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Application of high hydrostatic pressure for the reduction of STEC on raw ground beef patties and its impact on physicochemical properties: pH and color

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ABSTRACT

This study on raw ground beef patties evaluated: resistance of STEC strains inoculated at 6 log CFU/g (O157:H7, O26, O45, O103, O111, O121, and O145) subjected to 350, 450 and 600 MPa, for 5 min at 10 °C; effectiveness of pressure for initial loads (2, 3 and 4 log CFU/g) of O157:H7; and general impact of high hydrostatic pressure (HHP) on raw patties. For 600 MPa treatments and 6 log CFU/g load of STEC, ~5 log CFU/g reductions were obtained with all strains. For 450 MPa, differences in baroresistance among strains became evident. When inoculating O157:H7 at different loads, counts after 600 MPa fell below quantification limits, though virulence genes were detected for the two highest loads. Additionally, HHP reduced native aerobic microbiota to <1 log CFU/g and slightly affected a* and b* color values. Montecarlo simulation was used to estimate potential initial counts of STEC that allow compliance with existing regulatory limits after applying HHP, showing that absence of STEC studied can be achieved in 65 g of patties provided initial loads are ~2 log CFU/g. Finally, HHP treatments at 600 MPa and mild temperatures can be considered a valid non-thermal processing technology to achieve 5 log CFU/g reductions.

1. Introduction

Escherichia coli is part of the normal flora of the intestinal tract of several animals including humans and most strains do not cause gastrointestinal disorders, although same have been identified as being responsible for life-threatening cases of severe diarrhea. Strains of *E. coli* responsible for food outbreaks have been classified into seven different pathotypes, one of them being enterohemorrhagic *E. coli* (EHEC) — also defined as Shiga-toxin producing *E. coli* (STEC) (Estrada-Garcia, Hodges, Hecht, & Tarr, 2013; INEI-ANLIS, 2011; Koutsoumanis et al., 2020). The severity of the consequences depends on the virulence factors identified for STEC strains which are given by the Shiga toxin subtype gene (*stx*) and/or the presence of the *eae* gene that encodes for intimin, among others.

Within STEC, *E. coli* O157:H7 is the most significant one in relation to public health and is highly associated with the development of severe symptoms (hemorrhagic colitis and hemolytic uremic syndrome). Recently, several non-O157 STEC strains proved to be responsible for foodborne outbreaks, outnumbering the infections caused by O157 STEC (Adams et al., 2017; CDC, 2018; WHO, 2018). Within STEC serogroups associated with human diseases, 70–83% were originated by any of these six serogroups: O26, O45, O103, O111, O121 and O145.

Ground beef patties (minced beef or pork meat with <20% fat matter, with the addition or not of salt, spices, and authorized additives) are a widely consumed meat product often linked to STEC outbreaks and thus a food matrix of concern for the food industry (Rangel, Sparling, Crowe, Griffin, & Swerdlow, 2005; Reel, 2016). The product is sold under freezing temperature and consumed cooked domestically, as well as in retailers (fast food restaurants), where one of the main consumers are children.

High hydrostatic pressure (HHP) technology is a non-thermal process that has the potential to maintain the sensory quality (colors, flavors)

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and nutrients of foods, to a greater extent than traditional thermal processing, making them similar to fresh food products, microbiologically safe and with extended shelf life (Huang, Hsu, & Wang, 2020; Hygreeva & Pandey, 2016). Since HHP consists in subjecting the packaged sample to pressures of 200–1400 MPa (Cheftel, 1995; Evelyn & Silva, 2019), post-processing recontamination hardly occurs. However, changes are expected in the structure of large protein molecules (denaturation or modification), enzymes (activation or deactivation), polysaccharides, fats and nucleic acids (Butz & Tauscher, 2002; Cheftel, 1995; Heremans, 1995; Omer et al., 2010). HHP inactivation depends on the amount of pressure applied, as well as the temperature and length of treatment. However, it is also influenced by other factors such as bacterial strains, the growth phase of the bacteria and their morphology, the presence of antimicrobial substances and the food matrix involved (Huang, Lung, Yang, & Wang, 2014; Rosario, Rodrigues, Bernardes, & Conte-Junior, 2020). Prior HHP studies on beef patties obtained relevant reductions of STEC strains (up to 5 log cycles) applying single or multiple cycles of moderate pressures (450–600 MPa) with moderate physicochemical changes (Black, Hirneisen, Hoover, & Kniel, 2010; Jiang, Scheinberg, Senevirathne, & Cutter, 2015). Operating in a single cycle is more efficient in terms of energy, equipment wear and downtimes, being more feasible to apply on an industrial scale (Yamamoto, 2017). HHP could be applied by food operators to reach the Performance Objectives (PO) and to prevent, minimize or reduce counts of STEC strains in order to achieve the Food Safety Objective (FSO) for Appropriate Level of Protection (ALOP) for consumers (ICMSF, 2018).

