COMBAT RATION NETWORK FOR TECHNOLOGY IMPLEMENTATION (CORANET II)

SHORT TERM PROJECT (STP) #2015

SHELF STABLE EGG-BASED PRODUCTS PROCESSED BY ULTRA HIGH PRESSURE TECHNOLOGY

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14. ABSTRACT

The U.S. Army and Marine Corps are in search of more satisfactory shelf stable, egg-based breakfast entrées for group or individual military rations. The provision of combat ration egg products with a fresh, familiar home-style taste, especially as breakfast food, has been a significant challenge for the military, as commercial retort processing typically results in foods with undesirable flavors, greenish-grey discoloration, and detrimental changes in texture and syneresis. In this project, High Pressure Thermal Sterilization (HP/TS), also known as pressure assisted thermal processing (PATP), is presented as an alternative to commercial sterilization techniques, as a promising approach to providing commercially sterile precooked egg products that can be stored at room temperature. The team focused on identifying the effect of HP/TS conditions on commercial and modified egg patty formulations. Two processing strategies were initially proposed for egg-based product commercial sterilization: a) to facilitate FDA approval, a standard thermal sterilization treatment at process temperature 121℃ accelerated by the application of 700MPa with holding time 3 min was evaluated; b) thermal HP/TS treatment using lower temperature than in a) with increased holding time. Furthermore, inactivation studies, incubation tests, packaging identification, and compression heating studies gave a deeper understanding of the process.

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TABLE OF CONTENTS

Project Participants **4**

Publications **6**

Awards **7**

1. BACKGROUND, OBJECTIVE, AND SCOPE 8
2. PRODUCT AND PROCESS DEVELOPMENT 8
2.1. FORMULATION SELECTION 8
2.2. PREPROCESSING 9
2.3. VACUUM PACKAGING LEVEL AND PATTY FORMING METHOD (INITIAL POROSITY) 10
2.4. PREHEATING 12
2.5. HIGH PRESSURE EQUIPMENT UTILIZED IN THIS STUDY 17
2.6. COMPRESSION HEATING CHARACTERISTICS 19
3. PACKAGING INTEGRITY 19
4. QUALITY ASSESSMENT 21
4.1. EFFECT OF PREHEATING TEMPERATURE ON EGG PATTIES' QUALITY 23
Color and appearance of patty formulation #1 24
Texture and syneresis of patty formulation #1 25
Flavor of patty formulation #1 27
4.2. EFFECT OF PRESSURE LEVEL ON EGG PATTIES' QUALITY 28
Color of patty formulation #1 29
Texture and weight loss of patty formulation #1 29
4.3. FORMULATION MODIFICATIONS 30
4.3.1. ADDITION OF XANTHAN GUM, EDTA AND NATURAL AND ARTIFICIAL FLAVORS *30*
Improved appearance and color 31
Improved texture, water retention, and mouthfeel 32
Improved flavor 35
4.3.2. ADDITION OF CHEDDAR CHEESE *36*
Overall acceptability 37
Appearance 38
Aroma and Flavor 38
Texture and mouthfeel 39
4.3.3. Diced egg patties, addition of annatto and carotenoids and liquid egg formulations *41*
Sensory evaluation at Natick 42
4.4. HP/TS VS. IN-POUCH RETORT TREATMENT 43
Overall acceptability 45
Appearance 47
Aroma and flavor 47
Texture and mouthfeel 48
5. MICROBIAL CHALLENGE STUDIES 49
5.1. INACTIVATION OF BACILLUS STEAROTHERMOPHILUS SPORES IN EGG PATTIES BY PRESSURE ASSISTED THERMAL PROCESSING 50
5.1.1. Inactivation of B. stearothermophilus spores *56*
5.1.2. Influence of egg matrix on B. stearothermophilus inactivation *62*
5.1.3. Modeling of inactivation curves *62*
5.2. INACTIVATION OF BACILLUS AMYLOLIQUEFACIENS SPORES IN EGG PATTY MINCE BY PRESSURE ASSISTED THERMAL PROCESSING 63
5.2.1. Inactivation of B. amyloliquefaciens spores *70*
5.2.2. Spore resistance during thermal processing or PATP *74*
5.2.3. Pressure and Thermal coefficients of B. amyloliquefaciens spores *75*
5.2.4. Influence of pressure and temperature on inactivation parameters of the Weibull model *76*

5.3. INCUBATION STUDIES AND SHELF LIFE TESTS 77
6. CONCLUSIONS.....80

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Publications

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Awards

Wannasawat Ratphitagsanti won "Developing Scientist" award during 2007 Annual meeting of International Association for Food Protection (IAFP) held at Lake Buena Vista, FL. She was awarded for her poster presentation: Influence of Pressurization Rate and Pressure Pulsing on Inactivation of Bacillus amyloliquefaciens Spores during Pressure-assisted Thermal Processing. Authors: Wannasawat Ratphitagsanti, J. Ahn, A.E. Yousef, and V.M. Balasubramaniam

Pablo Juliano of Washington State University received the Marvin "Byer Scholarship" Award in 2005 from Research and Development Associates (San Antonio, Texas) for his outstanding research and development of a meal-ready-to-eat (MRE) breakfast egg entrée for military foods.

