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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, 9.9 °C) and 600 MPa (10 min, 10.2 °C)] 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 chilled storage without this affecting sensory attributes.

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, & 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, & 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, & Heinz, 2012; Garriga, Grebol, Aymerich, Monfort, & Hugas, 2004; López-Caballero, Carballo, & 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, & 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, da Silva, Skibsted, & Orlien, 2012; Bolumar et al., 2016; Mariutti, Orlien, Bragagnolo, & Skibsted, 2008).

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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 the 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, & Bourke, 2008; Hayouni et al., 2008; Tajkarimi et al., 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). Then again, 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, & Zervas, 2008; Mariutti, Nogueira, & Bragagnolo, 2011; McCarthy, Kerry, Kerry, Lynch, & 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 & Pandey, 2016). Therefore, combined protection against both deteriorative actions, could help to extend the shelf life of additive-free meat products; this entails expanding logistic opportunities by allowing long-distance distribution in the global market, something that has been described as essential to ensure food safety (Bolumar et al., 2016). Therefore, 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, 9.9 °C) and 600 MPa (10 min, 10.2 °C)] and the antioxidant protection conferred by the incorporation of sage as a natural ingredient (0.3 and 0.6% in powder form), on prolonged chilled stability of beef burger prepared in a traditional manner.

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 in the open air in a dry, ventilated and shaded placed where the temperature was 26–30 °C), 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 kept (at room temperature) in hermetic jars of opaque glass, protected from light, and then 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 (away from light), followed by centrifugation (Beckman J2-MC USA) at 12000 × *g*, 5 °C. The antimicrobial activity of the sage extracts (stored at 2 °C, and within 24 h of arrival) 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 511 T, *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 °C) until use. For the preparation of burgers, meat packages were thawed (approx. 18 h 3 ± 2 °C, reaching between −3 and −5 °C) and minced again through a grinder with a 6 cm diam. Plate. Three different batches (5.0 kg) were prepared with 93.5% of beef (8.31% fat and 20.54% protein, pH 5.93) and containing 0% (control sample), 0.3% and 0.6% of added powdered sage (proportions selected based on prior sensory testing), 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. During preparation, the temperature of the burgers ranged between 3 and 5 °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. Treatment at 300 MPa: time to reach the pressure of 300 MPa was 45.5 s, initial temperature of the sample (pressure vessel) was 9.9 °C, and the temperature increased to 19 °C due to adiabatic heating during pressurization, at a pressurization rate of ~6.5 MPa/s. After 10 min the pressure was released. The vessel (sample) temperature after depressurization was 6.1 °C and the depressurization rate ~16.5 MPa/s. Treatment at 600 MPa: time to reach the pressure of 600 MPa was 90 s, initial temperature of the sample and the pressure vessel was 10.2 °C, and the temperature increased to 25.2 °C through adiabatic heating during pressurization, at a pressurization rate of ~6.6 MPa/s. After 10 min the pressure was released and the vessel temperature after depressurization was 2 °C and depressurization rate ~13 MPa/s.

Nine different treatments (at least 18 burgers for each one) 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 at least two burgers per day) at 1, 3, 6, 10, 24, 34, 44 and 60 days of chilled 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 burger composition.

2.5. Sensory evaluation

A semi-trained 48-member sensory panel, recruited among staff of the ICTAN-CSIC with previous experience, 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 & 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 using a 10-point hedonic scale from “dislike extremely” to “like extremely”. The assessors were provided with mineral water and bread to rinse their mouths between samples.

2.6. pH determination

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

2.7. Microbiological analysis

Samples were prepared in a vertical laminar-flow cabinet (model AV 30/70, Telstar, Madrid, Spain). Ten grams of each sample (from 2 pieces per sample) were taken and placed in a sterile plastic bag with 90 mL of peptone water (0.1%) (Panreac Química, S.A. Madrid, Spain). After 2 min. in a stomacher blender (Stomacher Colworth 400, Seward, UK), appropriate decimal dilutions were pour-plated (1 mL) on the following media: Plate Count Agar (PCA) for the total mesophile count (TMC) (30 °C for 72 h) and for Psychrotrophic bacteria (4 °C for 7–10 days); and Violet Red Bile GlucoseAgar (VRBG) for *Enterobacteriaceae* (37 °C for 24 h). All microbial counts were converted to logarithms of colony-forming units per gram (Log cfu/g).