The aim of the present work was to study the use of HHP (350, 450 and 600 MPa) to reduce plate counts of STEC strains (O157, O26, O45, O103, O111, O121 and O145) in raw ground beef patties. The second objective was to evaluate the effectiveness of the two most lethal pressure levels previously assayed, on *E. coli* O157:H7 inoculated into ground beef patties at three concentrations (2, 3 and 4 log CFU/g) in order to determine inactivation rates for different initial loads. Considering the prevalence and the association between the development of severe symptoms and its high resistance, *E. coli* O157 was selected for this trial. Finally, physicochemical changes induced by the application of HHP and the impact on the native mesophilic aerobic microbiota of raw patties were evaluated.

2. Materials and methods

2.1. Experimental design

Raw beef patties were irradiated (Irradiation unit of Laboratorio Tecnológico del Uruguay, LATU, Montevideo, Uruguay) at 10.1 ± 0.5 KGy, in order to eliminate vegetative forms of native microbiota and to achieve commercial sterilization (FDA, 2012). Samples were kept at −18 ± 2 °C until inoculation and treatment. Total aerobic count on Plate Count Agar - PCA (Oxoid, Hampshire, UK) was conducted to verify the PCA counts were below 10 CFU/g (Downes & Ito, 2001). Patties were then independently inoculated with seven different STEC strains and processed by means of high hydrostatic pressure. The reduction of different STEC strains with HHP and HHP processing of patty samples inoculated with three different loads of *Escherichia coli* O157:H7 was assessed. An independent series of patty samples were not irradiated nor inoculated in order to determine native mesophilic counts, pH and color changes only due to high pressure technology. Microbiological analyses were conducted at the Microbiology Department of Laboratorio Tecnológico del Uruguay, which performs its tests under the ISO/IEC 17025 guidelines, accredited by UKAS (National Accreditation Body for the United Kingdom).

2.2. Ground beef patty samples

100% ground beef patties, with 20% fat content, were obtained frozen from a local slaughterhouse. Three different batches were selected and samples for microbiological, physicochemical, and instrumental analysis were placed in sterile Whirl-Pak® Homogenizer Blender Filter Bags. The bags were stored at −18 ± 2 °C until they were analyzed or inoculated.

2.3. Microorganisms

Reference strains of *Escherichia coli* O26, O45, O103, O111, O121 and O145 (Staten Serum Institute, Copenhagen, DK) and *Escherichia coli* O157:H7 (ATCC 43895) were used to artificially contaminate the samples. The cultures were kept frozen at −80 ± 2 °C and they were activated by transferring an aliquot of the stock into nutrient broth, NB (Oxoid, Hampshire, United Kingdom) and incubating overnight at 37 °C on an orbital shaker at 100 RPM. For the preparation of the inoculum suspension (IS), successive dilutions were made in phosphate water to obtain the expected concentration for each stage of the study. The actual load of the IS was confirmed by making counts of the suspension with the automatic enumeration methodology TEMPO TVC (BioMérieux, Marcy-l'Étoile, France).

2.4. High pressure processing (HHP) treatment

HHP treatments were performed using a high-pressure unit Model S-IL-100-250-09W (HP Food Processor, Stansted Fluid Power, Ltd., Harlow, UK) located in LATU pilot food plant. The pressure chamber of 2 L volume has a 100 mm bore internal diameter, is 250 mm long and has the canister to hold the samples inside. The vessel body and the pressure transmitting fluid (water) were kept at treatment temperature by circulating water through an integral heat transfer jacket fitted to the outside of the high-pressure barrel assembly. The temperature of the pressure transmitting fluid was monitored with a thermocouple positioned in the bottom of the chamber. Before the treatment, frozen samples were individually vacuum packed in Cryovac® pouches as secondary packaging. Samples in the pressurization chamber and pressurizing fluid were both set at 10 °C. HHP processes were recorded by the data acquisition system of the device. Once the HHP treatment was completed, treated ground beef patties were removed from the chamber and immediately stored at −18 ± 2 °C, to replicate what is done on an industrial scale. All samples, including controls, were subjected to the same temperature conditions.

Pressure and temperature throughout all HHP processes were recorded and the data obtained is illustrated in Fig. 1.

