The International Journal of Engineering and Science (IJES)
|| Volume || 8 || Issue || 8 Series II || Pages || PP 01-09 || 2019 ||
ISSN (e): 2319 – 1813 ISSN (p): 23-19 – 1805

High pressure thermal processing for inactivation of Bacillus amyloliquefaciens and Clostridium sporogenes spores in a range of low acid commercial preparedfoods

Rachna Pandey[1], Sergio Ramos[2], Brijesh Tiwari[1], Silvia Garcia De la Torre[2]and Geraldine Duffy[# 1]

[1]*Teagasc Food Research Centre, Ashtown, Dublin 15, Ireland*
[2]*Centro Nacional de Tecnología y Seguridad Alimentaria (CNTA), Carretera NA-134, km 53. 31570-San Adrian, Navarra, Spain*
**Corresponding author: Rachna Pandey*

ABSTRACT

Bacterial spores (Bacillus and Clostridium) are common contaminants of food products andtheir germination and subsequent outgrowth may cause food spoilage or foodborne illness. High pressure thermal processing is an emerging technology with potential use in commercial food processing to obtain safe high quality food products with extended shelf life.The aim of this study was tocompare thermal treatment against high pressurethermal processing for inactivation of B. amyloliquefaciensDSM 7and C. sporogenes DSM 767 spores in a range of commercial prepared foods (vegetable soup, pea with ham and carrot, veal and sole). Spores (10^7 CFU/g) were inoculated into food productsand exposed to high temperatures (110 and 115 ºC) alone or in combination with high hydrostatic pressure (600 MPa) over a range of holding times. Survivor curves were non–linear with tailing populations and inactivation kinetics and D values were calculated using a Weibull modelling approach.At 110 ºC the D value for C. sporogenes ranged from 1.513 to 4.777min depending on the food matrix while at the same temperature B. amyloliquefacienswas more resistant with D values ranging between 3.015 and 6.434 min again varying with food matrix. At 115 ºC for both spores the D value ranged between 0.012 min and 1.472 min.When high pressure (600MPa) was combined with thermal (110 ºC) the D value for B. amyloliquefaciens was significantly reduced (0.029to 0.209 min) depending on the food and, further reduced with an increase in temperature to 115 º C(0.004 to 0.110 min). The results obtained in this study show that heat (110 or 115 ºC) in combination with high pressure (600 MPa, 300s) can reduce populations of B. amyloliquefaciens and C. sporogenes spores (4-5 Log) in prepared foods in significantly shorter process times than thermal alone. The study provides data to design process windows for application of high pressure thermal treatments.

Keywords:*Bacillus amyloliquefaciens, C. sporogenes, high pressure thermal treatment, Inactivation kinetics.*

Date of Submission: 07-08-2019 Date of acceptance: 23-08-2019

I. INTRODUCTION

Bacillus and Clostridium are spore-forming bacteria that are widely distributed in nature and can pose contamination problems for the food industry, leading to food spoilage and indeed food borne illness. Such sporesareparticularly resistant to treatments commonly used in food processing with high temperature processing (90 to 121 ºC)the primary method of spore inactivation; howeverthis temperature can potentially impact on the quality of the food.Recent advances in non- thermal food-processing technologies, such as high hydrostatic pressure has demonstrated strong potential for the delivery of a wide range of high quality chilled products with extended shelf life. Commercial high pressure processing of foods in a pressure range of 400–800 MPa at processing time up to 10 min depending on physicochemical properties of food is capable of achieving over 5 log reductions for pathogenic and spoilage vegetative cells, but with low or no inactivation rates of spores, since bacterial spore inactivation requires high pressures of at least 800-1700 MPa at room temperature, far in excess of what is commercially feasible (Farkas & Hoover, 2000; Leadley, 2005). Even foods with pH lower than 4.5 (the limit for Clostrium botulinum), require refrigerated storage and other preservation hurdles to prevent enzymatic degradation reactions and to inhibit spore germination. In early 1970s, studies on Clostridium species demonstrated the potential to combine pressure and heat to achieve spore inactivation (Sale, Gould & Hamilton, 1970; Heinz & Knorr, 2001). In the late 1990s; Rovere, Gola, Maggi, Scaramuzza, & Miglio, 1998; Reddy, Tetzloff, Solomon & Larkin, 2006; Heinz & Knorr, 2001), pressures in the range of 690 to 900 MPa

were combined with initial temperatures between 50 ℃ and 70°C showing the potential application of high pressure temperature processing (HPT) for commercial sterilization in the food industry.

An advantage of HPT treatment compared to conventional thermal processing is the potential for shorter processing time at lower temperatures to eliminate spore-forming microorganisms. Shorter processing time, uniform compression heating and rapid cooling on depressurization is reported to achieve complete inactivation of vegetable cells and spores while minimising thermal impacts on food (Li & Farid, 2016). Shorter process time and ultimate pressurization temperatures lower than 121°C have resulted in higher quality and nutrient retention in selected products. For example, better retention of flavour components has been reported in fresh basil, firmness in green beans, and colour in carrots, spinach and tomato puree have been found after HPHT processing (Krebbers, Matser, Koets, & van den Berg, 2002;
Krebbers, Matser, Hoogerwerf, Moezelaar, Tomassen & van den Berg 2003). Nutrientssuch as vitamins C and A have also shown higher retention after HPHT processing in comparison to retort methods (Matser, Krebbers, Berg & Bartels, 2004).

The aim of this research was to (i) study the impact of high temperatures and combination of high pressure and high temperature processing for inactivation of B. amyloliquefaciens DSM 7 and C. sporogenes DSM 767 spores in a range of commercially relevant prepared meals and,(ii) build a data set to support development of process window for food treatments with HPT.