2.8. Lipid stability evaluation

2.8.1. TBARs assay

Lipid oxidation was evaluated by changes in TBARs (thiobarbituric acid-reactive substances) in fresh burgers, pressurized and non-pressurized, during storage as described by Serrano, Cofrades, and Jiménez-Colmenero (2006) with slight modifications. Briefly, 5 g of each sample was homogenized in 35 mL of 7.5% trichloroacetic acid (Panreac) for 1 min at high speed in an Omnimixer blender (ES Homogenizer, OMNI International Inc., Gainsville, VA, USA). The blended sample was centrifuged (3000 g, 2 min) and 5 mL of the supernatant was mixed with 5 mL of 20 mM thiobarbituric acid; 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 (12 h) 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 6890 N gas chromatograph coupled to a (EI) 5973 N 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) (30 m × 0.25 mm × 0.25 μm) at constant pressure (12 psi) provided by a HS-40 Autosampler. The temperature was programmed isothermally at 50 °C for 7 min, then raised to 150 °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 & 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 homogenization, the sample was transferred to Erlenmeyer flasks and shaken for 30 min on a stir plate. Then, sample was filtered through Whatman No. 1 paper. 20 μl of filtrate was added to reagent kit 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, & 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, & 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*. Moreover, the lactic acid bacteria tested (*B. bifidum* and *L. acidophilus*) were not affected by the extracts, regardless of the solvent used. This is important because of the relationship (positive) of these bacteria with health. However, the aqueous extract was found to inhibit the growth of potentially pathogenic bacteria such as *A. hydrophila* and *Y. enterocolitica* (with inhibition halos of 7.1 ± 1.2 mm and 10.7 ± 1.1 mm respectively) and/or spoilers such as *S. putrefaciens* (12.9 ± 1.3 mm). The aqueous extracts were the ones that proved effective against sage-sensitive microorganisms; the ethanolic extract was effective only against *S. putrefaciens* (inhibition halo of 12.7 ± 1.1, similar to the aqueous extract). This may be due to the polarity of the compounds that exhibit antimicrobial activity, although alcoholic solvents could enhance the extraction of some polyphenol compounds not extractable by water. Makanjuola (2017) reported that the total phenol content in tea was higher in water than in ethanolic extracts, suggesting greater activity of the former; the particle size of the tea powder is also a very important factor.

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 with the addition of sage, and then only slightly.

3.3. Sensory evaluation

Overall, the sensory evaluation of beef burgers was unaffected by formulation (Table 2). Panellists were unable to distinguish ($P > 0.05$), in terms of flavour and odour acceptability and general acceptability, between different 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 (Mielnik, Sem, Egelandsdal, & Skrede, 2008). Similarly, Hayouni et al. (2008) reported that minced beef containing 1.5% of essential oil of *S. officinalis* was acceptable, but at higher concentrations it was unacceptable to the panellists, probably because sage essential oil has a strong, warm, spicy, herbaceous, and camphoraceous scent. This negative smell–taste effect is inherent in the use of essential oils (or their components) but is not evident when powdered leaves are used, even at 0.6% (Table 2). Since sensory attributes of the product are relevant in stability studies, further studies are needed for better understanding of effect the of the target variables during burger storage, taking into account the stability determined in the present experiment.

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	Flavour acceptability	Odour 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 ($P < 0.05$).

3.4. pH

The addition of sage to burgers did not affect ($P > 0.05$) pH levels either initially or during storage (Table 3). During storage of pork patties at 4 °C (9 days), the pH of patties containing sage was found to be quite variable (McCarthy et al., 2001). However, the same authors reported that the pH of those with ginseng and rosemary increased and those with fenugreek and mustard decreased. In cooked turkey meatballs, the addition of sage resulted in a decrease in pH (Karpinska-Tymoszczyk, 2007). Moreover, in the present case a slight increase in pH was observed after high-pressure treatment in all batches (Table 3). This behaviour was observed in dry fermented meat products after HPP (300 MPa) or raw sausages pressurized above 200 MPa, as a consequence of protein denaturation and the formation of new linkages (Mandava, Fernández, & Juillerat, 1994; Marcos, Aymerich, & Garriga, 2005). Moreover, Suzuki, Watanabe, Iwamura, Ikeuchi, and Saito (1990) attributed this effect particularly to conformational changes in histidine. Macfarlane, McKenzie, Turner, and Jones (1981) observed an increase in the pH of beef muscle caused by pressure treatment, attributed to a loss of free protons through redistribution of ions as a consequence of increased ionization at elevated pressures. Microbial metabolism did not appear to influence the pH of hamburgers during storage (Tables 3–4). Thus, the increase in the counts, especially in the unpressurized lots, could result in a pH increase due to the accumulation of basic compounds. Nevertheless, with small fluctuations, no significant differences were observed ($P > 0.05$) either through the effect of pressure or of the sage, as storage progressed (Table 3).

3.5. Microbial stability: considerations regarding the combined antimicrobial 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

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 ($P < 0.05$).

(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 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. (2011) reported that chicken breast fillets under 600 MPa/15 °C/5 min reduced counts of some previously-inoculated pathogenic organisms (*Salmonella thyphimurium* KCTC 1925 and *E. coli* KCTC1682 by 6–8 log cfu/g for 7–14 days and *L. monocytogenes* KCTC 3569 above 14 days). These authors found that at pressures of 300 MPa the reduction in counts was generally sustained at 1–2 log cycles. In our study, the psychrotrophic counts in burgers treated at 600 MPa

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

	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
Enterobacteria	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	–	–	–	–	–			
	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 ($P < 0.05$).

were < 6 log cfu/g at 60 days, showing the stability of the product over prolonged chilled storage (Table 4). Enterobacteria were inhibited by pressure (300 MPa or 600 MPa), remaining below the limit of detection during the experimental period. This is very important for purposes of improving hygiene during preparation of burgers and extending their shelf life. In this connection, a combined treatment of 0.3% sage and modified atmospheres (20% CO_2/80% N_2) in turkey meatballs has been found to prevent the appearance of coliforms (an effect not observed in batches under modified atmospheres only) (Karpinska-Tymoszczyk, 2010).