2.5. HHP processing inactivation of different STEC strains at ~6 log CFU/g

Independent samples were inoculated with 100 μL of the IS and thoroughly mixed, achieving ~6 log CFU/g of each of the seven STEC strains. The inoculated samples were then sealed and kept chilled for 1 h before being frozen again. Samples were processed at 0.1 (Controls), 350, 450 and 600 MPa for 5 min, representing the range of pressures most frequently used by the industry (Yordanov & Angelova, 2010). After treatment, all samples were kept at −18 ± 2 °C until microbiological analysis. Irradiated inoculated samples kept at atmospheric pressure were named Control samples. Irradiated, non-inoculated samples kept at atmospheric pressure were named Blank samples. STEC counts were assessed by means of total aerobic count on Plate Count Agar - PCA (Oxoid, Hampshire, UK). Samples were processed by triplicate. Reductions for every HHP treatment were obtained, comparing counts of HHP treated samples (N) with control samples (N_0), and calculated as $\log(N_0/N)$.

2.6. HHP processing of patty samples inoculated with three different loads of E. coli O157:H7

Considering that O157 is the most common STEC related to human

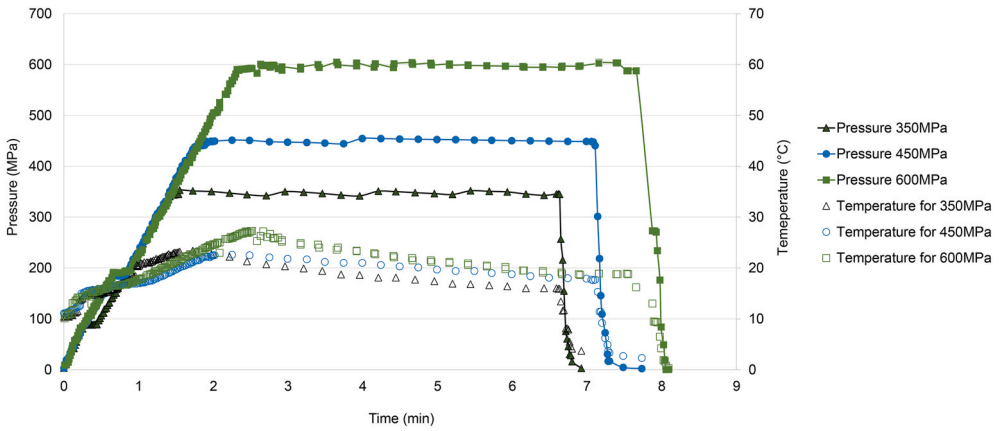

Fig. 1. Pressure profile and temperature profile of pressure transfer fluid (water). Black markers for treatment at 350 MPa, dark grey at 450 MPa and light grey at 600 MPa.

diseases (Koutsoumanis et al., 2020), at this stage, the effectiveness of HHP on this strain was studied at different initial loads. Samples were inoculated with three different loads of *E. coli* O157:H7: ~2, 3 and 4 log CFU/g, and intensively mixed. The samples were then sealed and kept chilled for 1 h before being frozen again. Inoculated samples were subjected to 0.1 (Control), 450 and 600 MPa. After treatment, all samples were kept at −18 ± 2 °C until microbiological analysis. *E. coli* O157:H7 counts were assessed by means of total aerobic count on Plate Count Agar - PCA (Oxoid, Hampshire, UK). Samples were processed by triplicate.

2.7. Microbiological counts of all STEC strains and detection of E. coli O157:H7

Counts of each STEC strain on irradiated and inoculated samples were followed by means of total aerobic counts performed on Plate Count Agar-PCA (Oxoid, Hampshire, UK) and incubated at 35 ± 1 °C for 2 days. This was possible because samples were previously irradiated before being inoculated with STEC. The detection limit was 1 log CFU/g for stage 2.5 and a lower dilution was prepared (1:4 instead of 1:10) to increase sensitivity to 0.6 log CFU/g for stage 2.6.

For the objective described in section 2.6, in addition to the microbiological counts of the three different loads of *E. coli* O157:H7 before and after the application of HHP, "BAX® System Real-Time PCR Assay - STEC Screening (*stx* and *eae*)" (Dupont, Delaware, USA) was used for the detection of *E. coli* O157:H7, after an enrichment in Modified Tryptone Soya Broth plus casamino acids – mTSB (Acumedia, Neogen, USA) at 1:4 dilution and incubated at 42 ± 1 °C for 15–22 h. The screening was made from wet pools (WP) of enrichment's lysates, with a <1 CFU/g detection limit (Mussio, Martínez, Soumastre, & Maquieira, 2014). Positive samples for both genes, *stx* and *eae*, were individually inoculated in CHROMagar ™ O157 (Chromagar, FR) to confirm the presence of viable cells in each sample. The plates were incubated at 37 ± 1 °C for 18–20 h.