II. MATERIALS AND METHODS

2.1 Bacterial strains and spore preparations

B. amyloliquefaciens DSM7and C. sporogenesDSM767strainswere used to prepare the respective spores. The strains were maintained on Protect^{TM} Stock Culture Beads (Technical Services Consultants Ltd, Lancashire, U.K) at -80 °C. Protect beads coated with respective strains were inoculated into the nutrient broth and cooked meat medium respectively, and incubated at 37°C for 24 hunder aerobic conditions for B. amyloliquefaciens DSM 7 and in anaerobic conditions for C. sporogenes DSM 767. The actively growing cells suspension was used to spread (100 µl) on to the surface of Campden Sporulation Agar (CSA:tryptone 5g, bacteriological peptone 5g, lab lemco 1g, yeast extract 2g, $CaCl_2$ 0.056g, $MnSO_4$:$4H_2O$ 0.082g, glucose 1g, agar 15g; / one litre water). The plates were incubated at 37°C for 6 days in aerobic conditions for B. amyloliquefaciensDSM 7and 12 days in anaerobic chamber for C. sporogenes DSM 767. After the respective incubation period, thespores were harvested by gently scraping the bacterial lawns from plates and washing the pellets with ice-cold sterile distilled water. The mixture of cells and spores were centrifuged at 8000 g for 15 min at 4°C. Pellets were washed with sterile distilled water. The resulting sporesuspensions were washed four to five times with sterile distilled water. To determine spore purity, selected samples were stained using the Shaeffer-Fulton staining method and through phase contrast microscope for phase bright spores. Spore suspension were generally >95% pure. The purified spore crop was stored at refrigeration temperature until used. To assess the number of colony forming unit (CFU), thespores were cultured by the pour plate technique ontonutrient agar and incubated at 37°C for 24 h under aerobic conditions (B. amyloliquefaciens) or for 3-4 days under anaerobic condition (C. sporogenes).

2.2 Sample preparationand inoculation

Four different preparedfoods were used in the study,i.e. vegetable soup, peas with ham, braised veal and sole were supplied by commercial food manufacturers. The main composition of each preparedfood product is described in Table 1. To facilitate inoculation, and insertion into the heating orhigh pressure thermal (HPT) apparatus, three of the prepared foods (peas with ham, veal and sole) were grinded with sterile water (30% volume) and mixed to get a homogenous paste. Thethree foods prepared as a pasteand the soup (2 g) were each inoculated with B. amyloliquefaciensorCl. sporogenesspores(~10^7cfu/g) and used in both thermal inactivation, and high pressure thermal treatment experiments.

2.3 Thermal inactivation

Thermal treatmentexperimentswere carried out in a thermoresistometer(Condón, Arrizubieta & Sala, 1993) for vegetable soup, veal and sole or glass capillary tubes (peas with ham). The temperature controlled thermoresistometer (Condón, Arrizubieta & Sala, 1993) consisted of a main heating vessel with a capacity of 400 ml made up of stainless steel (8.5 cm outer diameter × 12 cm high), a motor to enable the homogenization of the heating medium, a main control unit to control heating, sampling and agitation, an external pressure source, ports for injecting the microbial suspension and a fraction collector to allow for sampling in short duration experiments. The fraction collector enabled taking of samples at time intervals lower than 2 seconds. Before the start of the experiments the vessel was cleaned with detergent and alcohol and then sterilized with water by heating at 121°C for 20 min similar to autoclave. The vegetable soup,veal or sole (400 ml) was placed in the vessel of thermoresistometer and heating was turned on. Once the vegetable soup, veal and sole reached

the pre-set temperature (T ± 0.05 ºC) (110, 115ºC), it was inoculated with a microbial cell suspension (10^7 cfu/mL). After inoculation, samples were collected at different heating times and samples were immediately pour-plated onto nutrient agar and then incubated at 37 ºC for 24 h under aerobic conditions for B. amyloliquefaciens or 3-4 days underanaerobic conditions for Cl. sporogenes. After respective incubation time, colony forming units (CFU) were counted.For glass capillary tubes experiments, the inoculated food products were introduced into the capillary tubes and then heat sealed. After that, capillaries were placed in a water-bath at the appropriate temperature (T ± 0.05 ºC) (110, 115ºC) and removed at different time intervals. The capillaries were immersed in cold water to stop the heating of the food product in the capillary. Once the capillary was cooled they were crushed with a sterile glass rod and the samples were collected in sterile tubes andpour plated onto nutrient agar plate and incubated at 37 ºC for 24 h under aerobic conditions for Bacillus or for 3-4 days under anaerobic conditions for Clostridium. After respective incubation time, colony forming units (CFU) were counted.

2.4 High pressurethermal treatment(HPT)

The high pressure equipment used was a discontinuous isostatic system from Stansted Fluid Power FPG 11500 B (Stansted, Essex, United Kingdom). With this unit pressures up to 800 MPa and temperatures up to 130 ºC could be reached. The high-pressure transmitting medium was a mixture of propylene glycol (PPG)/water (70:30 v/v). The unit consists of one chamber with a volume of 30 mL. The pressure build-up rate was 240 MPa/min and pressure release time was less than 30 s regardless of the levels of target pressure. A circulating water bath was used to circulate temperature controlled PPG around the pressure vessel to regulate the shell temperature. Thecriteria used for these experimentswas600 MPa at temperatures of 110, 115ºCwith holding times ranging between 0 to 5 minutes.Low acidfoods prepared as described above were mixed with the spores (~$\log 10^7$ cfu g^{-1})and were filled inpolyoxymethylene acetale (PMA) bags (85 mm x12 mm x 3mm) and then sealed with a bag sealer.The filled sample bags were packed within another layer of the bags to avoid the leakage in the high pressure matching when heat and pressure of 600 MPa was applied. Following HPT treatment the samples were plated onto nutrient agar plate and incubated at 37 ºC for 24 h under aerobic conditions for Bacillus or for 3-4 days under anaerobic conditions for Clostridium. After respective incubation time, colony forming units (CFU) were counted.