CORANET STP #2015 FINAL REPORT

1. BACKGROUND, OBJECTIVE, AND SCOPE

The U.S. Army and Marine Corps are in search of more satisfactory shelf stable, egg-based breakfast entrées for group or individual military rations. The provision of combat ration egg products with a fresh, familiar home-style taste, especially as breakfast food**,** has been a significant challenge for the military, as commercial retort processing typically results in foods with undesirable flavors, greenish-grey discoloration, and detrimental changes in texture and syneresis. In this project, High Pressure Thermal Sterilization (HP/TS), also known as pressure assisted thermal processing (PATP), is presented as an alternative to commercial sterilization techniques, as a promising approach to providing commercially sterile precooked egg products that can be stored at room temperature.

The team focused on identifying the effect of HP/TS conditions on commercial and modified egg patty formulations. Two processing strategies were initially proposed for egg-based product commercial sterilization: a) to facilitate FDA approval, a standard thermal sterilization treatment at process temperature 121°C accelerated by the application of 700MPa with holding time 3 min was evaluated; b) thermal HP/TS treatment using lower temperature than in a) with increased holding time. Furthermore, inactivation studies, incubation tests, packaging identification, and compression heating studies gave a deeper understanding of the process.
Results of these studies are highlighted in the following sections.

2. PRODUCT AND PROCESS DEVELOPMENT

2.1. FORMULATION SELECTION

Michael Foods Egg Products Company (Gaylord, MN, USA) provided four types of commercial scrambled egg patties of 42.5 ± 7.1 g. Patties #1, #2 and #3 were round (88.9 ± 6.4 mm diameter)

and patty #4 was square (69.9 ± 6.4 mm x 76.2 ± 6.4 mm). Patty #1 (see table 1), the standard Michael Foods patty (code 46025-30020-00), had the following basic ingredients: whole eggs, water, soybean oil, modified food starch, whey solids, salt, nonfat dried milk, and citric acid. Patties #2 and #4 (code 03-1426-10) also had #1 patty basic ingredients, but natural and artificial flavors, xanthan gum, and EDTA were added. Patty #3 (code 46025-70019-00) had the same ingredients as #1 patty, plus 20% of pasteurized process Cheddar cheese granules.

Table 1. Characteristics of precooked scrambled egg patties

Sample code	Formulation	Dimensions (mm)
#1 46025-30020-00	Basic ingredients: whole eggs, water, soybean oil, modified food starch, whey solids, salt, nonfat dried milk, and citric acid	Round 88.9 dia
#2 03-1426-10	Basic ingredients + xanthan gum Added ingredients: natural and artificial flavors, xanthan gum, EDTA	Round 88.9 dia
#3 46025-70019-00	Basic ingredients + cheese Added ingredients: 20% pasteurized Cheddar process cheese granules	Round 82.6 dia
#4 03-1426-10	Basic ingredients + xanthan gum Added ingredients: natural and artificial flavors, xanthan gum, EDTA	square (69.9 × 76.2).
#5	Basic ingredients + cheese + annatto + carotenoids	Round 82.6 dia

2.2. PREPROCESSING

Preparation of precooked scrambled egg products has been reported in different patents developed by Michael Foods (Knipper and Beam, 2002; Merkle et al., 2003a,b). Whole eggs were mixed with dry and liquid ingredients; the mixture was then pumped into a mold on a flat cooking belt. Egg mixture portions were cooked (or formed) in a convection oven at 180-250ºC for a predetermined time, then frozen and packaged. Handling and shipping procedure for scrambled egg patties was performed according to industrial setting, where patties were stored in a frozen state before HPP treatment. Frozen samples from a single lot were received from Michael Foods and stored frozen at -30ºC.

2.3. VACUUM PACKAGING LEVEL AND PATTY FORMING METHOD (INITIAL POROSITY)

Other than formulation modification, the improvement of texture and water retention at levels comparable to controls was reached by modifying selected pretreatment steps before HP/TS treatment. Three vacuum packaging levels were used in addition to two forming methods for round and square patty shapes.

The effect of vacuum packaging level and patty forming method on TPA hardness and percentage of water loss are shown in table 2 and figure 1, respectively.

Table 2. TPA values of preheated (control) and HP/TS treated egg patties with 3 vacuum packaging levels and formulations # 2 and # 4.*

