).

701 Means ± standard deviation. Different letters (a,b,c) within the same column or numbers (1-3) in the same row indicate significant differences ( $P < 0.05$ ).

Table 5. Thiobarbituric acid-reactive substances (TBARS) concentration (mg MDA/kg sample) in burgers over storage.

Samples	Storage (days at 2 °C)							
	1	3	6	10	24	34	44	60
<b>0S</b>	0.43±0.01 <sup>C1</sup>	0.46±0.01 <sup>bc2</sup>	0.47±0.00 <sup>e2</sup>	0.51±0.01 <sup>cd3</sup>				
<b>0.3S</b>	0.25±0.06 <sup>ab1</sup>	0.28±0.08 <sup>a1</sup>	0.39±0.06 <sup>bcd2</sup>	0.43±0.01 <sup>bc2</sup>				
<b>0.6S</b>	0.26±0.10 <sup>ab1</sup>	0.26±0.07 <sup>a1</sup>	0.39±0.09 <sup>cde2</sup>	0.44±0.05 <sup>bc2</sup>				
<b>300/0S</b>	0.35±0.01 <sup>bc1</sup>	0.49±0.01 <sup>c3</sup>	0.40±0.01 <sup>cde2</sup>	0.73±0.01 <sup>e4</sup>	1.31±0.03 <sup>c5</sup>			
<b>300/0.3S</b>	0.24±0.01 <sup>a1</sup>	0.27±0.01 <sup>a12</sup>	0.33±0.01 <sup>abc123</sup>	0.35±0.11 <sup>ab23</sup>	0.39±0.09 <sup>a3</sup>			
<b>300/0.6S</b>	0.20±0.03 <sup>a1</sup>	0.22±0.02 <sup>a1</sup>	0.25±0.00 <sup>a12</sup>	0.32±0.07 <sup>ab23</sup>	0.39±0.10 <sup>a3</sup>			
<b>600/0S</b>	0.35±0.01 <sup>c1</sup>	0.39±0.00 <sup>b12</sup>	0.46±0.01 <sup>de3</sup>	0.62±0.00 <sup>de4</sup>	0.89±0.01 <sup>b6</sup>	0.60±0.01 <sup>b45</sup>	0.43±0.01 <sup>c23</sup>	0.67±0.01 <sup>b5</sup>
<b>600/0.3S</b>	0.26±0.04 <sup>ab12</sup>	0.26±0.04 <sup>a12</sup>	0.31±0.05 <sup>ab2</sup>	0.32±0.09 <sup>ab2</sup>	0.37±0.06 <sup>a2</sup>	0.18±0.11 <sup>a1</sup>	0.24±0.02 <sup>b12</sup>	0.26±0.04 <sup>a12</sup>
<b>600/0.6S</b>	0.21±0.00 <sup>a123</sup>	0.25±0.03 <sup>a234</sup>	0.28±0.01 <sup>a34</sup>	0.30±0.05 <sup>a34</sup>	0.38±0.03 <sup>a4</sup>	0.12±0.15 <sup>a12</sup>	0.11±0.09 <sup>a1</sup>	0.23±0.05 <sup>a123</sup>

Control burger: non-pressurized (0S) and pressurized at 300 and 600 MPa (300/0S and 600/0S, respectively). Burger containing 0.3% of sage: non-pressurized (0.3S) and pressurized at 300 and 600 MPa (300/0.3S and 600/0.3S, respectively). Burger containing 0.6% of sage: non-pressurized (0.6S) and pressurized at 300 and 600 MPa (300/0.6S and 600/0.6S, respectively).

Means ± standard deviation. Different letters (a,b,c) within the same column or numbers (1-3) in the same row indicate significant differences ( $P < 0.05$ ).



Table 6. Hexanal concentration ( $\mu\text{g/g}$  sample) in burgers over storage

Samples	Storage (days at 2 °C)						
	1	6	10	24	34	44	60
<b>0S</b>	0.22±0.01 <sup>c1</sup>	0.25±0.04 <sup>ab2</sup>	0.29±0.03 <sup>a3</sup>				
<b>0.3S</b>	0.04±0.01 <sup>a1</sup>	0.50±0.20 <sup>bc3</sup>	0.27±0.02 <sup>a2</sup>				
<b>0.6S</b>	0.05±0.01 <sup>ab1</sup>	0.38±0.09 <sup>abc3</sup>	0.25±0.02 <sup>a2</sup>				
<b>300/0S</b>	0.09±0.02 <sup>b1</sup>	0.63±0.01 <sup>c2</sup>	0.50±0.07 <sup>b2</sup>	0.62±0.11 <sup>b2</sup>			
<b>300/0.3S</b>	0.04±0.02 <sup>a1</sup>	0.31±0.04 <sup>ab2</sup>	0.39±0.04 <sup>ab3</sup>	0.39±0.04 <sup>a3</sup>			
<b>300/0.6S</b>	0.04±0.01 <sup>a1</sup>	0.39±0.04 <sup>a23</sup>	0.31±0.07 <sup>a2</sup>	0.43±0.03 <sup>a3</sup>			
<b>600/0S</b>	0.24±0.02 <sup>c1</sup>	0.25±0.16 <sup>ab1</sup>	0.72±0.05 <sup>c2</sup>	0.64±0.03 <sup>b2</sup>	0.39±0.02 <sup>b1</sup>	0.23±0.02 <sup>a1</sup>	0.30±0.04 <sup>b1</sup>
<b>600/0.3S</b>	0.04±0.01 <sup>a1</sup>	0.19±0.06 <sup>a2</sup>	0.32±0.08 <sup>a34</sup>	0.43±0.02 <sup>a4</sup>	0.21±0.03 <sup>a23</sup>	0.19±0.05 <sup>a2</sup>	0.15±0.00 <sup>a12</sup>
<b>600/0.6S</b>	0.04±0.00 <sup>a1</sup>	0.18±0.01 <sup>a2</sup>	0.36±0.03 <sup>ab3</sup>	0.32±0.04 <sup>a3</sup>	0.21±0.01 <sup>a2</sup>	0.21±0.01 <sup>a2</sup>	0.17±0.01 <sup>a2</sup>