2.8. Effects of high pressure technology in commercial beef patties

Four units of three different batches of frozen raw beef patties (non-irradiated) were evaluated to study the effect of HHP on native microbiota, color, and pH. One unit of each batch was subjected to 350, 450 and 600 MPa for 5 min (9 samples) and the remaining unit of each batch was kept untreated. After HHP treatment, samples were stored at −18 ± 2 °C for microbiological analysis and at 4 ± 2 °C for physicochemical analysis.

2.8.1. Microbiological reductions

Total aerobic counts on Plate Count Agar - PCA (Oxoid, Hampshire, UK), was carried out before and after the application of HHP to determine the effect of such treatment on native aerobic mesophilic microbiota (procedure stated in 2.7).

2.8.2. Physicochemical changes

Color analysis was performed on a Hunterlab LabScan® XE colorimeter (Hunter Associates Laboratory Inc., Reston, Virginia, USA) with illuminant A/10 and an open cell of 44 mm. Parameters L* (lightness), a* (redness) and b* (yellowness) were obtained.

pH measurements were made with a pH Meter (SevenMultiTM, METTLER TOLEDO, Greifensee, Switzerland), equipped with a temperature sensor and a combined penetration electrode previously calibrated with pH 4.00 and 7.00 buffer solutions.

2.9. Statistical analyses

Stages 2.5 and 2.6 were performed in triplicate with one production batch of patties, while 2.8 was carried out in duplicate with one batch for microbiota aerobic count, and three different batches for physicochemical properties (duplicate for pH and triplicate for color).

For total aerobic counts, results were converted to log CFU/g. Whenever the count was below the detection limit, such value was used for calculations and statistical analyses. In order to be more conservative, the worst-case scenario was considered, though this could underestimate the efficiency of the treatment.

Data of microbiological reductions were subjected to analysis of variance (ANOVA) using the statistical software Infostat version 2014e. A post-hoc Tukey test was used to obtain paired comparisons among sample means and differences were significant at $p < 0.05$. For instrumental color, results for different batches and processing conditions were analyzed through multivariate analysis of variance (MANOVA) using Pillai's trace as test statistic. When significant differences were observed, mixed models were used to evaluate each parameter, considering pressure as fixed effect and lot as random effect. A mixed model was also used to evaluate results of pH, with the same fixed and random effects. Analyses were performed using R software (v. 4.0.2) and the significance level was 0.05.

2.10. Estimation of potential initial counts of STEC strains that allow compliance with existing regulatory limits after the application of HHP to patties

In order to evaluate the application of this technology, a set of values of Hypothetical Initial Counts (HIC, expressed as log CFU/g) were determined, from which it would be highly possible to reach existing regulatory limits (for beef patties), after applying the three different

levels of HHP (350, 450 and 600 MPa).

HIC were estimated via Monte Carlo simulation, using @RISK 8.0 (Palisade Corporation, Ithaca, New York) as follows. Existing regulatory limits define absence in 65g (<1 CFU in 65g) of STEC in patties (<−1.81 log CFU/g) (Argentina, 2017; Uruguay, 2015) and absence in 325g (<1 CFU in 325g which corresponds to < −2.51 log CFU/g) for trimmings (USDA, 2019). Such limits were used to fix a target value.

The reductions obtained for each strain and pressure applied (mean and standard deviation in log, from data generated in 2.5) were used as parameters to define a normal distribution. Random values obtained from such distribution were added to the target value, to calculate HIC distribution, as described by the following equation:

$$HIC - reduction = target\ value \rightarrow HIC = reduction + target\ value$$

where *reduction* was defined as a normal distribution (μ, σ) for each strain-pressure combination (e.g., for O157 at 600 MPa, μ of reduction was 5 log CFU/g and σ was 0.4 log CFU/g), and the *target values* were −1.81 log CFU/g (absence in 65g) and −2.51 log CFU/g (absence in 325g). For absence in 65g, the formula would be:

$$HIC = N(5,\ 0.4) - 1.81$$

After 5000 iterations and transforming the log results back into CFU, distributions of HIC were obtained for each combination of strain-pressure, and target value. For each distribution, percentiles 1, 5 and 10, represent the value of the initial count to reach the regulatory limit with 99%, 95% and 90% probability, respectively.