Patty #	Vacuum level	Hardness (N)	Cohesiveness (10^2)	Adhesiveness (N. mm, 10^1)	Springiness (10^2)	Resilience (10^2)
2	Control	10.8 ± 2.7 Aa	52.9 ± 0.9 Aa	-1.99 ± 0.82 Aa	91.3 ± 0.3 Aa	19.7 ± 0.2 Aa
2	HP/TS no vacuum	33.0 ± 1.9 Ba	73.5 ± 0.6 Ba	-3.24 ± 0.58 Aa	90.6 ± 0.2 Aa	33.6 ± 0.2 Ba
2	HP/TS 400 mbar	35.5 ± 1.9 BCa	73.0 ± 0.6 Ba	-4.63 ± 0.58 Aa	88.6 ± 0.2 Aa	28.9 ± 0.2 Ba
2	HP/TS 10 mbar	40.7 ± 1.9 Ca	70.8 ± 0.6 Ca	-5.00 ± 0.58 Aa	87.1 ± 0.2 Aa	28.9 ± 0.2 Ba
4	Control	15.9 ± 2.7 Aa	70.7 ± 0.9 Ab	-4.58 ± 0.82 Aa	94.5 ± 0.3 Ab	30.5 ± 0.2 Ab
4	HP/TS no vacuum	21.6 ± 1.8 ABb	76.2 ± 0.6 Bb	-3.44 ± 0.54 Aa	96.9 ± 0.2 Ab	32.9 ± 0.1 Aa
4	HP/TS 400 mbar	27.4 ± 2.1 Bb	75.3 ± 0.7 Bb	-3.59 ± 0.64 Aa	96.7 ± 0.2 Ab	31.4 ± 0.2 Aa
4	HP/TS 10 mbar	26.4 ± 1.9 Bb	77.1 ± 0.6 Bb	-3.56 ± 0.58 Aa	93.2 ± 0.2 Ab	35.7 ± 0.2 Ab

*Different letters indicate significant differences ($p < 0.05$) between vacuum levels (capital letters) and patty porosity (lowercase).

HP/TS treated square patty #4 showed significantly lower hardness (26 to 35%) than HP/TS-treated round patty #2 at each vacuum level condition. During egg patty production, two different systems deposited the liquid egg mixture at different levels of injected air into the molds, yielding patty #2 with clearly visible and homogeneous pores and patty #4 with a gel-like

structure. One of the effects of high pressure processing is the displacement of air bubbles trapped in a solid matrix. After HP/TS treatment, patty #2 had higher air loss, which allowed for pore collapse and the formation of a denser structure. Because patty #4 had smaller pores than patty #2, there was less air removal and subsequent structural modification. This decreased the extent of gelation during pressurization and provided hardness values closer to the controls. These results for #4 patties were supported by the 29% lower water loss values observed at no or intermediate vacuum packaging conditions.

For patty #2, hardness was increased by 7 and 19% with vacuum packaging levels at 400 and 10 mbar, respectively, compared with no vacuum conditions. This can be explained as an additive compression effect during hydrostatic pressurization, in which vacuum and hydrostatic pressures add up and enhance the contact between proteins, resulting in greater gelation and greater hardness.

Preliminary studies showed that vacuum packaging did not affect the hardness after preheating, showing recovery of the structure after vacuum packaging for a short time. However, high vacuum packaging had a negative effect on texture after HP/TS treatment as shown by an increase in hardness.

Hardness in square patty #4 at no vacuum conditions did not differ from the control HP/TS. Furthermore, no significant vacuum level effect was seen after HP/TS treatment for patty #4. In this case, hardness of a more compact and less porous egg matrix like #4 might be less affected by the combination of pressure and high temperature than #2.

Vacuum packaging and patty performing method effects on hardness presented significant interactions ($P<0.05$). However, no vacuum packaging and patty performing method effects on adhesiveness were found before and after HP/TS. Springiness was only significantly affected by patty performing method, not by vacuum packaging and HP/TS processing. Patty #4 was more elastic than patty # 2 before and after HP/TS treatment. This difference in springiness can be directly related to the egg patty porosity, where patty #2 had more pores than patty #4 within the structure.

Syneresis in egg patties #2 and # 4 increased significantly after HP/TS treatment with respect to controls (figure 1). Patty #4 had the highest weight lost percentage at the highest vacuum condition, 10 mbar, which was 35% higher than low vacuum and HP/TS treated patty #4, whereas no influence of the initial vacuum condition was seen for patty #2.