Control burger: non-pressurized (0S) and pressurized at 300 and 600 MPa (300/0S and 600/0S, respectively). Burger containing 0.3% of sage: non-pressurized (0.3S) and pressurized at 300 and 600 MPa (300/0.3S and 600/0.3S, respectively). Burger containing 0.6% of sage: non-pressurized (0.6S) and pressurized at 300 and 600 MPa (300/0.6S and 600/0.6S, respectively).

Means  $\pm$  standard deviation. Different letters (a,b,c) within the same column or numbers (1-3) in the same row indicate significant differences ( $P < 0.05$ ).

Table 7. Antioxidant capacity of burgers over storage (mg eq trolox /mg sample)

Samples	Storage (days at 2 °C)							
	1	3	6	10	24	34	44	60
<b>0S</b>	0.13±0.01 <sup>a1</sup>	0.18±0.01 <sup>ab2</sup>	0.19±0.01 <sup>a2</sup>	0.18±0.02 <sup>ab2</sup>				
<b>0.3S</b>	0.22±0.02 <sup>b1</sup>	0.29±0.01 <sup>c2</sup>	0.29±0.01 <sup>b2</sup>	0.28±0.00 <sup>c2</sup>				
<b>0.6S</b>	0.34±0.00 <sup>c1</sup>	0.52±0.02 <sup>e2</sup>	0.60±0.02 <sup>e3</sup>	0.52±0.02 <sup>d2</sup>				
<b>300/0S</b>	0.13±0.00 <sup>a1</sup>	0.21±0.00 <sup>b3</sup>	0.20±0.01 <sup>a3</sup>	0.20±0.00 <sup>b3</sup>	0.15±0.01 <sup>a2</sup>			
<b>300/0.3S</b>	0.18±0.00 <sup>ab1</sup>	0.32±0.01 <sup>c3</sup>	0.27±0.01 <sup>b2</sup>	0.32±0.01 <sup>c3</sup>	0.32±0.01 <sup>c3</sup>			
<b>300/0.6S</b>	0.56±0.03 <sup>d12</sup>	0.51±0.02 <sup>e1</sup>	0.52±0.00 <sup>d1</sup>	0.51±0.02 <sup>d1</sup>	0.59±0.03 <sup>e2</sup>			
<b>600/0S</b>	0.10±0.00 <sup>a1</sup>	0.17±0.01 <sup>a4.5</sup>	0.16±0.01 <sup>a3.4.5</sup>	0.13±0.01 <sup>a1.2.3.4</sup>	0.12±0.01 <sup>a1.2</sup>	0.17±0.00 <sup>a5</sup>	0.13±0.02 <sup>a1.2.3</sup>	0.12±0.02 <sup>a1.2</sup>
<b>600/0.3S</b>	0.35±0.01 <sup>b2.3</sup>	0.36±0.00 <sup>d3</sup>	0.36±0.02 <sup>c3</sup>	0.30±0.03 <sup>c2</sup>	0.26±0.01 <sup>b1</sup>	0.28±0.00 <sup>b1.2</sup>	0.30±0.01 <sup>b2</sup>	0.27±0.02 <sup>b1.2</sup>
<b>600/0.6S</b>	0.45±0.08 <sup>c1</sup>	0.58±0.03 <sup>f2</sup>	0.48±0.05 <sup>d1</sup>	0.54±0.01 <sup>d12</sup>	0.50±0.00 <sup>d1.2</sup>	0.43±0.00 <sup>c1</sup>	0.49±0.01 <sup>c1.2</sup>	0.52±0.01 <sup>c1.2</sup>

Control burger: non-pressurized (0S) and pressurized at 300 and 600 MPa (300/0S and 600/0S, respectively). Burger containing 0.3% of sage: non-pressurized (0.3S) and pressurized at 300 and 600 MPa (300/0.3S and 600/0.3S, respectively). Burger containing 0.6% of sage: non-pressurized (0.6S) and pressurized at 300 and 600 MPa (300/0.6S and 600/0.6S, respectively).

Means ± standard deviation. Different letters (a,b,c) within the same column or numbers (1-3) in the same row indicate significant differences ( $P < 0.05$ ).

