High pressure processing to control *Salmonella* in raw pet food without compromising the freshness appearance: the impact of acidulation and frozen storage

Cristina Serra-Castelló, Arícia Possas[1], Anna Jofré, Margarita Garriga, Sara Bover-Cid*

IRTA, Food Safety and Functionality Programme - Finca Camps i Armet, E-17121 Monells, Spain

***Corresponding author:**

Sara Bover-Cid

sara.bovercid@irta.cat

[1]Present address: Arícia Possas, Department of Food Science and Technology, Faculty of Veterinary, Agrifood Campus of International Excellence (ceiA3), University of Cordoba, 14014, Córdoba, Spain.

Abstract

The trend of feeding dogs and cats with raw pet food claiming health benefits poses health concerns due to the occurrence of pathogenic bacteria. High pressure processing (HPP) allows the non-thermal inactivation of microorganisms, preserving the nutritional characteristics with minimal impact on organoleptic traits of food. The present study aimed to evaluate and model the effect of HPP application (450-750 MPa for 0-7 min) on the inactivation of *Salmonella*, endogenous microbiota and colour of raw pet food formulated with different concentrations of lactic acid (0-7.2 g/kg) as natural antimicrobial. Additionally, the effect of a subsequent frozen storage of pressurised product was assessed.

Salmonella inactivation ranged between 1 and 9 log, depending on the combination of conditions. According to the polynomial model obtained, the effect of pressure was linear, while a quadratic term was also included for holding time (depicting the occurrence of a resistant tail at *ca*. 4 to 6 min). The effect of lactic acid was dependent on the pressure level, being most relevant for treatments below 600 MPa. Frozen storage after HPP prevented the pathogen recovery and caused a further *Salmonella* inactivation enhanced by lactic acid in most of the treatments. Endogenous microbial groups were significantly reduced by HPP to below the detection level in several conditions. In general, little effect of HPP on the instrumental colour parameters was observed, except for a slight increase in lightness, which was hardly appreciable from visual observation.

High pressure processing emerges as a relevant technology for the control of *Salmonella* spp. and to manage the microbiological safety of raw pet food. The mathematical model can be used as decision support tool to design safer raw pet food, while keeping the desired freshness appearance of the products.

Keywords (4-6 max): raw pet food, high hydrostatic pressure, mathematical modelling, predictive microbiology, salmonellosis**.**

1. Introduction

Raw meat-based diets (RMBD) for pets are mainly composed by uncooked animal products or by-products, vegetables, fruits and/or grains (Nüesch-Inderbinen et al., 2019). They can be home-prepared or commercially supplied on their fresh, frozen or freeze-dried form or as premixes intended to be complemented with raw meat (van Bree et al., 2018; Davis et al., 2019; Nüesch-Inderbinen et al., 2019). Feeding cats and dogs with RMBD has become a popular practice by pet owners, due to their more "natural" and fresh characteristics and the perceived healthier benefits, including improvement of skin and coat and increase in oral health of pets, compared with cooked (sterilised) or dry pet food options (Weese et al., 2005; Fredriksson-Ahomaa et al., 2017; Davis et al., 2019).

Despite the claimed benefits of feeding pets with RMBD, this practice may pose health risks to animals, as raw materials may be contaminated with enteric pathogens such us *Salmonella* (Fredriksson-Ahomaa et al., 2017; Giacometti et al., 2017). In surveys conducted to evaluate the presence of bacterial pathogens in Dutch and Canadian commercially available RMBD, *Salmonella* was present in 20 % of raw pet food samples (Weese et al., 2005; van Bree et al., 2018). Whole genome sequencing approach found clinical isolates of *Salmonella* obtained from sick cats and dogs to be closely related to *Salmonella* strains isolated from raw pet food (Jones et al., 2019). In this context, current regulations require that commercial suppliers must ensure *Salmonella* is not detected in raw pet food (European Commission, 2011; FDA, 2013).

The supplementation of pet food with lactic acid has demonstrated to promote oral health in cats, inhibiting dental plaque, calculus and tooth stain accumulation (Scherl et al., 2019). Besides the health benefits, it has been demonstrated that the acidulation with lactic acid can be effective to control pathogenic bacteria such as *Salmonella*, *Escherichia coli* and *Listeria monocytogenes* in raw pet food samples (Serra-Castelló et al., 2022).

High Pressure Processing (HPP) technology is an emerging strategy being implemented by pet food producers as a killing step to assure compliance with current microbiological regulations (Anonymous, 2019). The application of high levels of pressure during few minutes can inactivate microorganisms in foods, with a minimal impact on their organoleptic and nutritional characteristics (Bover-Cid et al.,

2017; Possas et al., 2017). In addition, frozen storage after HPP application has shown to enhance the inactivation of pathogens in some types of foods, including strawberry puree (Huang et al., 2013) and ground beef (Black et al., 2010).

The combination of preservation technologies such as HPP with other preservation factors like acidulation and frozen storage to produce safe, stable and high quality food products has been designated as the "hurdle concept" (Leistner and Gorris, 1995). To date, the impact of combining these hurdles on the microbiological quality of RMBD has not been evaluated.

This work aimed at evaluating the inactivation of *Salmonella* and endogenous microbiota in raw pet food intended for cats, treated by HPP associated with acidulation with lactic acid and its subsequent frozen storage. The effects of these hurdles on raw pet food instrumental colour were also evaluated.

2. Material & Methods

2.1. Experimental design

A Central Composite Design (CCD) was performed to evaluate the impact of pressure level (450-750 MPa), holding times (0-7 min) and lactic acid concentrations (0-7.2 g/kg), on the efficacy of HPP treatments to inactivate *Salmonella* spp. and endogenous microbiota in raw pet food samples. The experimental layout performed is depicted in Table 1. The ranges set for the pressurization parameters, *i.e.* pressure levels and holding times, were set based on previous studies that demonstrated the effectiveness of HPP treatments at pressure levels of 450-750 MPa and holding times of 0 (i.e. a pulse of pressure come-up followed by immediate release) up to 7 min to inactivate pathogenic bacteria in foods (Bover-Cid et al., 2015; 2017). Additional experiments were conducted at the central point of the CCD to enable the evaluation of the experimental error and the lack-of-fit of the model. The trials were randomly performed to minimize the systematic bias due to disturbing effects of environmental conditions (Robinson, 2000; Barba et al., 2014).

2.2. Raw pet food preparation/formulation

The raw ingredients for pet food intended for cat were provided by Affinity Petcare SA. and included: chicken, plant based-ingredients, salmon and spices. Pet food was prepared at the pilot plant according to a commercial recipe and procedure and stored frozen at -20 ºC until being used. The proximal composition of the raw pet food was: moisture (70 %), protein (12 %), fat (6 %), ash (2 %) and fibre (1%).