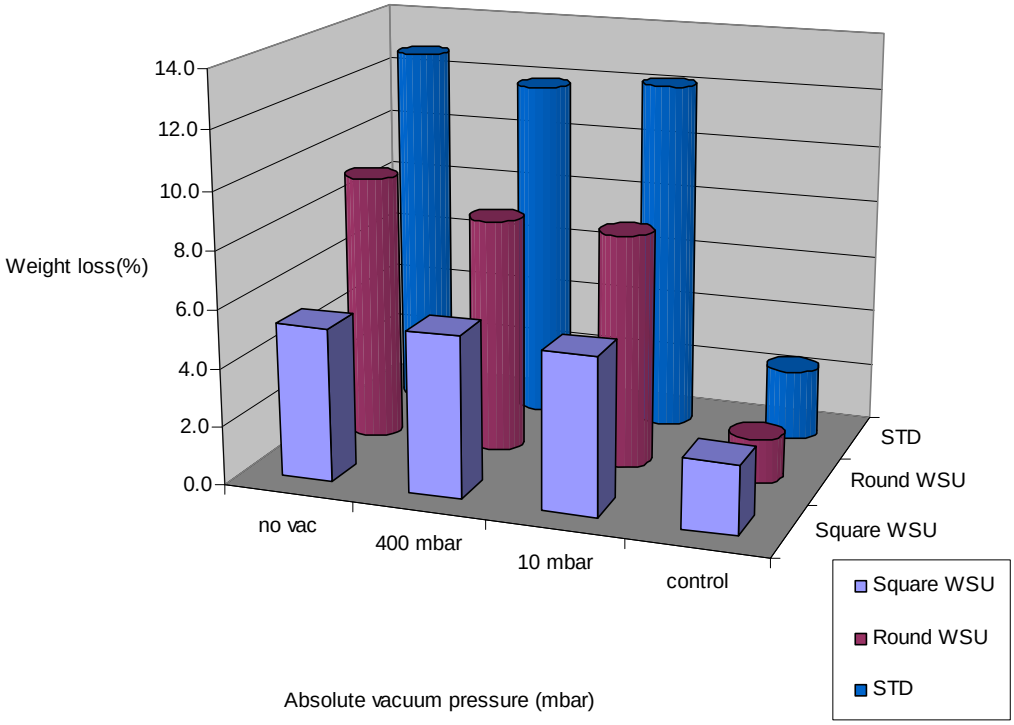

Figure 1. Percentage of weight loss in preheated (control) and HP/TS treated egg patties for formulations #2 (round) and #4 (square) as influenced by vacuum packaging level.

2.4. PREHEATING

During HPTS treatment, the process of preheating is extremely important to ensure that the initial temperature throughout the food samples matches (or is higher than) the initial pressure system temperature. Two heating systems at two respective temperatures were tested to evaluate the heat penetration rate and its consequence on texture and syneresis.

Preheating studies were conducted with water baths in a tilting steam kettle (DLT-40-1EG Groen, DI Food Service Companies, Jackson, MS, USA) at 80 and 90°C, using conventional pilot scale retort (design RDSW; Lee Metal Products Co., Philipsburg, PA, USA) at 90°C and

boiling temperature of 98°C (corresponding to 715 m above sea level). The water in the retort was injected with steam at approximately 207 kPa and air pressure at approximately 138 kPa. Temperature of the patties was measured using a thermocouple (T-type, Omega Engineering Inc., Stamford, CT, USA) fixed in place with a stuffing box (Ecklund Harrison Tech., Fort Meyer, FL, USA) at the center of the egg patties (figure 2). For this experiment, the initial temperature of the patties was 20°C and preheating times were measured until temperature of the egg patties reached 80°C. Heat penetration was evaluated by finding the heating rate index f_h and heating lag factor j_h according to Holdsworth (1997). Factors were calculated using the temperature of the heating medium as the reference temperature.

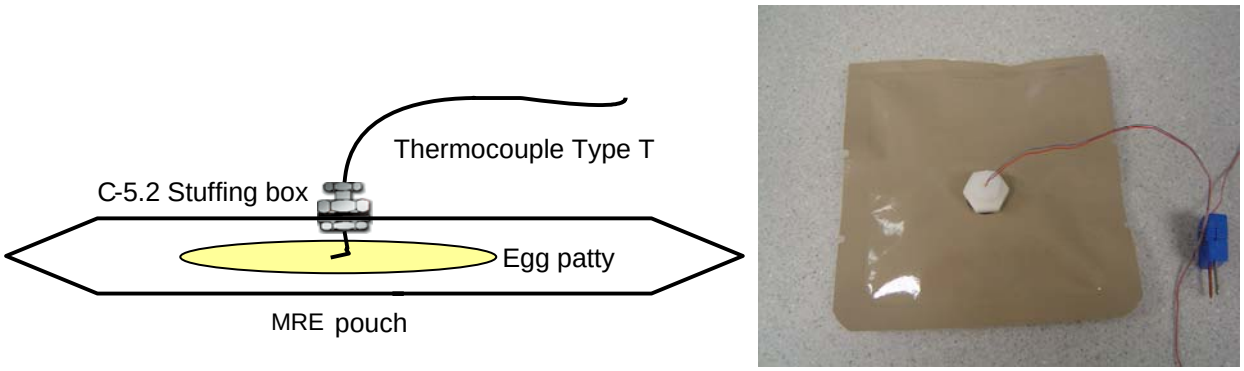

Figure 2: Flexible pouch with attached thermocouple.

Figure 3 shows the temperature profiles inside patty #1 obtained using each preheating method.

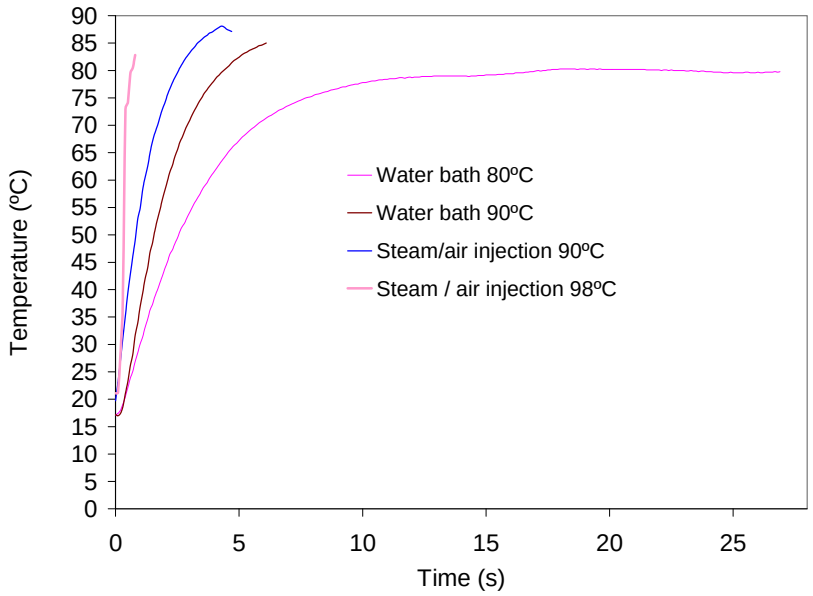

Figure 3: Typical preheating curves obtained using each tested condition for patty #1.