2.5 Statistical analysis

The number (CFU/g) of surviving B. amyloliquefaciens and C. sporogenes spores was plotted against time following each thermal or HPT treatment in the four processed foods. The inactivation kinetics of both spores was non-linear. The non-linear behaviour of the inactivation curves was explained by the Weibull model (Eq. 1) and GInaFiT tool was employed to perform the regression analysis of the microbial inactivation data (Geeraerd, Valdramidis, & Van Impe, 2005).

$$\mathrm{Log}_{10}[N_t] = \mathrm{Log}_{10}[N_0] - \left[\frac{t}{D}\right]^{\beta} \quad [1]$$

where N_t (cfu/mL$^-$) is the number of spores at time t (min), N_0 (cfu/ mL) is the initial number of spores at t=0, D (min) is the time for the first decimal reduction (90% spore inactivation) and β [-] is the shape parameter of the inactivation curve.

The numerical values of D and βwere used to calculate a desired log reduction. For example, the time required to obtain X log reduction (t_{xd}) can be calculated using Eq. 2. For this case study x was equal to 5.

$$t_{5D} = D \times [x]^{1/\beta} \quad [2]$$

III. RESULTS

The inactivation of B. amyloliquefaciens and C. sporogenesfollowing thermal or HPT treatments in the four prepared foods are presented in Figure 1 and 2, respectively. It was noted that the inactivation curves were non-linear with a generally concave shape with significant tailing effects, noted towards the end of the treatment times.A Weibull modelling approach gave a generally good fit for the data with R^2 values ranging from 0.881 to 0.999 for all treatments with B. amyloliquefaciens, while for C. sporogenes the fits were also generally good with R^2 0.702-0.991. For all treatments the RMSE fits values ranged from 0.13 to 1.31 (Table 2). The β Shape factor ranging from 0.25 to 0.66 across all treatments, reflecting the concave shape of the fitted curve.

As expected, the D value for both sporeswere higher when thermal treatment alone was applied. For C. sporogenes, at 110 ºC the D values ranged from 1.513 to 4.77 min depending on the food matrix while at the same temperature B. amyloliquefaciens was more resistant with D values ranging between 3.015 and 6.434 again varying with food matrix. At 115 ºC for both spores the D value was shorter (0.092 min to 1.472 min) for C. sporogenes and 0.012 to 0.167 min for B. amyloliquefaciens. Such differences may be due to variability across experiments and correlated with the different food matrices, which had different levels of fat (2.9 %, vegetable soup) to 12.8 % (green peas and ham); and protein (1.2% vegetable soup) to 10.2% (sole) which

might have been expected to impact on heat transfer. Acorrelation analysis showed some influence of fat content on the D value (r = 0.60) and β value (r = 0.52) for C. sporogenesbut no significant correlation coefficient in the case of B. amyloliquefaciens (Appendix 1 and 2).

When high pressure (600 MPa) was combined with thermal (110 ºC), nonlinear inactivation patterns were also observed for all treatments, with populations showing a fast decline initially and a slope tailing at the end of the process. This observation suggests that the remaining survivors are less pressure-temperature sensitive owing to the presence of heterogeneous resistant spores, resulting in an extended tailing phenomenon. A considerable effect on spore inactivation was observed after pressure come-up time (1 s pressure holding time). This effect increased when the process temperature was increased to 115 ºC. When high pressure (600 MPa) was combined with thermal (110 ºC) the D value for B. amyloliquefaciens were significantly reduced from thermal alone ranging from 0.008 to 0.209 min depending on the food. A generally similar result was observed for C. sporogenes. Correlation analysis did not show any significant association of temperature with D values or the shape factor for the prepared meals processed using high pressure in combination with temperature.

Figure 3 shows the 4D values for B. amyloliquefaciens and C. sporogenes spores in the four prepared meals using heat (110 ºC) alone versus high pressure processing (600MPa) in combination with heat (110 ºC) and, demonstrates the significantly reduced process times needed to achieve this target reduction when high pressure and temperature were applied in combination (Appendix 1 and 2).

IV. DISCUSSION

This study described the inactivation of Bacillusamyloliquefaciens and Clostridiumsporogenes spores in four commercially relevant prepared meals, all of which were low acid, following thermal or high pressure thermal treatments. In all cases the survivor curves for the spores over the treatment time showed significant tailing populations at the end of the treatment times.Thus the use of a classical thermal destruction line equation (log linear) which has been commonly used to describe the inactivation of spores over time/temperature combinations, with D values calculated based on the negative reciprocals of the slope was not a good fit for the full set of inactivation data. Resistant populations surviving at the end of process treatments may impact on food stability/spoilage during shelf life and should be accounted for in the predictive model. Survival curves can be modelled using non-linear models such as the Weibull model which describethe inactivation behaviour of microbial population withshape and tailing effect (Mafart, Couvert., Gaillard, & Leguerinel, 2002)

and for the data set from this study the Weibull model was shown to be a good fit . The β Shape factor which describes the shape of the inactivation curve showed a concave trend which is in an indication of bacterial resistance to thermal stress (Coroller,Leguerinel, Mettler, Savy, &Mafart, 2006) and this trend was observed for all treatments (β value ranging 0.25 to 0.66).When thermal treatment alone was applied, the length of heat time to achieve a 4D reduction in level of spores ranged from 29.6 to 64.1 min for B.amyloliquefaciens and 39.0 to 55.3 min for C. sporogenes at 110 ºC depending on the food matrices. Such length heating times highlight the opportunity for high pressure thermal technology to achieve longer shelf life in considerably shorter treatment durations with 4 D value at 600 MPA, 110 ºC reduced to 0.7 to 6.8min for B. amyloliquefaciens or 2.4 to 7.6 min for C. sporogenes. Significant reductions in process time were also noted at 115 ◦C with 600 MPa.