Lactic acid was added to samples at the concentrations set in the CCD (Table 1) by adding the appropriate amount of a 71 % lactic acid solution kindly provided by Corbion® (Amsterdam, The Netherlands). This procedure was conducted 24 hours before pressurization in order to allow the stabilization of the pH.

2.3. *Salmonella* strains, culture preparation and inoculation

Samples were inoculated with a three-strain *Salmonella* cocktail composed of equal amounts of *Salmonella* Derby CTC1022, isolated from pork meat, and *Salmonella* Typhimurium GN0085 and *Salmonella* Enteritidis GN0082, isolated from chicken meat. Strain selection was based on previous HPP-resistance studies (Serra-Castelló et al., 2022). For the preparation of the cocktail, individual cultures of the selected strains were prepared as reported in Serra-Castelló et al. (2022). Briefly, a loopful of the frozen stock culture (-80 ºC) was streaked on Plate Count Agar (PCA, Merck, Darmstadt, Germany) at 37 ºC overnight (18 h). An individual pure colony was spread in a new plate of PCA and grown at 37 ºC overnight to reach the stationary growth phase, which makes *Salmonella* more resistant than in the exponential growth phase. Bacterial biomass on the surface of the PCA plate was collected, resuspended with a cryoprotectant solution (0.3% of beef extract (Difco Laboratories, Detroit, MI, USA), 0.5% of Tryptone (Oxoid Ltd., Basingtok, Hampshire, UK) and 20% of glycerol) and frozen at -80 ºC until being used. The frozen culture is representative of the status of the strain in raw materials usually stored frozen to produce the raw pet food and, in addition, it is known to protect pathogens from HPP, making this procedure a conservative approach to cover worst-case scenarios (Hereu et al., 2014).

Samples were inoculated with the *Salmonella* cocktail (1% v/w) just before pressurization.

2.4. High pressure processing and storage conditions

Twenty-five-gram samples of the inoculated raw pet food were vacuum-packed in PA/PE plastic bags (oxygen permeability of 50 $cm^3/m^2/24$ h and a low water vapor permeability of 2.8 $g/m^2/24$ h; Sistemvac, Estudi Graf S.A., Girona, Spain) and pressurised at the target time-pressure combinations established by the CCD (Table 1). For pressures up to 600 MPa, the equipment used was a Wave 6000 from Hiperbaric S.A. (Burgos, Spain), while a pilot equipment from Thiot ingenierie – Hiperbaric (Bretenoux, France – Burgos, Spain) was used for pressure levels above 600 MPa. The average pressure come-up time was 191 MPa/min, while the pressure release was almost immediate (< 5s). The initial temperature of the pressurization fluid was set at 9 ºC. Compression heating was expected to be about 3 ºC/100 MPa (Patazca et al., 2007), therefore no thermal effect was expected. HPP samples were stored frozen (-18 ºC) for 14 days.

2.5. Sampling and microbiological determinations

Microbiological determinations of samples inoculated with *Salmonella* and non-inoculated samples were conducted in triplicate for each trial of the CCD before HPP, immediately after the HPP and after 14 days of frozen storage. Frozen-stored samples were thawed at 4 ºC for 24 hours before microbiological analysis in order to reproduce the recommendations of the raw pet food manufacturer regarding storage and thawing at household environments prior to consumption.

Raw pet food samples inoculated with *Salmonella* were ten-fold diluted in 0.1 % Bacto Peptone (Difco Laboratories, Detroit, MI, USA) with 0.85 % NaCl (Merck, Darmstadt, Germany) and homogenized for 60 seconds in a Smasher™ bag blender (bioMérieux, Marcy-l'Étoile, France). The homogenates of inoculated samples were serially diluted and plated onto *Salmonella* Plus chromogenic medium (SPCM, CHROMagar™ *Salmonella* Plus; Scharlab, S.L., Sentmenat, Spain). Colonies were enumerated after incubation at 37 °C for at least 48h (i.e. number of colonies were checked daily up to 5 days) to allow the recovery of cells sublethally injured due to the HPP treatments. For expected counts below the enumeration limit (< 2.5 cfu/g; no colony after spreading 4

ml of 1:10 dilution), the presence or absence of *Salmonella* spp. was determined after an enrichment of the homogenate in Rappaport-Vassiliadis (RV) broth (Oxoid Ltd., Basingstoke, Hampshire, UK) for 48 h at 42 °C. The enriched homogenate was streaked onto SPCM plates. The presence of *Salmonella* in the enriched homogenates was confirmed by PCR using the PrepSEQ™ Rapid Spin Sample Preparation Kit (Applied Biosystems) and MicroSEQ™ *Salmonella* spp. Detection Kit (Applied Biosystems).

Non-inoculated raw pet food samples were used to determine the levels of endogenous microbiota i.e. total aerobic mesophilic bacteria, *Enterobacteriaceae*, *Pseudomonas* spp. and lactic acid bacteria (LAB) before and after HPP. Raw pet food samples were ten-fold diluted in 0.1 % Bacto Peptone (Difco Laboratories, Detroit, MI, USA) with 0.85 % NaCl (Merck, Darmstadt, Germany) and homogenized for 60 seconds in a Smasher™ blender (bioMérieux, Marcy-l'Étoile, France). *Enterobacteriaceae* were enumerated on VRBD (Violet Red Bile Dextrose) agar (Merck Life Science S.L.U, Madrid, Spain) incubated for 24 hours at 37 ºC. *Pseudomonas* spp. were enumerated on Pseudomonas CFC selective agar (Oxoid S.A., Madrid, Spain) incubated at 25 ºC for 48 hours. Total aerobic mesophilic bacteria was plated on PCA (Plate Count Agar; Merck Life Science S.L.U, Madrid, Spain) and incubated at 30 ºC for 72 hours. LAB was plated on MRS (de Man, Rogosa and Sharpe) agar (Merck Life Science S.L.U, Madrid, Spain) and incubated at 30 ºC for 72 hours under anaerobiosis (AnaeroGen 2.5l, Thermo Scientific-Oxoid).

2.6. Physico-chemical and instrumental colour measurements

The a_w of the samples was measured with an Aqualab™ equipment (Series 3, Decagon Devices Inc., Pullman, WA, USA) and the pH was measured with a PH25 pHmeter (Crison Instruments S.A., Alella, Spain) before and after HPP treatments.

