Antimicrobial and antioxidant effects of combined high pressure processing and sage in beef burgers during prolonged chilled storage

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Abstract

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

Industrial relevance

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

Highlights

- Sage powder was an effective antioxidant in burgers over prolonged chilling storage.
- HPP of beef burgers did not induce lipid oxidation during prolonged chilling storage.
- Sensory attributes were unaffected by added sage powder.
- Burgers exposed to 600 MPa showed acceptable microbial quality after 60 days.

Key words

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

1. INTRODUCTION

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

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

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

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

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

2. MATERIAL AND METHODS

2.1. Sage preparation

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

2.1.1. Preparation of extracts and measurement of antimicrobial activity

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

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

2.2. Burger preparation

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

2.3. High pressure processing (HPP) of burger

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

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

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

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

2.4. Proximate analysis

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

2.5. Sensory evaluation

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

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

2.8.2. Hexanal assay

Lipid oxidation was also analysed by changes in hexanal content. Minced samples (3 g) and 7 mL of a 0.2% EDTA water solution were dispensed in glass vials and thoroughly mixed for 3 min. The vials were then sealed with Teflon-face silicone septums and aluminium caps. The vials were frozen at -80 °C until use, when they were thawed overnight (12h) at 4 °C, and resuspended by stirring for 30 s. Prior to injection into the Gas chromatography-mass spectrometer (CG–MS), sample was heated to 80 °C for 15 min following preconcentration for 2 cycles in an active carbon cap (carbopack), desorbing at 300 °C. Samples were injected into a CG–MSusing TurboMatrix HS 40 Trap Automated headspace sampler (Perkin Elmer, Massachusetts, USA). CG–MS analysis of sample headspace was carried out using an Agilent system (Waldbronn, Germany) consisting of a 6890N gas chromatograph coupled to a (EI) 5973N quadrupole mass spectrometer and a HP computer. The interface and the source temperature were 240°C and 230°C respectively. Electron impact mass spectra were recorded in SIM mode at an ionization energy of 70 eV. Separation was performed on a fused–sillica bonded phase capillary column HP5MS (J&W Scientific, Folson, CA, USA) (30m x0.25mm x0.25µm) at constant pressure (12 psi) provided by a HS-40 Autosampler. The temperature was programmed isothermally at 50°C for 7 min, then raised to150°C at 20°C min^{-1} and to 240°C at 50°C min^{-1}; this temperature was held for 5 min. Blank analyses were carried out with the same trapping material and following the same procedure, starting from distilled water as the sample.

2.8.3. Antioxidative activity by photochemiluminescence (PCL)

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

2.9. Statistical analysis

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

3. RESULT AND DISCUSSION

3.1. Antimicrobial activity of the sage extracts

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

3.2. Proximate composition

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

3.3. Sensory evaluation

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

297 (Mielnik, Sem, Egelandsdal 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

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

3.5. Microbial stability: Considerations regarding the antimicrobial combined effect of HPP and sage

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

Pressurization at 300 MPa/10 min, 9 ºC, reduced counts of psychrotrophic and mesophilic bacteria (P < 0.05) by at least two log cycles, and these differences were observed up to 10 days. Similar results have been reported by Jung, Nam, Ahn, Kim and Jo (2013) in ground beef pressurized at 300 MPa for 5 min at 15 ° C. Sage showed no activity in burgers at any of the concentrations studied (0.3% and 0.6%). Application of higher pressures (600 MPa) caused mesophilic and 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_2 / 80% N_2) 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 0S 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 (300/0S and 600/0S), 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 ± 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, Grzegorczyk, 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 ◦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

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

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

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

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

4. CONCLUSIONS

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

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Table 1. Proximate analysis (%) of burgers

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

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

Table 2. Sensory evaluation of burgers

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

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 ($P < 0.05$).

Table 3. pH of burgers over the storage time

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

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 ($P < 0.05$).

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

	600/0S	-	-	1.48±0.00 ^{b1}	2.50±0.00^{b2}	4.99±0.03 ^{b2}	5.48±0.00 ^{a3}	6.14±0.09 ^{b4}	5.96±0.17 ^{a4}
	600/0.3S	-	-	1.00±0.00 ^{a1}	2.68±0.08 ^{b2}	5.23±0.01 ^{b4}	5.33±0.07 ^{a4}	4.80±0.28 ^{a3}	7.57±0.03 ^{b5}
	600/0.6S	-	-	1.39±0.55 ^{ab1}	2.16±0.06 ^{a2}	3.43±0.04 ^{a3}	5.56±0.06 ^{a4}	5.80±0.28 ^{b4}	5.90±0.08 ^{a4}
		Day1	**Day3**	**Day 6**	**Day 10**	**Day24**	**Day34**	**Day44**	**Day 60**
	0S	4.30±0.09 ^{a2}	3.66±0.64 ^{a1}	4.12±0.39 ^{a12}	4.48±0.01 ^{b2}				
	0.3S	4.37±0.31 ^{a1}	4.33±0.17 ^{b1}	3.99±0.14 ^{a1}	3.82±0.01 ^{a1}				
	0.6S	4.25±0.24 ^{a1}	4.45±0.04 ^{b1}	4.17±0.09 ^{a1}	4.09±0.01 ^{ab1}				
	300/0S	-	-	-	-	-			
ENTEROBACTERIA	**300/0.3S**	-	-	-	-	-			
	300/0.6S	-	-	-	-	-			
	600/0S	-	-	-	-	-	-	-	-
	600/0.3S	-	-	-	-	-	-	-	-
	600/0.6S	-	-	-	-	-	-	-	-