Table 3 shows the heating rate index f_h and heating lag factor j_h determined using equations (1) and (2) for the tested preheating conditions.

$$\log u = \log\left(\frac{T_R - T}{T_R - T_0}\right) = -\frac{t}{f_h} + \log j \qquad (1)$$

where u is the reduced temperature, T is the temperature at the geometric center of the package, T_0 is the initial product temperature, T_R is the reference temperature of the heating medium, and j is the extrapolated lag factor. The corrected heating lag factor j_h can be determined from 58% come up time (t_{58}), which corresponds to an additional 42% of come up time needed to reach the target temperature.

$$j_h = 10^{-\frac{t_{58}}{f_h}} + \log j \qquad (2)$$

Table 3. Heating rate index f_h and heating lag factor j_h of tested preheating conditions.*

Preheating method	f_h (min)	j_h (10^1, dimensionless)
Water bath at 80°C	5.86 ± 0.28 a	0.20 ± 0.30 a
Water bath at 90°C	4.00 ± 0.35 b	3.14 ± 0.34 b
Steam/air injection at 90°C	3.54 ± 0.30 b	3.44 ± 0.28 b
Steam/air injection at 98°C	2.35 ± 0.35 c	5.16 ± 0.31 c

*Different letters indicate significant differences ($P<0.05$) between treatments.

The heating rate index depends on the thermal properties, particularly on the thermal diffusivity, and dimensions of the food product being heated (Holdsworth, 1997). At the same time, thermal diffusivity is related to the thermal conductivity of the food, the specific heat, and the food density. At 98°C, the mixture of injected steam and air accelerated the convective rate of heat transfer to the packaging material and then to the food. The headspace inside the pouch, as well as the internal vapor pressure increased due to higher temperature, and also influenced the j_h value due to convective currents in contact with the egg patty surface. For this experiment, single packages were located separated in each carrier during preheating. However, the configuration of multiple pouches stacked together needs also to be considered during preheating because it will decrease the heating rate.

The lag factor j_h, related to lag time needed to reach uniform heating rate values, was significantly lower for the 80°C water bath and significantly higher at 98°C in comparison to other temperatures. Part of the lag at 80°C was due to slow temperature come up in the water.

The TPA descriptors hardness, adhesiveness, springiness, and resilience showed no significant differences between preheating methods before and after HPTS treatment. However, as shown in table 4, cohesiveness was increased with higher temperatures and by the injection of air and steam. Contrary to what was found for hardness and resilience, cohesiveness only increased due to HPTS processing after being preheated in an 80°C water bath. Thus, a faster preheating might retain the cohesive structure of the egg patty after HPTS treatment.

Table 4. Comparison of texture profile analysis (TPA) significant descriptors and syneresis after different preheating conditions in control and HP/TS-treated samples.**

Treatment	Preheating method*	Hardness (N, ±1.4)	Cohesiveness (dimensionless, 10^2, ±0.6)	Resilience (dimensionless, 10^2, ±1.0)	Weight loss (%, ±0.4)
Control	WB 80°C	26.5 Aa	65.4 Aa	23.7 Aa	2.6 Aa
Control	WB 90°C	22.7 Aa	68.1 Ab	25.7 Aa	2.0 Aa
Control	SAI 80°C	22.7 Aa	70.6 Ac	25.2 Aa	2.0 Aa
Control	SAI 90°C	24.2 Aa	70.4 Ac	26.0 Aa	2.7 Aa
HP/TS2	WB 80°C	49.0 Bb	70.7 Ba	31.3 Bb	12.3 Bb
HP/TS2	WB 90°C	44.2 Bb	69.7 Aa	30.7 Bb	12.6 Bb
HP/TS2	SAI 80°C	44.2 Bb	71.0 Aa	30.2 Bb	12.6 Bb
HP/TS2	SAI 90°C	45.4 Bb	69.6 Aa	29.7 Bb	12.2 Bb

*WB = water bath; SAI = steam/air injection

**Different letters indicate significant differences ($p < 0.05$) between process (capital letters) and preheating method (lowercase).

Even though the preheating conditions tested provided significantly different penetration rates, this was not reflected in textural changes after HPTS process. The same occurred with syneresis values expressed as % weight loss (table 4). Because different preheating rates provide similar texture parameter values (except for cohesiveness), as well as water retention before and after HPTS treatment, variation in preheating rates/periods to reach temperatures around 60 to 80°C will probably not affect the texture and water retention of patties of this size. This allows for the possibility of preheating in two stages (e.g., initial equilibration of egg patties at 60°C using water bath and then fast heating up to 80°C), which will decrease initial temperature gradients within the product, minimizing cold spots.