Instrumental colour was assessed as the most determinant and sensitive measurement of potential changes of the product appearance due to HPP. Instrumental colour measurement consisted of L^* (lightness), a^* (redness) and b^* (yellowness) before (L_0^*, a_0^* and b_0^*) and after (L^*, a^* and b^*) the HPP treatment using a colorimeter (Minolta Chroma Meter CR-400, Tokyo, Japan) with illuminant

D65 with 2 º viewing angle and calibrated using a standard white tile. Measurements were conducted in triplicate for each condition of the CCD. To provide the relevance of the difference seen between the colour before and after HPP, the total colour change (Δ E) was calculated according to Eq-1.

$$\Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \quad \text{(Eq. 1)}$$

2.7. Data analysis & modelling

Inactivation of *Salmonella* spp. and endogenous microbiota in pet food samples was expressed in terms of logarithmic reductions as the difference between counts after HPP treatments (N) and before treatments (N_0), *i.e.*, log (N/N_0). For modelling purposes, *Salmonella* positive results below the detection limit were recorded as -1.40 log cfu/g. For the colour the change of the colour parameters (ΔL^*, Δa^*, Δb^*) was quantified as the difference between measurements after (L^*, a^* and b^*) and before (L_0^*, a_0^* and b_0^*) HPP treatments, i.e., $L^* - L_0^*$, $a^* - a_0^*$, $b^* - b_0^*$.

The effect of pressure level, holding time, lactic acid concentration and their possible interactions on the inactivation of *Salmonella* spp., endogenous microbiota and colour in raw pet food was investigated by using the Response Surface Methodology (RSM). The "rsm" package for R software (R Core Team, 2019) was used for stepwise backward regression.

To obtain the polynomial equation that best fitted the experimental data without compromising parsimony, only the significant terms derived from each factor were kept in the final model as indicated by an ANOVA test ($p \leq 0.05$). The ANOVA was performed to estimate the coefficients of the final equation. The goodness-of-fit was evaluated by means of the root mean square error (RMSE) that measures the differences between the fitted and observed inactivation values. The statistical significance of the model was evaluated through the significance of the *p*–values derived from the *F*–test. Response surface graphs were drawn in which the value of the not shown independent variable was kept at the central point of the CCD.

3. Results and Discussion

3.1. *Salmonella* inactivation due to HPP and lactic acid

HPP inactivation of *Salmonella*, expressed as log reductions, for each combination of the CCD immediately after the HPP treatment is shown in Table 1.

The highest *Salmonella* inactivation (9.08 log reduction) was observed in trial 23 where the highest pressure (750 MPa) was applied. On the contrary, in trials 2 (shortest HPP treatment) and 6 (samples without lactic acid) the lowest *Salmonella* inactivation were recorded (1.11 and 1.10 log, respectively), indicating that the three parameters studied in the present work (pressure level, holding time and lactic acid concentration) were relevant to explain the inactivation of *Salmonella* in raw pet food due to the HPP. However, lactic acid alone was not capable to reduce *Salmonella* counts as the level of contamination before HPP was very similar in all trials in agreement with the target inoculation level. For instance, counts of *Salmonella* before HPP were 8.26 ± 0.07 Log_{10} cfu/g in samples without lactic acid (trial 7) and 8.23 ± 0.04 Log_{10} cfu/g in the samples with the highest tested lactic acid amount (7.2 g/kg in trial 17).

The pressure-resistance of *Salmonella* in raw pet food observed in the present study was higher than that reported in simpler matrixes such as laboratory media (Alpas et al., 2000) and fresh raw chicken (Tananuwong et al., 2012), which is in line with the recognised protective effect of complex food matrixes on the microbial inactivation during HPP (EFSA BIOHAZ et al., 2022).

According to the modelling results, the pressure effect on *Salmonella* inactivation was almost linear, indicating that the inactivation of the pathogen was directly proportional to the level of pressure applied (Table 2). In this line, Cap et al. (2020) also reported higher inactivation of *Salmonella* with increasing pressure levels (100-600 MPa) in frozen chicken breast. The holding time parameter contributed to the *Salmonella* inactivation model with a linear and a quadratic term. These results indicated that after a rapid linear-based decrease on *Salmonella* levels during the first minutes of treatment, approximately 4-6 min, there was a slowing down on inactivation due to a strong reduction of the pressure- inactivation rate. This phenomenon, is compatible with the occurrence of a tail of

225 resistant cells, as already observed in the inactivation kinetics of the same *Salmonella* strains during
226 pressurization at 600 MPa in chicken based raw pet food (Serra-Castelló et al., 2022). *Salmonella*
227 resistance tails were empirically observed in a wide range of pressure levels and lactic acid
228 concentrations.

229 The addition of lactic acid in raw pet food at concentrations ranging from 0 to 7.2 g/kg yielded
230 samples with pH varying from 6.80 to 5.55, respectively (Table 1). Significant differences between the
231 pH of the samples before and after HPP treatments were not detected ($p > 0.05$). The a_w of samples
232 was neither affected by HPP application and was ≥ 0.99 in all cases. The increase in lactic acid
233 concentrations enhanced the lethal effect of HPP treatment. The membrane damage in bacterial cells
234 induced by HPP could enable the entry of antimicrobial substances that can enhance lethality of
235 pressure treatments (García-Graells et al., 1999). Moreover, Jung et al. 2013 reported that increasing
236 levels of lactic acid (pH 4.0-6.0) enhanced the inactivation of *L. monocytogenes* after pressurization at
237 300 MPa for 5 min.

238 The coefficient of the quadratic term of lactic acid (Table 2, Eq.1), indicated that the enhancing HPP
239 effect was stabilized at concentrations above 4.25 g/kg, as higher lactic acid concentration did not
240 result in additional *Salmonella* inactivation at pressures equal or above 600 MPa (Figure 1a). These
241 results were relevant since the increase in the production costs due to the addition of higher amounts
242 of lactic acid would not increase the safety of the product. This can be seen in Table 1 in the HPP
243 treatment at 600 MPa for 3.5 min where *Salmonella* inactivation was 6.83 log in a product with 7.2
244 g/kg of lactic acid (Trial 17) compared to the mean of *Salmonella* inactivation (6.66 log) of the central
245 points of the CCD with 3.6 g/kg of lactic acid (Trials 8-16). This difference was below 0.5 log and
246 thus, was not relevant from a microbiological point of view. Since the industrial HPP equipment
247 currently available can achieve maximum working pressures of 600 MPa, the addition of lactic acid in
248 products intended to be pressurized could be an effective strategy to enhance the level of safety and to
249 ensure the compliance with current regulations for raw pet food concerning *Salmonella*.

3.2. Survival of *Salmonella* after HPP during subsequent frozen storage

The storage of pressurized samples at -18 ºC for 14 days resulted in additional *Salmonella* inactivation, though the extent of further inactivation during the frozen storage varied depending on the HPP parameters and lactic acid concentration used in each trial (Table 1). These results indicate that bacterial cells sub-lethally damaged by HPP were more susceptible to subsequent frozen storage. Similar results were reported for *E. coli* O157:H7 in ground beef submitted to HPP and subsequently frozen (Black et al., 2010; Zhou et al., 2016).