3.6. Lipid stability

TBARs values were affected ($P < 0.05$) by formulation, HPP and storage (Table 5). Initially, samples containing sage had lower ($P < 0.05$) TBARs values than 0S burgers irrespective of sage concentration. Lipid oxidation increased during storage, but those differences generally persisted after HPP and throughout storage (10 days for non-pressurized samples). Comparison of TBARs values in samples with/without added sage showed that these were generally little affected by pressurization during storage (Table 5). Lipid oxidation increased ($P < 0.05$) during storage in the pressurized control samples (300/0S and 600/0S), while the increase of TBARs values was proportionately smaller in burgers containing sage. The fact that the TBARS values of burgers with added sage were significantly lower over storage indicates a lower lipid oxidation rate. The decrease found after 34 days in long-term storage samples (burgers pressurized at 600 MPa) could be the result of further reactions between secondary lipid oxidation products (TBARs) and other meat macromolecules or compounds, such as proteins, as reported by Utrera, Morcuende, and Estevez (2014).

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

Antioxidant activity of 87.87 ± 5.08 mg eq trolox/mg sample was registered for the sage extract, much greater than the activity recorded in the burgers, which was affected ($P < 0.05$) by formulation, HPP and storage (Table 7). Martins et al. (2014) reported antioxidant activity in various sage extracts (aqueous, methanol/water) obtained by decoction or infusion. Also, Grzegorczyk, Matkowski, and Wysokinska (2007) reported antioxidant potential in methanol and acetone extracts prepared from organs (shoots and hairy roots) and undifferentiated elements (cell and callus) in in-vitro cultures of *S. officinalis*. In the present case antioxidative activity was greater ($P < 0.05$) in burger samples containing sage than in the control (0S); this behaviour correlated directly with sage concentration, regardless of pressurization and storage. Significant differences were noted in some cases, but pressurization level and storage generally had a relatively small effect on the antioxidative activity of the burgers, with no clear trend (Table 7).

TBARS, hexanal and PCL are all methods that provide information about the oxidative status of the system and the progress of lipid oxidation in meat products such as burgers, and so it is possible to establish a level of correlation among them. When all the experimental data (irrespective of formulation and storage time) were collated, significant correlations were found for TBARs/PCL (−0.502, $P < 0.01$) and TBARs/hexanal (0.661, $P < 0.01$), but for PCL/hexanal the correlation was not significant (−0.209, $P > 0.01$). This means that there is an inverse relationship between the progress of lipid oxidation and the radical quenching capacity of the system. Also, there is a direct relationship between the parameters used to evaluate the formation of secondary compounds from lipid oxidation in beef burgers with different formulations, and processing. Rey, Hopia, Kivikari, and Kahkonen (2005) also found a direct relationship between TBARs and hexanal content in cooked burgers after 3 days of refrigerated storage at 4 °C and with different plant extracts as natural antioxidants. Cofrades et al. (2011) found a significant correlation for TBARs/PCL in frankfurters enriched with n-3 fatty acids and containing antioxidants such as butylhydroxytoluene (BHT) and hydroxytyrosol (Hyt). However, other authors have reported no significant correlation between lipid oxidation and antioxidant capacity in fresh meat (Descalzo et al., 2008) and fish muscle (Medina, Gallardo, Gonzalez, Lois, & Hedges, 2007).

These results invite two main considerations: a) the antioxidant activity of sage, and b) the absence of prooxidant activity of HPP under the studied conditions. The antioxidative effect of sage demonstrated in this experiment is consistent with the results reported by various authors, although they used sage in different forms and on different matrices. In this regard, sage has been used in different forms, including essential oils (Fasseas et al., 2008; Mohamed et al., 2011; Unal, Babaoglu, & Karakaya, 2014), extracts (McCarthy et al., 2001) and dried powders (Mariutti et al., 2008; Mariutti et al., 2011), to study the oxidative stability of minced meat from different species (beef, pork, chicken) and as affected by cooking and/or chilled and/or frozen storage. For example, the addition of 3% sage essential oil inhibited lipid

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 ($P < 0.05$).

oxidation in raw pork and in cooked bovine meat (Fasseas et al., 2008). Addition of 0.1% dried sage to minced chicken meat effectively minimized and delayed the oxidation of lipids and cholesterol during thermal processing and storage at −18 °C (Mariutti et al., 2011). There are no reports in the literature associating the demonstrated natural antioxidant activity of sage with conditions of use in minced meat, but it seems that the presence of phenolic compounds (rosmarinic acid and carnosic acid, among others) contributes to its antioxidant 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, & 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; Ma & Ledward, 2013; Mariutti et al., 2008), which is consistent with the results observed in the samples treated at 300 MPa (Tables 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, & 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 chilled 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 chilled 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 chilled storage. Beef burgers did not undergo lipid oxidation during prolonged chilled 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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