If the patties were of larger dimensions, the differences found in heating rate index j_h and preheating time for each tested condition would be more pronounced. The same would apply when a high number of pouches placed in the carriers are touching. Reduction of preheating time by means of a higher preheating rate for a larger scale egg patty (e.g., in an institutional type of pouch) would provide less exposure to heat, especially at the food's surface, and would possibly improved texture.

As seen before, higher hardness, resilience, and % weight loss were found after HPTS with respect to the preheated control. However, springiness and adhesiveness (data not shown) did not change significantly after HPTS.

The advantage of HPTS with respect to retort processing is that once the initial temperature is reached throughout the entire product, temperature increase during pressurization will be uniform within the whole volume. This benefit can also be used when scaling up the process for larger sized products. In this case, a media with larger heat transfer coefficient, such as water injected with steam/air mixtures can help make the process shorter by decreasing come up time, and can eventually yield products with improved texture and water retention. The heat transfer properties of the packaging material might also play a role in the heating rate of penetration and the lag factor.

2.5. HIGH PRESSURE EQUIPMENT UTILIZED IN THIS STUDY

Figures 4, 5 and 6 show the high pressure units available for the project at the different facilities

Figure 4: DUST 35L machine (QUINTUS Food Autoclave Type 35L-600 Avure Technologies, Kent, WA, USA) at National Center for Food Safety and Technology.

Figure 5. 1.5L (Quintus Food Processor-6, Flow Autoclave systems, Columbus, OH, USA) at The Ohio State University.

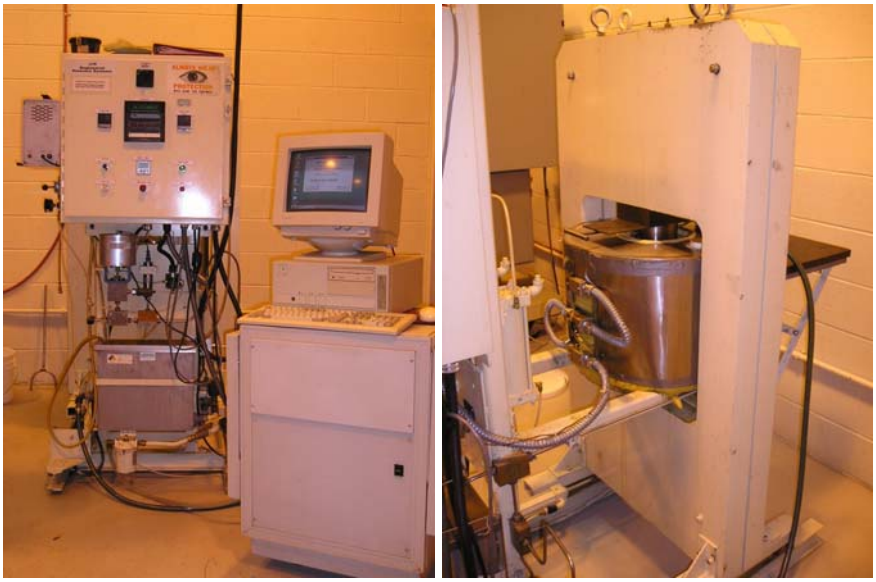

Figure 6. 1.7L (Engineered Pressure Systems, Inc., model #914-100, Haverhill, MA, USA) at Washington State University.

2.6. COMPRESSION HEATING CHARACTERISTICS

During high pressure processing the compression heating rates were determined to assure that the target temperature was reached. Several food components provide variable compression heating rates, however, among the existing precooked egg products, scrambled egg patties have been identified as an adequate product for high pressure processing because of their semisolid homogenous structure (Juliano et al., 2004; 2006).

Determination of compression heating properties of egg mixtures showed no significant differences with water. The compression heating factor of egg patties ranged from 3.3°C/100 MPa to 4.8°C/100 MPa at the initial process temperatures of 25 and 80°C, respectively (Juliano, 2006). Therefore, to reach 105 and 121°C during pressurization at 700 MPa the egg samples were preheated to 75 and 90°C, respectively (figure 7).

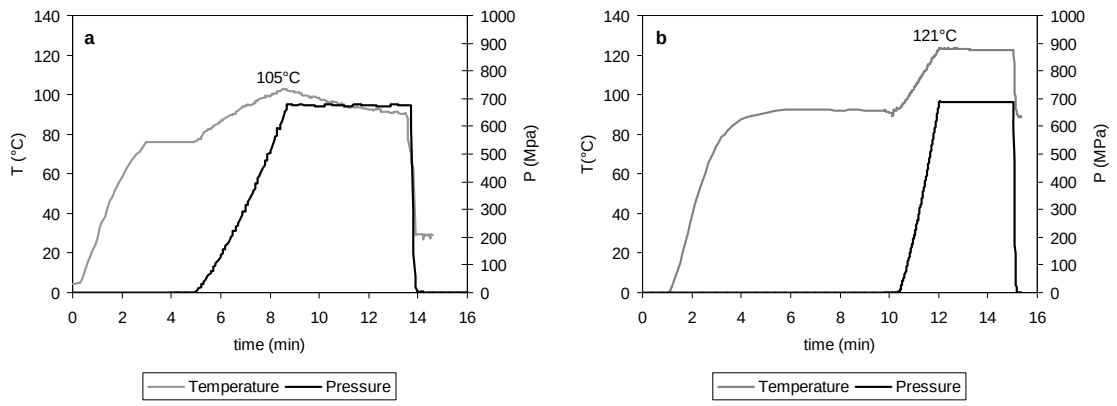

Figure 7. Typical temperature and pressure profiles during HP/TS processing in water/egg patty system at (a) 700MPa/105°C/5min and (b) 700 MPa/121°C/3min.