With the frozen storage, lactic acid exerted a quantitatively more noticeable effect throughout the tested range of lactic acid concentrations, being linear and interactive with pressure as shown by the polynomial model obtained (Table 2). Therefore, the presence of lactic acid, not only prevented the recovery of sub-lethally damaged cells after HPP, but also contributed to the loss of viability during the storage at -18 ºC after HPP. These results were in accordance with the results reported by King et al. 2012 in which greater reductions of *Salmonella* of at least 1 log were observed in frozen-stored pork meat samples treated with lactic acid in comparison with those non-treated with the acid. Moreover, the interaction between lactic acid concentration and pressure (Table 2, Eq. 2) indicated that the enhancement of the lethality of the HPP effect by the lactic acid was dependent on the level of pressure. Therefore, and in accordance with the results reported in Section 3.1, the inactivation of *Salmonella* could be enhanced at lower pressure levels in acidulated products after the frozen storage, while at higher pressure levels, the pressure would be sufficient to damage and inactivate *Salmonella* even without the addition of lactic acid.

In a previous work (Serra-Castelló et al., 2022) dealing with the kinetics of HPP inactivation of *Salmonella* in raw pet food, the storage under refrigeration after HPP allowed the recovery of sublethally injured cells, though the addition of lactic acid minimised the recovery. As a result, about 2-log higher levels of *Salmonella* could be counted when samples were analysed after being stored for 24h at 4 ºC after being pressurised at 600 MPa for 7 min. On the contrary, the results of the present work indicate that the storage of raw pet food intended for cat (formulated without and with lactic

acid) stored under frozen conditions after the HPP treatment could be a feasible and effective control measure applied by manufacturers to avoid the recovery of sublethally-injured *Salmonella* cells.

3.3. Endogenous microbiota of raw pet food

Counts of endogenous microbiota in non-inoculated raw pet food samples before HPP were 2.81 ± 0.73 log cfu/g for *Enterobacteriaceae*, 2.16 ± 0.45 log cfu/g for *Pseudomonas* spp., 4.63 ± 0.20 log cfu/g for total aerobic mesophilic bacteria and 4.34 ± 0.19 log cfu/g for LAB. Due to the relatively low initial levels of *Enterobacteriaceae* and *Pseudomonas* spp., the HPP effect on both groups could not be evaluated as they were reduced to levels below the plate detection limit in the majority of the trials performed (data not shown). Argyri et al. (2019) reported that the HPP treatment of chicken fillets at 500 MPa for 10 min resulted in a reduction of the inoculated *Enterobacteriaceae* and *Pseudomonas* levels of approximately 6 log, indicating the high susceptibility of both bacterial groups to HPP.

Total aerobic mesophilic bacteria showed to be the bacterial group less affected by HPP treatments (Table 1). Reductions from 0.92 to 2.73 log were recorded in the different trials (Table 1). The inactivation of total aerobic mesophilic microorganisms depended on the three technological parameters studied, i.e., pressure, holding time and concentration of lactic acid (Table 2, Eq. 3). The pressure exerted a linear effect, though the interaction with holding time and with the concentration of lactic acid reflected that pressure also modulated the effect of these two parameters. Thus, inactivation increased almost linearly over time, with the appearance of a slight resistance tail at the central pressure value of the CCD (around 600 MPa). The effect of the lactic acid was statistically significant, so it was included in the mathematical model. However, from the microbiological perspective, in most of the trials it was hardly relevant because differences in inactivation between concentrations of lactic acid in treatments below 600 MPa and/or 5 min were < 0.5 log units. On the other hand, the significance of the quadratic and interaction terms with pressure was surprising, indicating that pressure levels above 600 MPa lead to lower inactivation levels in the presence of increasing amounts of lactic acid. Among the plausible hypotheses that could explain these results, there is the fact that the

group of total aerobic mesophilic microorganisms is formed by a great variety of genera, species (including sporulated bacteria) and strains with different resistance against the studied factors.

For LAB, the significant and relevant factors determining the HPP inactivation were pressure and holding time (Table 2, Eq. 4). The pressure had a linear effect on LAB inactivation, being the impact of the quadratic factor not significant. At higher pressures (> 600 MPa), the levels of lactic acid bacteria were below the quantification limit in most of the trials. In contrast, the quadratic effect of the holding time was more pronounced and would describe the maximum inactivation values that could be quantified taking into account the initial levels of LAB. The effect of lactic acid was not significant, which can be explained by the fact that LAB are relatively tolerant to this acid as it is a product of their own metabolism.

3.4. HPP effect on raw pet food colour

The results of the evaluation of the instrumental colour of raw pet food on non-inoculated samples subjected to different HPP treatments according to the CCD are shown in Table 1. The HPP caused a slight decrease of redness (a^*) in most of the samples, while the yellowness (b^*) parameter generally slightly increased (Table 1). Nevertheless, when fitting models to colour data measurements, a lack of fit was obtained for a^* and b^* parameters, indicating that neither the pressure level, holding time or lactic acid contributed to explain the slight differences in redness and yellowness showed by the HPP-treated product.

An increase on the parameter L^* was detected, which means that the pressurized samples presented a slightly lighter (white) than the non-pressurized, which can be attributed to the denaturation of myofibrillar proteins (Kruk et al., 2011). These results are in accordance with published studies showing an increase in the L^* parameter after the pressurization of poultry meat (Yuste et al., 1999; Beltran et al., 2004; Mariutti et al., 2008; Del Olmo et al., 2010; Kruk et al., 2011; Omana et al., 2011; Cap et al., 2020).

Modelling the lightness (L^*) resulted in quadratic terms for pressure and time (Table 1, Eq. 5) indicating that the impact of these factors on the lightness of the matrix was evidenced in a relevant

way from a certain level of pressure (around 600 MPa) and treatment time (approximately 5 min). The presence of increasing concentrations of lactic acid would have a seemingly protective effect of the change in lightness, since for a certain level of pressure and/or treatment time the difference in lightness (L^*-L_0^*) before and after the treatment was reduced. This could be explained by the fact that the lightness value used to calculate the difference was determined in the product matrix once the lactic acid was incorporated. The total colour change (ΔE) was below 3 in most of the trials, except in trial 7 (without lactic acid), trial 19 and 21 (low lactic acid concentration and pressure level), showing ΔE higher than 3. The slight change of the lightness of the matrix when measured instrumentally was not perceived as a drawback from the commercial point of view as the visual colour appearance of the HPP product (Figure 2, comparing the product from trials with low and high ΔE) was considered to be within the reasonably foreseeable range of variability among production batches.

4. Conclusions

High pressure processing points out as a strategy that can be applied by manufacturers of chicken-based raw pet food as a technological measure to inactivate pathogenic and non-pathogenic bacteria without causing relevant negative effects in the appearance of the product. The formulation of chicken-based raw pet food with lactic acid as well as the subsequent frozen storage of pressurised products enhances the HPP lethal effects avoiding the recovery of pressure-injured cells during storage of chicken-based raw pet food and, in addition, promoted a further inactivation of *Salmonella*. The predictive models developed in this study constitute a useful decision support tool to help manufacturers of chicken-based raw pet food to increase the microbiological safety of their products by allowing the selection of most effective pressure level, holding time and lactic acid combinations to achieve target levels of bacterial inactivation.