Most of the studies investigating the combined effect or pressure and thermal treatment on the microbial inactivation have not taken into account the temperature increase in the samples during pressurization due to adiabatic heat (Wang,Wang, Li, Du, &Zeng , 2015). In this study, high pressure thermal processing was applied over a holding time of 0 to 300 seconds and even after a pressure holding time of 1 s (come-up time), a log reduction ranging from 0.55 (vegetable soup) to 2.14 log (green peas ham) was notedfor B. amyloliquefaciens after compression at 600 MPa and 110 ºC. At 115 ºC this effect was increased and was highest forC. sporogenes (3.78 log reduction) in braised veal. Several other authors have also reported significant levels of spore reduction during come-up time. Wang,Li, Zeng, Huang, Ruan, Zhu & Li, (2009) described reduction levels of B. coagulans spores in milk and buffer during come-up time of 0.37 and 1.77 log at 400 MPa/80 ºC and 600 MPa/80 ºC, respectively. While Ahn&Balasubramaniam(2007) reported significantreductions levels in different sporeforming bacteria, including C. tyrobutylicum ATCC 25755 (2.5 log), T. thermosaccharolyticum ATCC 27384 (2.1 log), C. sporogenes ATCC 7955 (3.3 log), B. amyloliquefaciens TMW 2479 Fad 82 (0.9 log), B. amyloliquefaciens TMW 2482 Fad 11/2 (0.8 log), Bacillus sphaericus NZ 14 (3.7 log) and B. amyloliquefaciens ATCC 49763 (2.5 log) after treating samples at 700 MPa at 105 ºC. This inactivation during come up time is largely related to the exposure to the temperatures and pressure during the come-up time with inactivation thought to occur due to a multi-stage mechanism beginning with sub-lethal injury of spores by heat and pressure (Margosch, Ehrmann, Buckow, Heinz, Vogel, & Gänzle, 2004) and/or Dipicolinic acid release due to the inner membrane losing its barrier function. The development of integrated process lethality models taking the come-up time, into consideration may provide more useful information for designing the entire high pressure temperature conditions.

During the pressure holding time, a fast rate of inactivation was noted between 30 s and 60 s for all the microorganisms at 110 ºC and 115ºC at 600 MPa depending on the prepared food product. The decline behavior was non-linear with a rapid initial inactivation, followed by moderate inactivation and a characteristic tailing during extended pressure holding time and as described earlier was best described by Weibull model which accounts for such kinetic behavior. The data also highlights the variability in the inactivation curves with both within treatment variation and also variation related to type of spore and food composition. In general, there was trend that B. amyloliquefaciens was more resistant than C. sporogenes with higher D values for all thermal and high pressure thermal treatments at 110 ºC and this was also observed in some of the treatments at 115 ºC. Several other authors have also reported differences in response to HPT between different species and between strains of the same species and even between (Ahn & Balasubramaniam, 2007; Lenz& Vogel, 2014; Margosch, Ehrmann, , Gänzle & Vogel, 2004, 2006; Olivier, Bull, Stone, van Diepenbeek, Kormelink, Jacops & Chapman, 2011; Paredes-Sabja, Gonzalez, Sarker Torres., 2007; Ramaswamy, Kabel, Schols, Gruppen, 2013; Reddy, Solomon, Fingerhut, Rhodehamel, Balasubramaniam, Palaniappan, 1999, Reddy, Tetzloff, Solomon, & Larkin 2006; Sevenich Sevenich, Kleinstueck, Crews, Anderson, Pye, Riddellova, Hradecky, Moravcova, Reineke, Knorr, 2014). Bacterial spores are resistant to pressure due to their morphology, however, it has been shown that germination of spores can be achieved at moderate pressures and subsequently application of heat can cause inactivation (Wimalaratne & Farid, 2008).

The heating matrice i.e. the food composition (level of fat, carbohydrate, and protein etc.)may have a protective effect on bacterial spore inactivation by thermal and high pressure thermal treatment (Solomon & Hoover, 2004). In this study, inactivation kinetics were noted to vary in different food types and composition. In particularC. sporogeneswas more resistant to both thermal and HPT treatments in green peas and ham. This food had the highest level of fat (12 %) and may have provided a protective effect to the high temperature pressure treatment. Molin &Snygg (1967) compared the heat resistance of spores of Bacillus megaterium, B. subtilis, B. cereus, B. stearothermophilus, and Clostridium botulinum type E in lipids with resistance of the spores in phosphate buffer solution. A high water content of the lipids used as heating menstruum lowered the heat resistance of the spores and was most notable for B. subtilis and C. botulinum type E. Although, Lynch & Potter (1988) in studies on Frankfurter particulates with fat contents of 12 and 26% showed no significant differences ($p>0.25$) in spore inactivation rates when retort processed at 105"C and a previous study on egg patties did not show a protective effect on the inactivation of G. stearothermophilus spores processed at 700 MPa and 105 ºC (Rajan,Ahn, Balasubramaniam, &Yousef, 2006).As food matrice may have an impact on inactivation kinetics, it highlights that the design and validation of process windows for commercial thermal and HPT treatments should be done under conditions as close as possible to the commercial conditions.

