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

Key external stakeholders:
Prepared consumer foods sector, regulators and policy
Practical implications for stakeholders:

Bacterial spores (*Bacillus* and *Clostridium*) are common contaminants of food products and their germination and subsequent outgrowth may cause food spoilage or foodborne illness. High Pressure Temperature Processing (HPT) is an emerging technology which involves the use of pressures in the range of 600 MPa or greater at temperatures of 90°C to 130°C and can inactivate spores. HPT thus has potential for use in commercial food processing to obtain safe high quality food products with extended shelf life. This study compared HPT with traditional thermal processing for inactivation of *B. amyloliquefaciens* and *C. sporogenes* spores in four different prepared meals. The results showed that HPT (110 or 115 ºC in combination with high pressure 600 MP) reduced populations of *B. amyloliquefaciens* and *C. sporogenes* spores by 4 -5 Log in prepared foods in significantly shorter process times than thermal (100 or 115 ºC) alone. The study provides data to design process windows for application of high pressure thermal treatments.

Main results:

- Spores (10^7 CFU/g) were inoculated into four commercial low acid food products (vegetable soup, pea with ham and carrot, veal and steamed sole. Thermal treatment at 110 ºC showed the D value (1 log reduction at a defined temperature) for *C. sporogenes* ranged from 1.51 to 4.78 min depending on the food matrix while at the same temperature *B. amyloliquefaciens* was more resistant with D values ranging between 3.01 to 6.43 min again varying with food matrix. At 115 ºC for both spores the D value ranged between 0.01 min and 1.47 min.

- When HPT (high pressure, 600MPa combined with thermal (110 ºC) was applied to the four inoculated foods, the D value for *B. amyloliquefaciens* was significantly reduced (0.03 to 0.21 min) depending on the food matrice and further reduced with an increase in temperature to 115 º C (0.004 to 0.11 min).

- When thermal treatment alone was applied, the length of heat time to achieve a 4D reduction in levels of spores in the four foods 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 matrice. When the combined high pressure thermal treatment at 600 MPA, 110 ºC the 4D treatment time was reduced to 0.7 to 6.8 min for *B. amyloliquefaciens* or 2.4 to 7.6 min for *C. sporogenes*.

Opportunity / Benefit:
The results obtained in this study show that HPT (600 MPa at 110 or 115 ºC) could 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.

Collaborating Institutions: Centro Nacional de Tecnología y Seguridad Alimentaria (CNTA)

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1. Project background

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 spores are particularly resistant to treatments commonly used in food processing with high temperature processing (90 to 121 ºC) the primary method of spore inactivation; however this 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 no inactivation 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. High Pressure Temperature Processing (HPT) is an emerging technology which involves the use of pressures in the range of 600 MPa or greater at temperatures of 90°C to 130°C. Both the high pressure and heat contribute to the process's microbial and spore lethality and particular combinations of these two parameters can inactivate spores of concern to food safety and spoilage. 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. HPT thus has potential for use in commercial food processing to obtain safe high quality food products with extended shelf life.

2. Questions addressed by the project:

What is the the impact of high pressure and high temperature processing (600MpA at 110 or 115 ◦C) compared with traditional high temperatures (110 and 115 ◦C) alone for inactivation of *B. amyloliquefaciens* DSM 7 and *C. sporogenes DSM 767* spores in a range of commercially relevant prepared meals (vegetable soup, pea with ham and carrot, veal and steamed sole) and to build a data set to support development of process window for food treatments with HPT.

3. The experimental studies:

- Spores (10^7 CFU/g) were inoculated into four commercial low acid food products food products (vegetable soup, pea with ham and carrot, veal and steamed sole)
- The inoculated foods were 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.

Main results:

- Spores (10^7 CFU/g) were inoculated into four commercial low acid food products (vegetable soup, pea with ham and carrot, veal and steamed sole. Following both thermal and treatments, survivor curves were non–linear with tailing populations and inactivation kinetics and D values were calculated using a Weibull modelling approach.
- Thermal treatment at 110 ºC showed the D value (1 log reduction at a defined temperature) for *C. sporogenes* ranged from 1.51 to 4.78 min depending on the food matrix while at the same temperature *B. amyloliquefaciens* was more resistant with D values ranging between 3.01 to 6.43 min again varying with food matrix. At 115 ºC for both spores the D value ranged between 0.01 min and 1.47 min.
- When HPT (high pressure, 600MPa combined with thermal (110 ºC) was applied to the four inoculated foods, the D value for *B. amyloliquefaciens* was significantly reduced (0.03 to 0.21 min) depending on the food matrice and further reduced with an increase in temperature to 115 º C (0.004 to 0.11 min).
- When thermal treatment alone was applied, the length of heat time to achieve a 4D reduction in levels of spores in the four foods 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 matrice. When the combined high pressure thermal treatment at 600 MPA, 110 ºC the 4D treatment time was reduced to 0.7 to 6.8

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min for *B. amyloliquefaciens* or 2.4 to 7.6 min for *C. sporogenes*.

4. Opportunity/Benefit:

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.

5. Dissemination:

Main publications:

Pandey, R., Ramos, S., Tiwari, B., Garcia De la Torre, S and Duffy G. (2018). High pressure thermal processing for inactivation of *Bacillus amyloliquefaciens and Clostridium sporogenes* spores in a range of low acid commercial prepared foods. *Emerging and Innovative Technologies.*

Duffy, G., Pandey, R and Tiwari, B. (2017). High Pressure High Temperature (HPT) Processing for inactivation of spores in ready to eat meals. Industry Workshop on High Pressure Processing to Control Pathogens in Ready-to-eat-Traditional cooked meat products with reduced-sodium, lower preservatives and no artificial colours or flavours (FIRM 11/F/031). UCC, June 23rd 2017.

Duffy, G., Pandey, R and Tiwari, B. (2017). Regulatory challenges in application of high pressure temperature processing (HPT) to foods. Food Industry workshop, CEMITEC, Noáin, Pamplona, Spain, November 17th 2017.

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