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6. Declaration of conflict of interests

Authors declare no conflict of interest. The funders provided the raw materials for preparing the raw pet food product used in the study. They had no responsibility on the design of experiments, data collection and analysis or decision to publish.

7. References

Alpas, H., Kalchayanand, N., Bozoglu, F., Ray, B. (2000). Interactions of high hydrostatic pressure, pressurization temperature and pH on death and injury of pressure-resistant and pressure-sensitive strains of foodborne pathogens. *International Journal of Food Microbiology, 60*, 33–42.

Anonymous. (2019). Raw pet food - caring for their nutrition is caring for them. Hiperbaric-Blog. [cited 2022 April 13]. Available from: http://blog.hiperbaric.com/en/raw-pet-food?utm_source=Salesforce+Hiperbaric&utm_campaign=4217f418b1-EMAIL_CAMPAIGN_OCTOBER_COPY_01&utm_medium=email&utm_term=0_6821c8620a-4217f418b1-118454799

Argyri, AA., Papadopoulou, OS., Sourri, P., Chorianopoulos, N., Tassou, CC. (2019). Quality and safety of fresh chicken fillets after high pressure processing: survival of indigenous *Brochothrix thermosphacta* and inoculated *Listeria monocytogenes*. *Microorganisms*, *7*, 520.

Barba, FJ., Criado, MN., Belda-Galbis, CM., Esteve, MJ., Rodrigo, D. (2014). Stevia rebaudiana Bertoni as a natural antioxidant/antimicrobial for high pressure processed fruit extract: Processing parameter optimization. *Food Chemistry, 148*, 261–7.

Beltran, E., Pla, R., Capellas, M., Yuste, J., Mor-Mur, M. (2004). Lipid oxidation and colour in pressure- and heat-treated minced chicken thighs. *Journal of the Science of Food and Agriculture, 84*, 1285–9.

Black, EP., Hirneisen, KA., Hoover, DG., Kniel, KE. (2010). Fate of *Escherichia coli* O157:H7 in ground beef following high-pressure processing and freezing. *Journal of Applied Microbiology, 108*, 1352–60.

Bover-Cid, S., Belletti, N., Aymerich, T., Garriga, M. (2015). Modeling the protective effect of a_w and fat content on the high pressure resistance of *Listeria monocytogenes* in dry-cured ham. *Food Research International, 75*, 194–9.

382 Bover-Cid, S., Belletti, N., Aymerich, T., Garriga, M. (2017). Modelling the impact of water activity
383 and fat content of dry-cured ham on the reduction of *Salmonella enterica* by high pressure
384 processing. *Meat Science, 123*, 120–5.

385 Cap, M., Paredes, PF., Fernández, D., Mozgovoj, M., Vaudagna, SR., Rodriguez, A. (2020). Effect of
386 high hydrostatic pressure on *Salmonella* spp. inactivation and meat-quality of frozen chicken
387 breast. *LWT Food Science and Technology, 118,* Article: 108873.

388 Davies, RH., Lawes, JR., Wales, AD. (2019). Raw diets for dogs and cats: a review, with particular
389 reference to microbiological hazards. *Journal of Small Animal Practice, 60,* 329–339.

390 Del Olmo, A., Morales, P., Ávila, M., Calzada, J., Nuñez, M. (2010). Effect of single-cycle and
391 multiple-cycle high-pressure treatments on the colour and texture of chicken breast fillets.
392 *Innovative Food Science and Emerging Technologies, 11,* 441–4.

393 EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis, K., Alvarez-Ordóñez, A.,
394 Bolton, D., Bover-Cid, S., Chemaly, M., Davies, R., De Cesare, A., Herman, L., Hilbert, F.,
395 Lindqvist, R., Nauta, M., Peixe, L., Ru, G., Simmons, M., Skandamis, P., Suffredini, E., Castle,
396 L., Crotta, M., Grob, K., Milana, MR., Petersen, A., Roig Sagu´es, AX., Vinagre Silva, F.,
397 Barthélémy, E., Christodoulidou, A., Messens, W., Allende, A. (2022). Scientific Opinion on
398 the efficacy and safety of high-pressure processing of food. EFSA Journal 2022;20(3):7128,
399 195. https://doi.org/10.2903/j.efsa.2022.71

400 European Commission Regulation (EU). No 142/2011 of 25 February 2011 implementing Regulation
401 (EC) No 1069/2009 of the European Parliament and of the Council laying down health rules as
402 regards animal by-products and derived products not intended for human consumption and
403 implementing Council Directive 97/78/EC as regards certain samples and items exempt from
404 veterinary checks at the border under that Directive. (2011). *Official Journal of the European*
405 *Union, L54,* 1-254

406 FDA. (2013). Compliance Policy Guide Sec. 609.800 *Salmonella* in Food for Animals. *Federal*

Register, 78, Article: 42526.

Fredriksson-Ahomaa, M., Heikkilä, T., Pernu, N., Kovanen, S., Hielm-Björkman, A., Kivistö, R. (2017). Raw Meat-based diets in dogs and cats. *Veterinari Sciences, 4,* 33.

García-Graells, C., Masschalck, B., Moonjai, N., Michiels, C. (1999). High Pressure Inactivation and Survival of Pressure-Resistant *Escherichia coli* Mutants in Milk BT , *Advances in High Pressure Bioscience and Biotechnology*. In: Ludwig H, editor. Berlin, Heidelberg: Springer Berlin Heidelberg. pp. 133–6.

Giacometti, F., Magarotto, J., Serraino, A., Piva, S. (2017). Highly suspected cases of salmonellosis in two cats fed with a commercial raw meat-based diet: health risks to animals and zoonotic implications. *BMC Veterinary Research, 13,* 224. https://doi.org/10.1186/s12917-017-1143-z

Hereu, A., Dalgaard, P., Garriga, M., Aymerich, T., Bover-Cid, S. (2014). Analysing and modelling the growth behaviour of *Listeria monocytogenes* on RTE cooked meat products after a high pressure treatment at 400 MPa. *International Journal of Food Microbiology, 186,* 84–94.

Huang, Y., Ye, M., Chen, H. (2013). Inactivation of *Escherichia coli* O157:H7 and *Salmonella* spp. in strawberry puree by high hydrostatic pressure with/without subsequent frozen storage. *International Journal of Food Microbiology, 160,* 337–43.

Jones, JL., Wang, L., Ceric O., Nemser, SM., Rotstein, DS., Jurkovic, DA., Rosa, Y., Byrum, B., Cui, J., Zhang, Y., Brown, CA., Burnum, AL., Sanchez, S., Reimschuessel, R. (2019). Whole genome sequencing confirms source of pathogens associated with bacterial foodborne illness in pets fed raw pet food. *Journal of Veterinary Diagnostic Investigation, 31,* 235–40.