V. CONCLUSION

The results obtained in this study show that heat (110 or 115 ºC) in combination with high pressure (600 MPa, 300s) can yield a 4 log reductions in B. amyloliquefaciens and C. sporogenes spores in significantly shorter process times than thermal alone. The study provides data that will support the design of process windows for application of HPT treatments.

ACKNOWLEDGMENTS

This work was supported in part by the EU Horizon 2020 project on “Deployment of High Pressure and Temperature processing for sustainable, safe, nutritious and high quality foods” (HIPSTER).

REFERENCES

[1]. Ahn, J., &Balasubramaniam, V.M.(2007). Physiological responses of Bacillus amyloliquefaciens spores to high pressure.Journal Microbiology Biotechnology;17(3):524-529.

[2]. Condón, S., Arrizubieta, M. J., & Sala, F. J. (1993). Microbial heat-resistance determinations by the multipoint system with the Thermoresistometer TR-SC – Improvement of this methodology. J. Microbiological Methods 18, 357–366.

[3]. Coroller,.L, Leguerinel, I., Mettler, E., Savy, N., &Mafart, P. (2006). General model, based on two mixed weibull distributions of bacterial resistance, for describing various shapes of inactivation curves. Applied and Environmental Microbiology; 72(10):6493-502.

[4]. Farkas, D. & Hoover, D. (2000). High pressure processing: kinetics of microbial iactivation for alternative food processing technologies. Journal of Food Science (Supplement): 65: 47-64.

[5]. Heinz, V., &Knorr. D. (2003). Non-thermal food preservation and its potential in meat processing.Forum Nutr. 2003;56:369-70.

[6]. Krebbers, B., Matser, A.M., Koets, M., & van den Berg R.W., (2002). Quality andStorage-Stability of High-Pressure Preserved Green Beans, J Food Eng. **54**(1):27-33.

[7]. Krebbers, B., Matser, A.M., Hoogerwerf, S.W., Moezelaar, R., Tomassen, M.M.M.,& van den Berg R.W. (2003).Combined High-Pressure and Thermal Treatmentsfor Processing of Tomato Puree: Evaluation of Microbial Inactivation and QualityParameters. Innovative Food Science Emerging Technology. **4**(4):377–85.

[8]. Leadley, C., (2005). High Pressure Sterilisation: A Review. Campden & Chorleywood Food

[9]. Research Association **47**:1–42.

[10]. Krebbers, B., Matser, A.M., Hoogerwerf, S.W., Moezelaar, R., Tomassen, M.M.M.,&van den Berg R.W. (2003).Combined High-Pressure and Thermal Treatmentsfor Processing of Tomato Puree: Evaluation of Microbial Inactivation and QualityParameters. Innovation Food Science Emerging Technology. **4**(4):377–85.
[11]. Lenz, C.A.,& Vogel, R.F. (2014). Effect of sporulation medium and its divalent cation content on the heat and high pressure resistance of Clostridium botulinum type E spores. Food Microbiology 44, 156-167.
[12]. Li, X., & Farid, M. (2016). A review on recent development in non-conventional food sterilization technologies. Journal of Food Engineering, 182(Supplement C), 33-45.
[13]. Lynch, D.J. &Potter, N.N. (1998). Effects of Acidification and Processing Variables on Thermal Inactivation of Bacillus Coagulans Spores in Meat Particulates. Journal of Food Protection, Vol. 52, No. 5, Pages 320-328
[14]. Mafart, P., Couvert, O., Gaillard, S., & Leguerinel, I. (2002). On calculating sterility in
[15]. thermal preservation methods: application of the Weibull frequency distribution
[16]. model. International Journal of Food Microbiology, 72(1e2), 107e113.
[17]. Margosch, D., Ehrmann, M.A., Gänzle, M.G., &Vogel, R.F. (2004). Comparison of pressure and heat resistance of Clostridium botulinum and other endospores in mashed carrots. Journal of Food Protection 67, 2530-2537.
[18]. Molin, N.&Snygg, B.G. (1967). Effect of lipid materials on heat resistance of bacterial spores.Applied Microbiology. 15(6):1422-6.
[19]. Matser A.M., Krebbers B., Berg R.W. & Bartels P.V. (2004). Advantages of high pressure sterilisation on quality of food products. Trends in Food Science and Technology 15(2): 79-85.
[20]. Olivier, S.A., Bull, M.K., Stone, G., van Diepenbeek, R.J., Kormelink, F., Jacops, L.& Chapman, B.(2011). Strong and consistently synergistic inactivation of spores of spoilage-associated Bacillus and Geobacillus spp. by highpressure and heat compared with inactivation by heat alone.Appliedand Environmental Microbiology. 77(7):2317-24
[21]. Paredes-Sabja, D., Gonzalez, M., Sarker, M.R. & Torres, J.A.(2007). Combined effects of hydrostatic pressure, temperature, and pH on the inactivation of spores of Clostridium perfringens type A and Clostridium sporogenes in buffer solutions.Journal Food Science 72(6):M202-6
[22]. Rajan, S., Ahn, J., Balasubramaniam, V.M. &Yousef, A.E. (2006) Combined pressure-thermal inactivation kinetics of Bacillus amyloliquefaciens spores in egg patty mince. Journal Food Protection. 69(4):853-60.
[23]. Ramaswamy, U.R., Kabel, M.A., Schols, H.A., Gruppen, H. (2013). Structural features and water holding capacities of pressed potato fibre polysaccharides. Carbohydrates Polymers. 2;93(2):589-96.
[24]. Ramirez, N. & Abel-Santos, E. (2010). Requirements for germination of Clostridium sordelliispores in vitro.Journal Bacteriology 192(2):418-25
[25]. Reineke, K., Mathys, A., Heinz, V., Knorr, D. (2013). Mechanisms of endospore inactivation under high pressure.Trends in Microbiology. 21(6):296-304
[26]. Solomon, E.B.&Hoover, D.G. (2004). Inactivation of Campylobacter jejuni by high hydrostatic pressure.Letters in Applied Microbiology 38(6):505-9.
[27]. Rovere, P., Gola, S., Maggi, A., Scaramuzza, N., & Miglioli L. (1998). Studies on
[28]. Bacterial Spores by Combined Pressure-Heat Treatments: Possibility to Sterilize Low-
[29]. Acid Foods, in: High Pressure Food Science, Bioscience and Chemistry, N.S. Isaacs
[30]. (ed.), The Royal Society of Chemistry, Cambridge, pp. 354–63.
[31]. Sale, A.J.H., Gould, G.W., & Hamilton W.A., 1970, Inactivation of Bacterial Spores by
[32]. High Hydrostatic Pressure, Journal General Microbiology**60**:323–334
[33]. Sevenich, R., Kleinstueck, E., Crews, C., Anderson, W., Pye, C., Riddellova, K., Hradecky, J., Moravcova, E, Reineke, K., &Knorr, D. (2014).High-pressure thermal sterilization: food safety and food quality of baby food puree.Journal Food Science.;79(2):M230-7.
[34]. Wang, B., B. Li, Q. Zeng, J. Huang, Z. Ruan, Z. Zhu, & L. Li. (2009). Inactivation kinetics and reduction of Bacillus coagulans spores by the combination of high pressure and moderate heat. JournalFood Process Engineering. 32:692–708.
[35]. Wang BS, Li BS, Du JZ, Zeng QX. (2015).Combined pressure-thermal inactivation effect on spores in lu-wei beef--a traditional Chinese meat product.Journal Applied Microbiology.;119(2):446-54.
[36]. Wimalaratne, S. K., & Farid, M. M. (2008). Pressure assisted thermal sterilization. Food and Bioproducts Processing, 86(4), 312-316.