3. Results and discussion

3.1. HHP processing inactivation of different STEC strains at ~6 log CFU/g

Blank samples that were irradiated at 10.1 ± 0.5 KGy were tested to confirm the reduction of vegetative forms of native microbiota. As shown in Fig. 1, temperature throughout all HHP proccesses turn out to be between 10 to 25°C, more frecuently in the range of 15 to 25°C. The results obtained for microbial counts of HHP treated and untreated samples are shown in Table 1. In order to show the individual behavior of each strain at different pressure levels, Fig. 2 depicts all the reductions obtained.

As observed in both Table 1 and Fig. 2, HHP reduced the load of all strains when increasing the applied pressure. For 350 MPa, reductions from 1.33 ± 0.67 to >4.17 log were observed, strain O103 being the most sensitive one, followed by strain O145. Regarding the use of 450 MPa (5 min, at temperature ranging from 10 to 25 °C), reductions for STEC strains used in the present study ranged from 2.69 ± 0.36 for strain O157:H7 up to > 5.07 log CFU/g for strain O103. Taking into account that the fat content of the beef patties was approximately 20%, these reductions are in line with those observed by Jiang et al. (2015) in beef patties. These authors used four 60 s cycles of 400 MPa at approximately 17 °C to inactivate strains O103, O111, O26, O145, O121, O45 and O157:H7, and the reductions found ranged from 2.35 to 3.88 and 2.26 to 4.31 log CFU/g in 20% fat and 10% fat patties, respectively. Strain O103 was also the one most sensitive to high pressure while strain O157:H7 proved to be the most baroresistant. Similar results were obtained by Black et al. (2010) for *E. coli* O157:H7 in ground beef after 10 min at 400 MP and 20 °C; in addition, reductions increased after storing their samples at −20 °C for 5 days. For 600 MPa treatments, nearly 5 log CFU/g of reduction were achieved for the STEC strains assessed. For validation of nonthermal technologies, the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) guidelines state the need of a 5 log CFU/g reduction for pathogenic *E. coli* in meats using HHP (NACMCF, 2006). Thus 600 MPa can be considered an efficient pressure level for the reduction of pathogens and production of safer raw patties, contributing to achieving FSO for this food matrix.

In order to compare the reduction behavior of O157 vs non-O157, the mean reduction for all non-O157 was calculated as the average of the triplicate counts of the six strains (n = 18). For 350 MPa, the high dispersion of the data prevented the comparison between both means; this is probably due to the fact that counts were carried out individually, as well as to different baroresistance among strains that appears more where inactivations are lower. The reduction obtained at 450 MPa was approximately 45% higher in non-O157 than in O157 (data not shown). However, at 600 MPa, the average reduction for O157 and non-O157 strains was ~5 log CFU/g. This behavior had been reported by Hsu et al. (2015) who observed that the sensitivity of *E. coli* O157 increased above 450 MPa.

3.2. HHP processing of patty samples inoculated with three different loads of E. coli O157:H7

As described in section 3.1, irradiated and non-inoculated samples were tested in order to confirm the elimination of vegetative forms of native microbiota. Table 2 depicts the results obtained for microbial counts on HHP treated and untreated samples inoculated with approximately 2, 3 and 4 log CFU/g of *E. coli* O157:H7. The selected pressure levels were 450 and 600 MPa; 350 MPa was not used due to the low reductions observed in all 7 strains. For 450 MPa, reductions obtained for a ~4 log CFU/g initial load were 1.64 ± 0.29 log CFU/g, lower than those of experiments carried out in section 3.1 with ~6 log CFU/g loads (2.69 ± 0.36 CFU/g). It can be argued that there was a small fraction of baroresistant cells that survived. This phenomenon has been frequently reported for HHP processing showing different pressure tolerance between cells of the same strain (Tassou, Panagou, Samaras, Galiatsatou, & Mallidis, 2008; Tay, Shellhammer, Yousef, & Chism, 2003). For 600 MPa treatments, it can be observed that *E. coli* O157:H7 initial loads ≤3.79 log CFU/g resulted in almost complete destruction. This evidences the positive effect of HHP for inactivating one of the most resistant STEC strains and enhancing food safety.

Evaluation or confirmation of the presence of the *stx/eae* virulence genes in all treated samples, including those in which counts were below the detection limit was conducted, since the detection method for Shiga toxin *Escherichia coli* includes the detection of these genes at the first stage (ISO/TS 13136, 2012; USDA, 2019). These results are shown in Table 3.

Samples that were positive for the virulence gene screening, confirmed the presence of viable cells when inoculated in chromogenic media. In the samples inoculated with the lowest concentration (2 log

Table 1
Mean values ± standard deviation for each *E. coli* strain count (log CFU/g) performed on control patties and treated with 350 MPa, 450 MPa and 600 MPa.