Jung, L-S., Lee, H-Y., Lee, SH., Kim, S., Ahn, J. (2013). Assessment of pressure-induced inactivation of *Listeria monocytogenes* exposed to low pHs. *Food Science and Biotechnology, 22,* 99–105.

King, AM., Miller, RK., Castillo, A., Griffin, DB., Hardin, MD. (2012). Effects of lactic acid and commercial chilling processes on survival of *Salmonella, Yersinia enterocolitica,* and *Campylobacter coli* in Pork Variety Meats. *Journal of Food Protection, 75,* 1589–94.

Kruk, ZA., Yun, H., Rutley, DL., Lee, EJ., Kim, YJ., Jo, C. (2011). The effect of high pressure on microbial population, meat quality and sensory characteristics of chicken breast fillet. *Food Control, 22,* 6–12.

Leistner, L., Gorris, LGM. (1995). Food preservation by hurdle technology. *Trends in Food Science and Technology, 6,* 41–6.

Mariutti, LRB., Orlien, V., Bragagnolo, N., Skibsted, LH. (2008). Effect of sage and garlic on lipid oxidation in high-pressure processed chicken meat. *European Food Research and Technology, 227,* 337–44.

Nüesch-Inderbinen, M., Treier, A., Zurfluh, K., Stephan, R. (2019). Raw meat-based diets for companion animals: a potential source of transmission of pathogenic and antimicrobial-resistant Enterobacteriaceae. *Royal Society Open Science, 6,* 191170.

Omana, DA., Plastow, G., Betti, M. (2011). The use of β-glucan as a partial salt replacer in high pressure processed chicken breast meat. *Food Chemistry, 129,* 768–76.

Patazaca, E., Koutchma, T., Balasubramaniam, VM. (2007). Quasi-adiabatic temperaature increase during high pressure processing of selected foods. *Journal of Food Engineering, 80,* 199-205.

Possas, A., Pérez-Rodríguez, F., Valero, A., García-Gimeno, RM. (2017). Modelling the inactivation of *Listeria monocytogenes* by high hydrostatic pressure processing in foods: A review. *Trends in Food Science and Technology, 70,* 45–55.

R Core Team. (2019). R: A language and environment for statistical computing. Vienna: *R Foundation for Statistical Computing*. Available from: https://www.r-project.org/

Robinson GK. (2000). Plan a single experiment. *In: Practical strategies for experimenting*. West Sussex, England: John Wiley & Sons. pp. 113–40.

Scherl, DS., Coffman, L., Davidson, S., Stiers, C. (2019). Two randomized trials demonstrate lactic acid supplementation in pet food inhibits dental plaque, calculus, and tooth stain in cats.

Journal of Veterinary Dentistry, 36, 129–34.

Serra-Castelló, C., Possas, A., Jofré, A., Garriga, M., Bover-Cid, S. (2022). Enhanced high hydrostatic pressure lethality in acidulated raw pet food formulations was pathogen species and strain depedent. *Food Microbiology, 104*, Article: 104002.

Tananuwong, K., Chitsakun, T., Tattiyakul, J. (2012). Effects of high-pressure processing on inactivation of *Salmonella* Typhimurium, eating quality, and microstructure of raw chicken breast fillets. *Journal of Food Science, 77*, 321-7.

van Bree, FPJ., Bokken, GCAM., Mineur, R., Franssen, F., Opsteegh, M., van der Giessen, JWB., Lipman, LJA., Overgaauw, PAM. (2018). Zoonotic bacteria and parasites found in raw meat-based diets for cats and dogs. *Veterinary Record, 182*, 50.

Weese, JS., Rousseau, J., Arroyo, L. (2005). Bacteriological evaluation of commercial canine and feline raw diets. *Canadian Veterinary Journal, 46*, 513–516.

Yuste, J., Mor-Mur, M., Capellas, M., Guamis, B., Pla, R. (1999). Mechanically recovered poultry meat sausages manufactured with high hydrostatic pressure. *Poultry Science, 78*, 914–21.

Zhou, Y., Karwe, M.V., Matthews, KR. (2016). Differences in inactivation of *Escherichia coli* O157:H7 strains in ground beef following repeated high pressure processing treatments and cold storage. *Food Microbiology, 58*, 7–12.

476 **Figure captions**

477

478 **Figure 1.** Response surface graphs of HPP-induced inactivation of *Salmonella* spp. in raw pet food
479 according to the developed model: (a) pressure and holding time effects; (b) pressure and lactic acid
480 effects; (c) holding time and lactic acid effects. The factors not included in each graph were
481 maintained at the central value of the central composite design (lactic acid = 3.6 g/kg in graph (a),
482 time = 3.5 min in graph (b), pressure = 600 MPa in graph (c)).

483

484 **Figure 2.** Visual colour appearance of raw pet food formulated with lactic acid before (No-HPP) and
485 after pressurization (Post-HPP) at (a) 450 MPa for 3.5 min (with 3.6 g/kg lactic acid, Trial 1) and at
486 (b) 689 MPa for 5.6 min (with 1.5 g/kg lactic acid, Trial 21).

487

488

Table 1. *Salmonella*, aerobic mesophilic and lactic acid bacteria inactivation (log reduction) and changes in the colour lightness (L^*), redness (a^*) and yellowness (b^*) on raw pet food samples due to high pressure processing (HPP) treatments at each combination of the Central Composite Design.