Table 1. The main composition components (g/100 g) for the four prepared meals

Composition	Vegetables soup[1]	Green peas and ham[2]	Braised Veal[3]	Sole[4]
Fat	2.9	12.8	6.2	8.6
Carbohydrates	5.9	5.1	6.7	5.7
Fiber	1.3	3.9	1.5	1.7
Protein	1.2	5.1	7.6	10.2
Salt	1	1.1	3.69	2.95
pH	5,82	5.87	6.53	6.75
a_w	0.96	0.98	0.98	0.98

The man ingredient of the four prepared foods were
[1] Peas, potatoes, onion, courgette, olive oil, salt
[2] Peas, carrots, ham, olive oil, salt
[3] Veal
[4] Sea perch fish, bokchoi, mushrooms, parsnip, mussel gravy, beet root, cream, vegetable oil, sugar, yoghurt, shallots, butter, salt, potato flakes, leek, carrot, rib celery, fennel, lemon/lime juice, bread crumbs, aroma, vinegar, herbs, spices

Table2. Inactivation kinetics from Weibul modelling approach for B. amyloliquefaciensandC. sporogenesin four different types of prepared foods following exposure to thermal treatment or high pressure thermal processing (HPT).

Processing conditions	Prepared Meal	B. amyloliquefaciens				C. sporogenes			
		D value (min)	β (Shape factor)	RMSE	R^2	D value (min)	β (Shape factor)	RMSE	R^2
110 °C	Vegetable soup	3.015	0.607	0.354	0.96	2.658	0.514	0.3	0.961
	Steamed sole	5.314	0.603	0.262	0.966	3.21	0.487	0.331	0.941
	Green peas ham	4.848	0.62	0.409	0.925	4.777	0.66	0.377	0.945
	Braised veal	6.434	0.603	0.209	0.975	1.513	0.412	0.461	0.930
115 °C	Vegetable soup	0.167	0.332	0.295	0.971	0.737	0.356	0.38	0.950
	Steamed sole	0.152	0.249	0.622	0.876	0.747	0.369	0.427	0.926
	Green peas ham	0.139	0.298	0.415	0.958	1.472	0.441	0.41	0.949
	Braised veal	0.012	0.189	0.459	0.927	0.092	0.247	0.351	0.955
600MPa at 110 °C	Vegetable soup	0.088	0.365	0.423	0.945	<1 sec	0.105	0.131	0.991
	Steamed sole	0.008	0.314	0.618	0.956	<1 sec	0.133	0.274	0.97
	Green peas ham	0.029	0.254	0.577	0.881	0.101	0.321	0.725	0.833
	Braised veal	0.209	0.449	0.411	0.947	0.088	0.418	0.596	0.913
600 MPa at 115 °C	Vegetable soup	0.004	0.252	0.764	0.905	<1 sec	0.108	0.438	0.925
	Steamed sole	0.008	0.294	1.082	0.881	0.036	0.243	1.318	0.705
	Green peas ham	0.074	0.522	0.13	0.999	0.056	0.392	1.578	0.716
	Braised veal	0.11	0.475	0.475	0.978	0.051	0.299	1.18	0.702

FIGURE LEGENDS

Figure1. Effect of thermal and high pressure processing in combination with temperature for inactivation of B. amyloliquefaciens spores in (a) Vegetable soup, (b) Steamed sole, (c) Braised veal and (d) Green peas ham.
Figure2. Effect of thermal and high pressure processing in combination with temperature for inactivation of C. sporogenes sporesin (a) Vegetable soup, (b) Steamed sole, (c) Braised veal and (d) Green peas ham
Figure 3. Comparison of 4 D values for (a) B. amyloliquefaciens and (b) C. sporogenes spores inprocessed meals using heat alone (█) and high pressure processing (600MPa) in combination with heat (█).