3. PACKAGING INTEGRITY

Shelf stability of the egg-based products will not only depend on the HP/TS treatment applied but also on the barrier provided by the selected packaging material. Packaging materials selected for shelf stable egg products must meet a number of requirements in terms of overall integrity,

and barrier properties to oxygen and water vapor. Seal strength is also a critical point in HP/TS processing of flexible food pouches.

Even though numerous barrier materials are used in the food industry, there are only a few that can be used for the HP/TS application. They include nylon, ethylene-vinyl alcohol (EVOH), polyethylene (PE), polyethylene terephthalate (PET), polypropylene (PP), cast polypropylene (CPP), aluminum oxide (AlOx) coating, and aluminum or metallized layer. Several multilayered films produced from those materials are commercially available.

Throughout the first phase, project partners worked with different packaging companies to identify suitable individual flexible pouches (clear and foil laminates). Selected plastic and foil laminated pouches from Pyramid, ALCAN, and Smurfit-Stone manufacturers (Schaumburg, IL) (figure 8) were screened for their ability to withstand HPTS and retort treatments. Overall packaging integrity, oxygen permeability (determined from a Mocon-Oxtran unit), and seal strength (determined from tensile tests using an Instron texture analyzer) were evaluated before and after treatments at NCFST facilities.

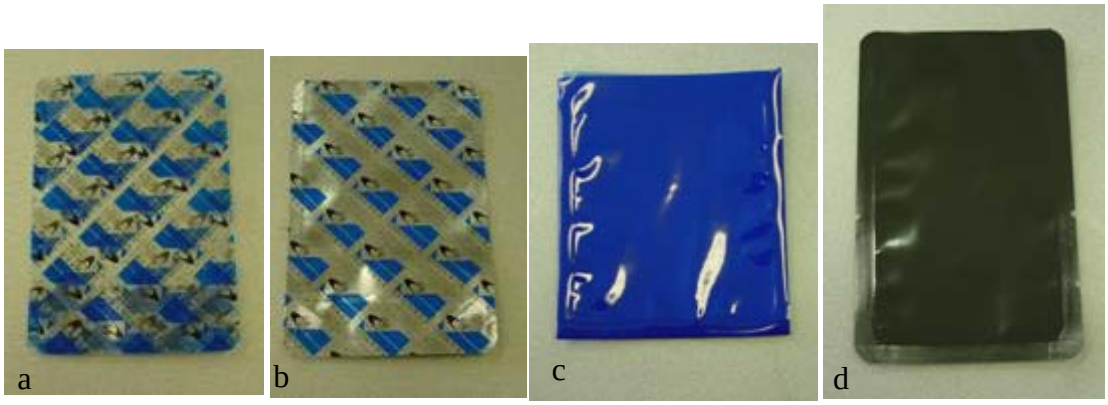

Figure 8.Selected packaging for HP/TS process. a) Pyramid plastic pouch. b) Pyramid foil laminated pouch. c) Alcan plastic pouch. d) MRE pouch Smurfit.

It was proven that foil laminates from Pyramid and Smurfit-stone (48 ga. Polyethylene/adhesive/0.0005″ Aluminum foil/adhesive/4 mL polyolefin) retained their barriers during steam injection preheating and HP/TS treatment, however, some sporadic blisters and delamination defects were found in several of both foil laminated pouches (figure 9). A

statistically significant loss of the barrier was found in clear plastic pouches from Pyramid without Aluminum foil. Seal strength of all packaging materials was not significantly affected by HP high temperature and HP low temperature treatments, while retort at 121°C decreased seal strength. Furthermore, WSU, in partnership with ALCAN Packaging, identified a pouch made of coextruded laminates that provided almost no blistering and low oxygen permeability after pressure under a worst case scenario condition (700MPa/121°C/3min). ALCAN packaging material composition consisted of 60 ga. Biaxial nylon/adhesive/5.0 mL ethylene vinyl alcohol (EVOH) coextruded sealant.

Figure 9. MRE pouch delamination.

4. QUALITY ASSESSMENT

Formulations and processing conditions were tested to gain understanding of their effect on the quality and acceptability of egg patties after HP/TS treatment. Table 5 shows the factorial design for the five experiments performed.

Table 5. Research design.