Pressure (MPa)	*E. coli* strain						
	O26	O45	O103	O111	O121	O145	O157
Control	6.24 ± 0.14^{d}	6.29 ± 0.18^{d}	6.17 ± 0.06^{c}	6.04 ± 0.19^{d}	5.88 ± 0.24^{d}	6.09 ± 0.10^{c}	6.21 ± 0.13^{d}
350	4.50 ± 0.32^{c}	4.65 ± 0.26^{c}	$<2.00^{b}$ *	4.44 ± 0.18^{c}	4.50 ± 0.43^{c}	3.51 ± 0.28^{b}	4.76 ± 0.25^{c}
450	2.32 ± 0.26^{b}	2.29 ± 0.30^{b}	$<1.10^{a}$ *	3.24 ± 0.15^{b}	2.69 ± 0.58^{b}	1.78 ± 0.28^{a}	3.52 ± 0.23^{b}
600	$<1.22^{a}$ *	$<1.00^{a}$ *	$<1.13^{a}$ *	$<1.22^{a}$ *	$<1.00^{a}$ *	$<1.37^{a}$ *	1.23 ± 0.24^{a}

Note. Means within a column which do not share a letter differ significantly ($p < 0.05$). *When at least one value was below the detection limit, the mean was calculated considering this value (detection limit: 1.00 log CFU/g) as absolute.

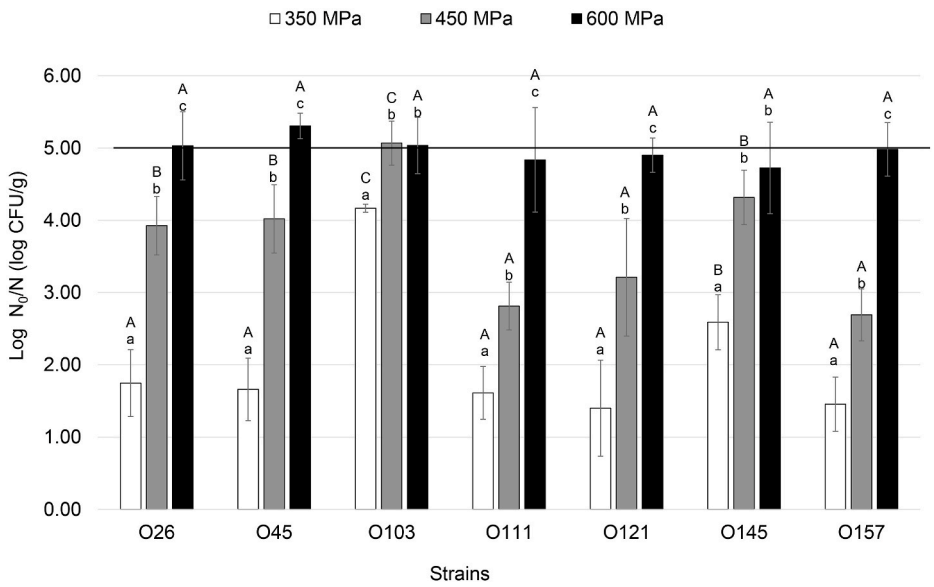

Fig. 2. HHP reduction for each STEC strain treated at different pressure levels. All values are expressed as mean ± standard deviation of three replications. N_0 stands for counts of control samples and N for treated samples. Different letters in reductions within the same strain (a, b, c) indicate significant differences ($p < 0.05$) among values. Different capital letters within the same pressure level (A, B, C) indicate significant differences ($p < 0.05$) between strains.

Table 2
Mean values ± standard deviation for *E. coli* O157:H7 microbiological counts (log CFU/g) performed on patties inoculated with approximately 2, 3 and 4 log CFU/g, HHP treated at 450 MPa and 600 MPa.

Pressure (MPa)	Initial count ~ 2 log CFU/g	Initial count ~ 3 log CFU/g	Initial count ~ 4 log CFU/g
Control	1.83 ± 0.19^{b}	3.04 ± 0.04^{b}	3.79 ± 0.12^{c}
450	$<0.60^{a}$ *	$<0.90^{a}$ *	2.15 ± 0.17^{b}
600	$<0.60^{a}$ *	$<0.60^{a}$ *	$<0.60^{a}$ *

Note. Means within a column which do not share a letter differ significantly ($p < 0.05$).
*When at least one value was below the detection limit, the mean was calculated considering this value (detection limit: 0.60 log CFU/g) as absolute.

Table 3
Results of the detection of the *stx/eae* virulence genes in composite samples (WP) of the 3 samples inoculated with 2, 3 and 4 log CFU/g of *E. coli* O 157:H7.