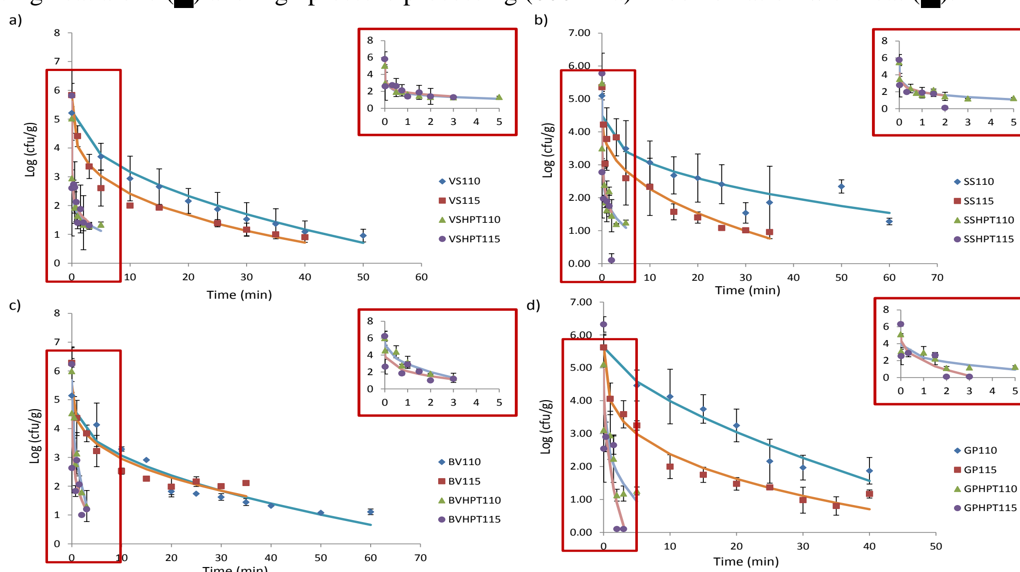

Fig 1

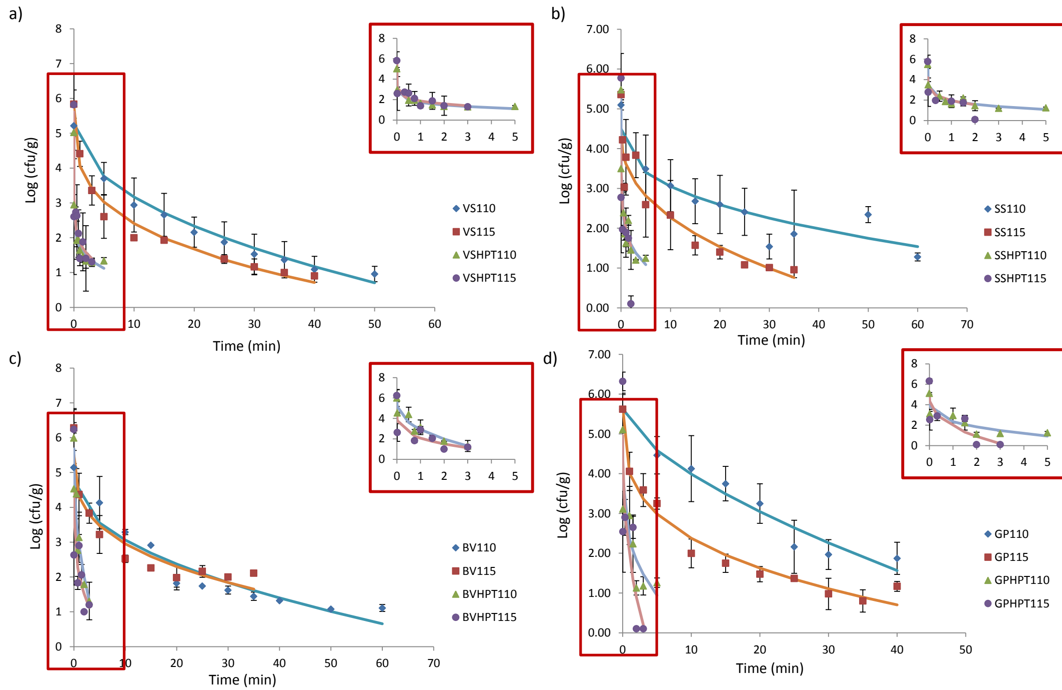

Fig 2

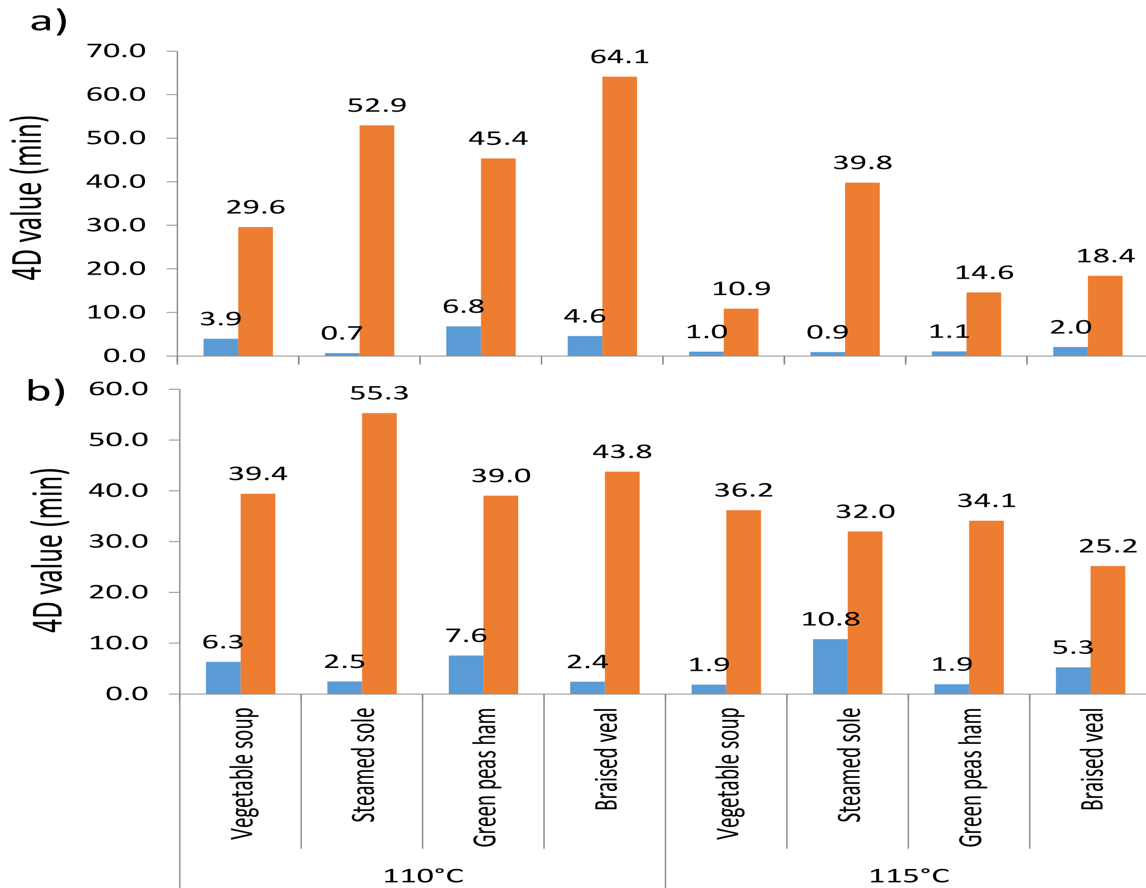

Fig 3

Appendix 1. Correlation analysis of microbial inactivation data of thermally processed ready meals with food composition analysis

	Temp (°C)	Dvalue (min) (BA)	β (Shape factor) (BA)	Dvalue (min) (CS)	β (Shape factor) (CS)	Moisture (%)	DM (%)	Fat (%)	CHO (%)	Protein (%)	Salt (%)	pH	aw
Temp (°C)	1.00	**-0.94***	**-0.98***	**-0.78***	**-0.72***	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Dvalue (min) (BA)		1.00	**0.92***	**0.68***	**0.61***	-0.20	0.20	0.00	-0.18	0.20	0.12	0.17	0.21
β (Shape factor) (BA)			1.00	**0.82***	**0.78***	0.10	-0.10	0.07	0.08	-0.13	-0.14	-0.14	-0.11
Dvalue (min) (CS)				1.00	**0.96***	-0.08	0.08	0.52	-0.18	-0.14	-0.55	-0.27	0.08
β (Shape factor) (CS)					1.00	0.01	-0.01	0.60	-0.12	-0.26	-0.67	-0.41	0.00
Moisture (%)						1.00	-1.00	-0.32	0.98	-0.90	-0.15	-0.71	-0.98
DM (%)							1.00	0.32	-0.98	0.90	0.15	0.71	0.98
Fat (%)								1.00	-0.51	-0.12	-0.83	-0.42	0.40
CHO (%)									1.00	-0.79	0.04	-0.55	-0.98
Protein (%)										1.00	0.50	0.95	0.82
Salt (%)											1.00	0.69	0.12
pH												1.00	0.60
aw													1.00

BA: B. amyloliquefaciens; CS: C. sporogenes; * significant at $P<0.05$