Trial	Lactic acid (g/kg)[a]	Pressure (MPa)	Time (min)	Microbial inactivation (log reduction)[b]				Colour changes post HPP[c]			
				Salmonella		Mesophilic bacteria	Lactic acid bacteria				
				Post-HPP	Post-HPP + Frozen storage	Post-HPP	Post-HPP	L^*- L_0^*	a^*- a_0^*	b^*- b_0^*	ΔE
1	3.6 [5.99 ± 0.04]	450	3.5	-2.61 ± 0.32	-3.10 ± 0.26	-1.30 ± 0.06	-1.32 ± 0.09	1.65 ± 0.26	-0.49 ± 0.19	0.05 ± 0.14	1.74 ± 0.24
2	1.5 [6.43 ± 0.02]	511	1.4	-1.11 ± 0.12	-2.01 ± 0.01	-1.05 ± 0.10	-1.52 ± 0.02	1.40 ± 0.24	0.12 ± 0.09	-0.30 ± 0.09	1.44 ± 0.22
3	5.7 [5.72 ± 0.03]	511	1.4	-3.30 ± 0.08	-5.74 ± 0.13	-1.47 ± 0.01	-1.55 ± 0.02	0.97 ± 0.33	1.40 ± 0.04	0.19 ± 0.07	1.73 ± 0.21
4	1.5 [6.43 ± 0.02]	511	5.6	-3.91 ± 0.05	-4.06 ± 0.09	-2.36 ± 0.16	-2.77 ± 0.02	1.42 ± 0.24	-0.51 ± 0.10	-0.26 ± 0.09	1.54 ± 0.18
5	5.7 [5.72 ± 0.03]	511	5.6	-5.72 ± 0.15	-7.67 ± 1.77	-2.54 ± 0.01	-2.89 ± 0.25	0.94 ± 0.08	-0.66 ± 0.23	0.04 ± 0.06	1.16 ± 0.19
6	3.6 [6.01 ± 0.04]	600	0.0	-1.10 ± 0.04	-2.07 ± 0.12	-0.92 ± 0.26	-0.50 ± 0.13	0.14 ± 0.35	-0.61 ± 0.04	-0.49 ± 0.08	0.85 ± 0.05
7	0.0 [6.80 ± 0.03]	600	3.5	-4.44 ± 0.16	-5.39 ± 0.26	-2.26 ± 0.15	-3.79 ± 0.49	3.52 ± 0.14	-2.08 ± 0.17	-0.32 ± 0.08	4.11 ± 0.10
8	3.6 [6.01 ± 0.04]	600	3.5	-6.60 ± 0.24	-7.46 ± 0.24	-2.20 ± 0.21	-3.75 ± 0.35	1.30 ± 0.22	-0.96 ± 0.02	-0.02 ± 0.04	1.62 ± 0.17
9	3.6 [6.01 ± 0.04]	600	3.5	-6.49 ± 0.82	-7.06 ± 0.36	-2.16 ± 0.02	-4.22 ± 0.81	1.12 ± 0.73	-0.76 ± 0.08	-0.19 ± 0.14	1.44 ± 0.48
10	3.6 [5.99 ± 0.05]	600	3.5	-7.59 ± 0.32	-7.79 ± 0.24	-1.82 ± 0.33	-4.90 ± 0.85	1.86 ± 0.32	-0.20 ± 0.09	0.00 ± 0.14	1.88 ± 0.30
11	3.6 [5.99 ± 0.05]	600	3.5	-7.31 ± 0.23	-6.82 ± 0.13	-1.91 ± 0.11	-5.39 ± 0.00	1.81 ± 0.21	1.17 ± 0.06	0.05 ± 0.09	2.16 ± 0.20
12	3.6 [6.05 ± 0.05]	600	3.5	-6.18 ± 0.28	-6.31 ± 0.61	-1.98 ± 0.05	-5.40 ± 0.00	1.00 ± 0.46	-0.49 ± 0.04	0.44 ± 0.09	0.99 ± 0.32
13	3.6 [6.05 ± 0.05]	600	3.5	-6.49 ± 0.07	-7.97 ± 1.47	-1.83 ± 0.71	-5.40 ± 0.00	2.22 ± 0.39	-0.01 ± 0.13	0.67 ± 0.16	2.00 ± 0.41
14	3.6 [6.05 ± 0.05]	600	3.5	-6.49 ± 0.28	-6.35 ± 0.34	-1.98 ± 0.20	-5.33 ± 0.00	0.99 ± 0.08	-0.74 ± 0.08	0.17 ± 0.06	1.25 ± 0.10
15	3.6 [6.05 ± 0.05]	600	3.5	-6.16 ± 0.29	-6.35 ± 0.29	-1.85 ± 0.07	-4.99 ± 0.58	0.38 ± 0.23	-1.59 ± 0.11	0.11 ± 0.09	1.64 ± 0.14
16	3.6 [6.05 ± 0.05]	600	3.5	-6.62 ± 0.45	-6.42 ± 0.10	-1.94 ± 0.28	-4.99 ± 0.58	0.75 ± 0.34	-1.13 ± 0.14	0.35 ± 0.11	1.44 ± 0.12
17	7.2 [5.55 ± 0.05]	600	3.5	-6.83 ± 0.41	-7.55 ± 0.42	-2.31 ± 0.19	-5.02 ± 0.00	1.61 ± 0.10	1.26 ± 0.21	0.17 ± 0.08	2.05 ± 0.21
18	3.6 [5.99 ± 0.04]	600	7.0	-6.95 ± 0.11	-8.10 ± 1.36	-2.21 ± 0.19	-5.07 ± 0.58	2.41 ± 0.34	0.45 ± 0.11	0.68 ± 0.09	2.21 ± 0.36
19	1.5 [6.43 ± 0.07]	689	1.4	-5.73 ± 0.13	-7.10 ± 0.37	-2.58 ± 0.28	-5.55 ± 0.00	3.43 ± 0.28	-1.20 ± 0.17	0.14 ± 0.09	3.64 ± 0.21
20	5.7 [5.66 ± 0.08]	689	1.4	-7.73 ± 0.00	-8.31 ± 1.16	-2.20 ± 0.24	-5.43 ± 0.00	0.96 ± 0.15	-1.97 ± 0.08	0.13 ± 0.02	2.20 ± 0.13
21	1.5 [6.43 ± 0.07]	689	5.6	-7.59 ± 0.35	-8.20 ± 1.36	-2.73 ± 0.06	-5.55 ± 0.00	4.82 ± 0.24	-0.14 ± 0.09	0.76 ± 0.03	4.88 ± 0.24
22	5.7 [5.67 ± 0.08]	689	5.6	-8.13 ± 1.46	-7.89 ± 1.54	-2.18 ± 0.03	-5.43 ± 0.00	2.08 ± 0.33	-0.95 ± 0.10	0.03 ± 0.05	2.29 ± 0.26
23	3.6 [6.09 ± 0.05]	750	3.5	-9.08 ± 0.94	-9.08 ± 0.94	-2.37 ± 0.18	-5.40 ± 0.00	2.91 ± 0.34	-0.27 ± 0.14	0.98 ± 0.15	2.76 ± 0.34

[a] Mean ± standard deviation of the pH of three replicates are reported between square brackets

[b] Mean ± standard deviation of three replicates.

[c] Mean ± standard deviation of three replicates. L_0^*, a_0^* and b_0^* indicate the measurements before the HPP treatment and L^*, a^* and b^* the measurements after the application of HPP on raw pet food. Delta E (ΔE) provides the insight into the difference seen betwee two colours.

Table 2. Results of the multivariate regression describing the effect of pressure, pressure-holding time and lactic acid concentration on the inactivation of *Salmonella* spp. (immediately after high pressure processing (HPP) and after 14 days of frozen storage), total aerobic mesophilic bacteria, lactic acid bacteria and lightness in raw pet food.