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 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**
0S	0.43±0.01^{C1}	0.46±0.01^{bc2}	0.47±0.00^{e2}	0.51±0.01^{cd3}				
0.3S	0.25±0.06^{ab1}	0.28±0.08^{a1}	0.39±0.06^{bcd2}	0.43±0.01^{bc2}				
0.6S	0.26±0.10^{ab1}	0.26±0.07^{a1}	0.39±0.09^{cde2}	0.44±0.05^{bc2}				
300/0S	0.35±0.01^{bc1}	0.49±0.01^{c3}	0.40±0.01^{cde2}	0.73±0.01^{e4}	1.31±0.03^{c5}			
300/0.3S	0.24±0.01^{a1}	0.27±0.01^{a12}	0.33±0.01^{abc123}	0.35±0.11^{ab23}	0.39±0.09^{a3}			
300/0.6S	0.20±0.03^{a1}	0.22±0.02^{a1}	0.25±0.00^{a12}	0.32±0.07^{ab23}	0.39±0.10^{a3}			
600/0S	0.35±0.01^{c1}	0.39±0.00^{b12}	0.46±0.01^{de3}	0.62±0.00^{de4}	0.89±0.01^{b6}	0.60±0.01^{b45}	0.43±0.01^{c23}	0.67±0.01^{b5}
600/0.3S	0.26±0.04^{ab12}	0.26±0.04^{a12}	0.31±0.05^{ab2}	0.32±0.09^{ab2}	0.37±0.06^{a2}	0.18±0.11^{a1}	0.24±0.02^{b12}	0.26±0.04^{a12}
600/0.6S	0.21±0.00^{a123}	0.25±0.03^{a234}	0.28±0.01^{a34}	0.30±0.05^{a34}	0.38±0.03^{a4}	0.12±0.15^{a12}	0.11±0.09^{a1}	0.23±0.05^{a123}

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 (μg/g sample) in burgers over storage

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

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 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**
0S	0.13±0.01^{a1}	0.18±0.01^{ab2}	0.19±0.01^{a2}	0.18±0.02^{ab2}				
0.3S	0.22±0.02^{b1}	0.29±0.01^{c2}	0.29±0.01^{b2}	0.28±0.00^{c2}				
0.6S	0.34±0.00^{c1}	0.52±0.02^{e2}	0.60±0.02^{e3}	0.52±0.02^{d2}				
300/0S	0.13±0.00^{a1}	0.21±0.00^{b3}	0.20±0.01^{a3}	0.20±0.00^{b3}	0.15±0.01^{a2}			
300/0.3S	0.18±0.00^{ab1}	0.32±0.01^{c3}	0.27±0.01^{b2}	0.32±0.01^{c3}	0.32±0.01^{c3}			
300/0.6S	0.56±0.03^{d12}	0.51±0.02^{e1}	0.52±0.00^{d1}	0.51±0.02^{d1}	0.59±0.03^{e2}			
600/0S	0.10±0.00^{a1}	0.17±0.01$^{a4.5}$	0.16±0.01$^{a3.4.5}$	0.13±0.01$^{a1.2.3.4}$	0.12±0.01$^{a1.2}$	0.17±0.00^{a5}	0.13±0.02$^{a1.2.3}$	0.12±0.02$^{a1.2}$
600/0.3S	0.35±0.01$^{b2.3}$	0.36±0.00^{d3}	0.36±0.02^{c3}	0.30±0.03^{c2}	0.26±0.01^{b1}	0.28±0.00$^{b1.2}$	0.30±0.01^{b2}	0.27±0.02$^{b1.2}$
600/0.6S	0.45±0.08^{C1}	0.58±0.03^{f2}	0.48±0.05^{d1}	0.54±0.01^{d12}	0.50±0.00$^{d1.2}$	0.43±0.00^{c1}	0.49±0.01$^{c1.2}$	0.52±0.01$^{c1.2}$

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 ($P < 0.05$).