Appendix 2. Correlation analysis of microbial inactivation data of HPP processed ready meals with food composition analysis

	Temp (°C)	Dvalue (min) (BA)	β (Shape factor) (BA)	Dvalue (min) (CS)	β (Shape factor) (CS)	Moisture (%)	DM (%)	Fat (%)	CHO (%)	Protein (%)	Salt (%)	pH	aw
Temp (°C)	1.00	-0.26	0.21	-0.15	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Dvalue (min) (BA)		1.00	0.70	0.47	0.60	-0.05	0.05	-0.25	-0.01	0.09	0.54	0.11	0.18
β (Shape factor) (BA)			1.00	0.27	0.61	-0.22	0.22	0.05	-0.23	0.15	0.26	0.08	0.34
Dvalue (min) (CS)				1.00	0.89	-0.49	0.49	0.51	-0.57	0.22	-0.06	0.00	0.64
β (Shape factor) (CS)					1.00	-0.57	0.57	0.45	-0.63	0.33	0.04	0.11	0.72
Moisture (%)						1.00	-1.00	-0.32	0.98	-0.90	-0.15	-0.71	-0.98
DM (%)							1.00	0.32	-0.98	0.90	0.15	0.71	0.98
Fat (%)								1.00	-0.51	-0.12	-0.83	-0.42	0.40
CHO (%)									1.00	-0.79	0.04	-0.55	-0.98
Protein (%)										1.00	0.50	0.95	0.82
Salt (%)											1.00	0.69	0.12
pH												1.00	0.60
aw													1.00

BA: B. amyloliquefaciens; CS: C. sporogenes; * significant at $P<0.05$

Rachna Pandey" High pressure thermal processing for inactivation of Bacillus amyloliquefaciens and Clostridium sporogenes spores in a range of low acid commercial preparedfoods" The International Journal of Engineering and Science (IJES), 8.8 (2019): 01-09