Pressure (MPa)	Initial count ~ 2 log CFU/g	Initial count ~ 3 log CFU/g	Initial count ~ 4 log CFU/g
	WP 1	WP 2	WP 3
450	+	+	+
600	–	+	+

CFU/g) and subjected to the highest pressure (600 MPa), *E. coli* O157:H7 was not recovered at the wet pool of the three enrichment broths of that inoculum. However, in the other two inoculums (~3 and ~4 log CFU/g) the presence of virulence genes (*stx* and *eae*) was detected. This is in accordance with Smelt (1998), who describe that increasing the pressure and lowering microbiological initial load, reduces the possibility for DNA to be detected.

3.3. Effects of high pressure technology in beef patties

3.3.1. Microbiological reductions

As observed in Table 4, the application of HHP gradually reduced the cell count of naturally present biota of all samples, with the lowest loads obtained in samples treated with the highest levels of pressure (450 and 600 MPa).

Table 4
Mean values ± standard deviation for cell count of native aerobic mesophilic microbiota (log CFU/g) on non-irradiated samples treated at different levels.

Pressure (MPa)	Mesophilic aerobic counts (log CFU/g)
Control	3.41 ± 0.36^{b}
350	2.69 ± 0.35^{b}
450	1.50 ± 0.23^{a}
600	$<1.00^{a}$ *

Note. Means within a column which do not share a letter differ significantly ($p < 0.05$).
*When at least one value was below the detection limit, the mean was calculated considering this value (detection limit: 1.00 log CFU/g) as absolute.

Other authors also found interesting results after submitting meat products to HHP. Treatments at 600 MPa for 6 min were effective in preventing the growth of yeasts and —off-flavors—generating *Enterobacteria*, delaying the growth of lactic acid bacteria and reducing the risk associated with *Salmonella* spp. and *Listeria monocytogenes* in marinated beef (Garriga, Grèbol, Aymerich, Monfort, &). Aymerich, Picouet, and Monfort (2008) concluded that it is possible to obtain a 5 log reduction of total mesophiles, *E. Coli* O157:H7 by applying pressures between 450 and 700 MPa at moderate temperatures. The results of the present work are well in line with these studies.

3.3.2. Physicochemical changes

Table 5 shows pH results obtained for samples of the three batches treated with different levels of HHP, after analysis using a mixed model (pressure as fixed effect and lot as random effect). No significant differences were observed for pH between all samples (treated and control), with a p-value of 0.06. This is in line with results obtained by Black et al. (2010), who found pH values of ground beef samples treated at pressure from 300 to 500 MPa did not differ significantly from the control samples.

Table 5 also presents mean color parameters obtained for all samples of the three batches treated with different levels of HHP. Results after conducting MANOVA (p-value < 0.05) indicated significant differences among color results (at least one statistically significant parameter), so the three parameters were studied independently (pressure being the fixed effect and lot the random effect).

Table 5
Results of pH and color parameters (L* = lightness; a* = redness; b* = yellowness) of samples treated with different levels of HHP.

Pressure (MPa)	pH	Color		
		L*	a*	b*
0	6.06 ± 0.04[a]	49.38 ± 1.64[a]	23.86 ± 2.24[c]	22.69 ± 1.10[b]
350	6.09 ± 0.01[a]	49.99 ± 1.83[ab]	16.27 ± 1.13[b]	17.34 ± 0.62[a]
450	6.09 ± 0.03[a]	50.88 ± 0.45[c]	14.34 ± 0.77[a]	17.15 ± 0.30[a]
600	6.08 ± 0.02[a]	50.29 ± 1.32[bc]	15.52 ± 0.24[ab]	17.52 ± 0.09[a]

Note. All results are expressed as mean ± sd. Means within a column which do not share a letter differ significantly ($p < 0.05$).

Results obtained for lightness values (L*) indicate significant differences (p-value = 0.0001) between control samples, and those subjected to the two highest pressures (450 MPa and 600 MPa). This is in accordance with Black et al. (2010), who observed a slight increase in L* values of ground beef samples after HHP processing.

Regarding a* value (green-red), the application of HHP yielded statistically lower values ($p < 0.05$). This was also observed by Szerman et al. (2011), who attributed this decrease in meat redness to an increase in oxidation (ferrous myoglobin decrease, increasing metmyoglobin).

Concerning the b* parameter (blue-yellow), no significant differences were observed between samples subjected to the different levels of pressure, and all of them had significantly lower values, than the control sample (shift to blue). A reduction on b* value was also observed in thawed carpaccio, as described by Szerman et al. (2011).

According to the statistical analysis of the mixed models (data not shown), L* and a* values were also affected by the batch itself, while no variability was detected for b* values between batches.