Microorganism / colour parameter	**Treatment**	**Model**[a]	**RMSE**
Salmonella spp.	HPP	$Log\ (N/No) = 27.92 - 0.06217 \cdot P - 3.3580 \cdot t - 1.051 \cdot LA + 0.00003 \cdot P^2 + 0.2013 \cdot t^2 + 0.06628 \cdot LA^2 + 0.00193 \cdot (P \cdot t) + 0.05765 \cdot (t \cdot LA)$ Eq. (1)	0.635
	HPP + frozen storage	$Log\ (N/No) = 22.9733 - 0.04152 \cdot P - 2.7381 \cdot t - 2.9986 \cdot LA + 0.1251 \cdot t^2 + 0.0022 \cdot (P \cdot t) + 0.0043 \cdot (P \cdot LA)$ Eq. (2)	1.021
Mesophilic bacteria	HPP	$Log\ (N/No) = 5.818 - 0.0123 \cdot P - 1.2350 \cdot t - 0.3692 \cdot LA + 0.0231 \cdot t^2 - 0.0331 \cdot LA^2 + 0.0015 \cdot (P \cdot t) + 0.0010 \cdot (P \cdot LA)$ Eq. (3)	0.208
Lactic acid bacteria	HPP	$Log\ (N/No) = 33.21 - 0.0936 \cdot P - 2.5040 \cdot t + 0.00006 \cdot P^2 + 0.1557 \cdot t^2 + 0.0017 \cdot (P \cdot t)$ Eq. (4)	0.702
Lightness (L*)	HPP	$L^* - {L_0}^* = 10.99 - 0.0393 \cdot P - 0.7910 \cdot t + 0.7056 \cdot LA + 0.00004 \cdot P^2 + 0.0971 \cdot LA^2 + 0.0017 \cdot (P \cdot t) - 0.0029 \cdot (P \cdot LA)$ Eq. (5)	0.507

[a]Where $Log\ (N/No)$ is the bacterial inactivation, P is pressure level, t is the holding time, LA is lactic acid and $L^* - {L_0}^*$ is the difference in product lightness due to the HPP treatment.

Figure 1.

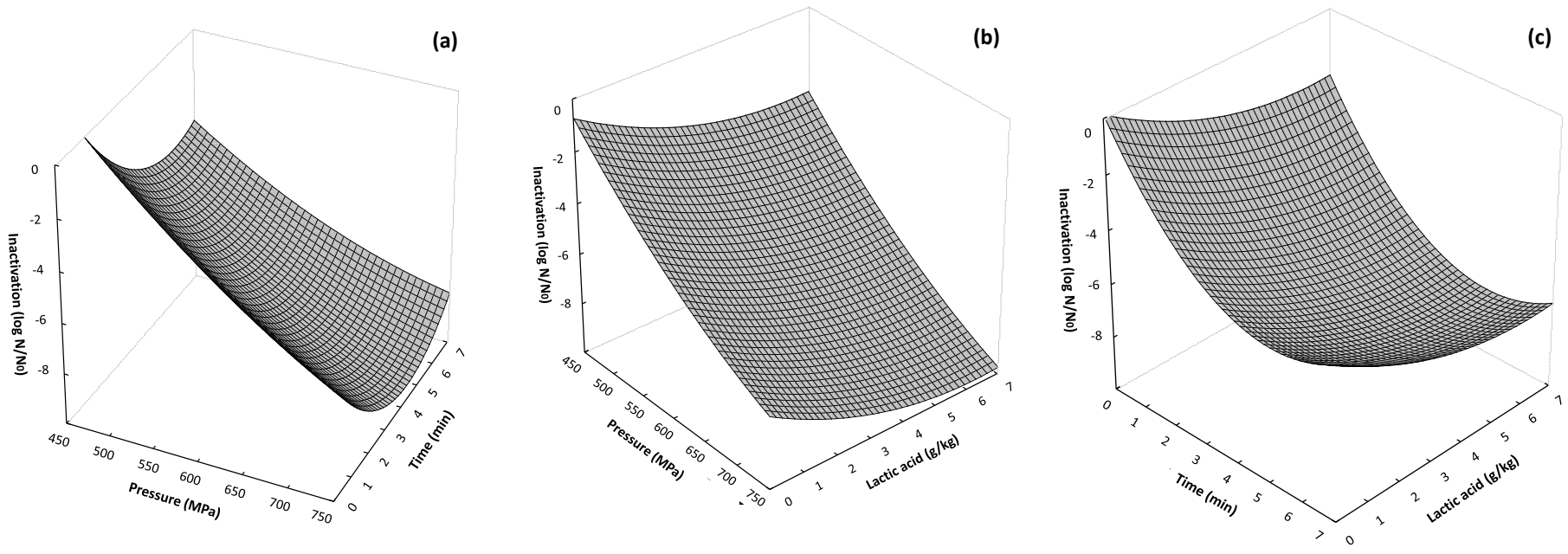
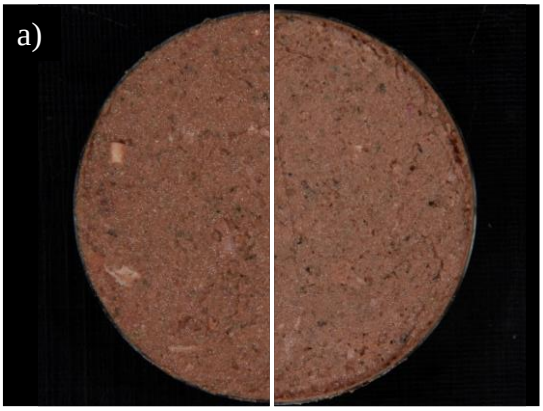
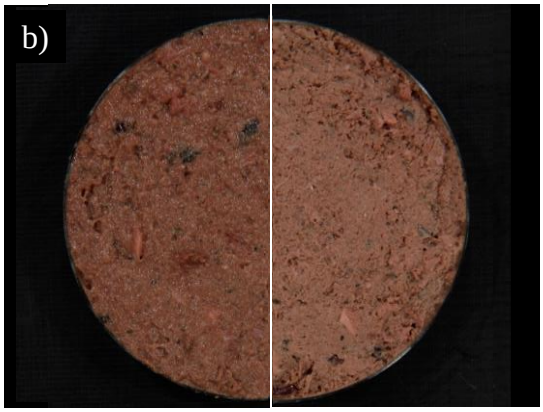

(a)
Inactivation (log N/No)
0
-2
-4
-6
-8
450
500
550
600
650
700
750
Pressure (MPa)
0
1
2
3
4
5
6
7
Time (min)
(b)
Inactivation (log N/No)
0
-2
-4
-6
-8
450
500
550
600
650
700
750
Pressure (MPa)
0
1
2
3
4
5
6
7
Lactic acid (g/kg)
(c)
Inactivation (log N/No)
0
-2
-4
-6
-8
0
1
2
3
4
5
6
7
Time (min)
0
1
2
3
4
5
6
7
Lactic acid (g/kg)

Figure 2.



Colour parameter	No-HPP	Post-HPP
L^1	45.81	47.46
a^2	13.68	13.19
b^3	11.69	11.74



Colour parameter	No-HPP	Post-HPP
L^1	43.17	47.99
a^2	14.43	14.29
b^3	11.39	12.15