Finally, though significant changes were observed in the three parameters, Hayes, Raines, DePasquale, and Cutter (2014) reported no obvious color differences in cooked patties subjected to HHP treatment (four cycles of 1 min at 400 MPa). However, further analysis should be conducted to reach a conclusion under the experimental conditions of this study.

3.4. Estimation of potential initial counts of STEC strains that allow compliance with existing regulatory limits after the application of HHP to patties

Table 6 presents the results estimated to obtain less than 1 CFU in 65 g of the sample, calculated after 5000 iterations. These values correspond to the HIC that warranted, with high probability, the reduction of STEC counts to achieve the existing regulatory limit for each combination of strain-pressure.

For patties subjected to 600 MPa it is possible to reach the regulatory limit (<1 CFU in 65 g) with 99% probability when the initial counts are in the range of 1.92–3.17 log CFU/g. Strain O45 used in the present study showed the highest value of HIC at 600 MPa, implying that even when the initial counts are approximately 3 log CFU/g, the application of the 600 MPa allows compliance with current regulations, with 99% probability. Finally, for 350 and 450 MPa, the strain O103 tested in the present study proved to be the most pressure sensitive one.

If absence in 325 g is set as a target (instead of absence in 65 g) the HIC that warrants reaching such target when patties are subjected to 600 MPa, is 2.48 log CFU/g (with 99% probability, data not shown). Thus, HHP contributes to the mitigation of these pathogenic strains and to achieve FSO.

4. Conclusions

For 600 MPa treatments and 6 log CFU/g initial load of STEC, ~5 log CFU/g reductions were obtained for all STEC strains studied (O26, O45, O103, O111, O121, O145 and O157:H7). For 450 MPa treatments, differences in baroresistance among strains became more evident, ranging from 2.69 ± 0.36 log CFU/g for strain O157:H7 to >5.07 log CFU/g for strain O103. For initial loads of 4 log CFU/g of strain O157:H7 studied, HHP at 450 MPa yielded lower reductions of 1.64 ± 0.29 CFU/g. For 600 MPa, *E. coli* O157:H7 counts always fell below quantification detection limits regardless of the initial load. The pH of HHP treated patties was unaffected by pressure, and regarding color parameters, only a* and b* were reduced by the application of this technology. Since this study was conducted in raw patties, a sensory evaluation should be conducted to determine whether there are significant color differences in cooked patties.

HHP treatments at 600 MPa and mild temperatures can be considered a valid non-thermal processing technology to warrant 5 log CFU/g reductions and an absence of *E. coli* O157:H7 in 65 g of uncooked 20% fat beef patties, provided that initial loads are ~2 log CFU/g. Considering the results from the simulation study, the former statement can also be applied to the other six STEC assessed in the present study with a 99% confidence level.

HHP improved the microbiological quality and safety of the product, achieving the established limits that contribute to the product's FSO and potentially increasing its shelf life.

Table 6
Maximum Hypothetical Initial Counts (HIC) for each strain-pressure that guarantee with 99%, 95% and 90% probability reducing counts of STEC to 1 CFU/65g. Results are expressed in log CFU/g.

Strain	Pressure (MPa)	HIC (log CFU/g) for each probability to reach 1 CFU in 65g of ground beef patties		
		99%	95%	90%
O26	350	-0.66	-0.49	-0.40
	450	1.47	1.66	1.76
	600	2.94	3.05	3.11
O45	350	-0.80	-0.62	-0.51
	450	1.21	1.47	1.61
	600	3.17	3.26	3.31
O103	350	2.25	2.28	2.30
	450	2.84	2.96	3.02
	600	2.68	2.84	2.92
O111	350	-0.74	-0.59	-0.49
	450	0.53	0.67	0.74
	600	2.05	2.33	2.48
O121	350	-1.22	-1.00	-0.89
	450	0.33	0.65	0.81
	600	2.67	2.79	2.85
O145	350	0.13	0.32	0.42
	450	1.96	2.13	2.22
	600	1.92	2.22	2.37
O157	350	-0.85	-0.70	-0.62
	450	0.44	0.57	0.63
	600	2.63	2.79	2.87

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CRediT authorship contribution statement

Inés Martínez Bernié: Conceptualization, Validation, Investigation, Formal analysis, Project administration, Writing – review & editing. **Paula Mussio:** Conceptualization, Validation, Investigation, Writing – review & editing. **Santiago Jorcin:** Investigation, Software, Formal analysis, Writing – review & editing. **Mikaela Rajchman:** Formal analysis, Writing – review & editing. **Tomás López-Pedemonte:**

Conceptualization, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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