Experiments	Design	Levels
1. Effect of preheating temperature on egg patties' quality	2 x 4 x 1 factorial three replicates	[Control, HP/TS] × [30ºC, 50ºC, 70ºC, 90ºC] × [formulation #1]
2. Effect of pressure level on egg patties' quality	2 x 3 x 1 factorial two replicates	[Control, HP/TS] × [0.1 MPa, 500MPa, 675MPa] × [formulation #1]
3. Formulation modification by adding xanthan gum, EDTA and natural and artificial flavors.	2 × 2 factorial two replicates	[Control, HP/TS1] × [formulations #1, #2]
4. Formulation modification by adding pasteurized Cheddar cheese.	2 × 3 factorial two replicates	[Control, HP/TS1] × [formulations #1, #3 & #4]
5. HP/TS *vs.* in-pouch retort treatment	4 × 1 factorial: two replicates	[Control, HP/TS1, HP/TS2, Retort] × [formulation #4]

HP/TS1: 700 MPa/105°C/5 min
HP/TS2: 700 MPa/121°C/3 min

- **Effect of preheating temperature on egg patties' quality**

In the first experiment the standard commercial formulation #1 was processed at different initial chamber temperatures at pressure of 675 MPa for 5 min. The main purpose was to identify the effect of selected temperatures on the sensory attributes and physical characteristics of a standard commercial egg patty under high pressure conditions, which when combined with selected temperatures, could pasteurize (at low temperature) or sterilize (at high temperature) egg products.

- **Effect of pressure level on egg patties' quality**

The second experiment was performed in the same way using formulation #1 at initial product/liner/chamber temperature 90ºC for different pressures (table 5). This experiment aimed to show the effect of pressure levels, lower than 675 MPa, on the final physical characteristics of egg patties when combined with a high initial temperature of 90ºC.

- **Formulation modification by adding xanthan gum, EDTA and natural and artificial flavors**

Experiment 3 was designed to improve the overall quality obtained for the standard egg patty formulation #1 by adding to the basic ingredients, xanthan gum, EDTA and natural and artificial flavors.

- **Formulation modification by adding pasteurized Cheddar cheese**

Experiment 4 was designed to study the effects of HP/TS processing on consumer acceptability of formulations #1, #3 and #4 to evaluate if cheese addition improved the sensory evaluation of the scrambled egg patties.

- **HP/TS *vs.* in-pouch retort treatment**

Experiment 5 aimed to compare quality after processing by novel in-pouch retort and HP/TS at a standard temperature (121ºC) and lower pressurization temperature (105ºC).

4.1. EFFECT OF PREHEATING TEMPERATURE ON EGG PATTIES' QUALITY

Michael Foods Egg Products Company provided a round commercial scrambled egg patty (#1, code 46025-30020-00). Frozen samples from a single lot were stored frozen at -30ºC. Each patty was cut in half and repackaged in retort pouches (Smurfit-Stone) that measured 6.0 x 10.3 cm. The vacuum packaging machine was a tabletop vacuum chamber (KOCH 15-EasyPack™, Kansas City, MO) used at 400 mbar absolute pressure.

Commercial egg patties were processed at varied initial chamber temperatures and 675 MPa for 5 min. Egg patties were preheated using a water bath at boiling temperature (98ºC) up to preset temperatures. Comparisons were made with nonpressure treated controls preheated up to the same initial product temperatures. Samples were preheated in a boiling water bath for 2.5 ± 0.2 min to reach 30ºC, 3.4 ± 0.2 min to reach 50ºC, 6.5 ± 0.5 min to reach 70ºC, and 15 ± 1.0 min to reach 90ºC inside the half patty.

Table 6 shows the liner temperatures before, during pressurization, and after pressure release.

Table 6. Temperature profile at different temperature-pressure combinations inside liner.

Pressure	Initial (ºC)	In-Process Initial[a] (ºC)	Final[b] (ºC)
675 Mpa	30.1 ± 1.5	50.2 ± 1.9	27.6 ± 1.9
675 Mpa	51.5 ± 0.5	74.9 ± 1.5	47.4 ± 1.2
675 MPa	70.4 ± 3.2	98.4 ± 3.6	66.8 ± 2.1
675 MPa	91.0 ± 2.4	121.0 ± 3.5	83.3 ± 0.1

[a]Initial temperature at 675 MPa

[b]Temperature after pressure was released

Product analyses

This section describes the appearance, texture/mouthfeel, and flavor/aroma profiles of egg patties formulation #1 after HP/TS treatment at low and high temperature conditions.

Color and appearance of patty formulation #1

The descriptive panel did not detect significant differences ($p>0.05$) in gloss and green between #1 egg patties treated at 30ºC/675 MPa and the control. This coincided with lightness L* values and *Chroma* found with the colorimeter (table 7). Since egg patties were previously heat formed, it is not surprising that there were no significant differences ($p>0.05$) detected after pressure treatment.

When pressurized at 70ºC/675 MPa, the descriptive panel did not find differences in gloss with respect to the control, further supported by no significant ($p>0.05$) changes in L* value. However, the descriptive panel found slight, though significant ($p<0.05$), differences in green color when patties were treated at HP/TS conditions (>70ºC/675 MPa).

The #1 egg patties treated at 30ºC/675 MPa gave *Chroma* values not significantly different ($p>0.05$) from the control. *Chroma* values, however, were slightly but significantly decreased ($p<0.05$) at pressure chamber temperature 70ºC or higher (table 3). Changes in *Chroma* of egg

patty formulation #1 after treatment at HP/TS conditions (≥70ºC/675 MPa) agreed with the color ratings by the descriptive panel.

Table 7. Color and appearance of patty formulation #1 as indicated by colorimeter (L*, *chroma*) and descriptive panel.**

Treatment	Color (Analytical)		Color (Sensory)		Appearance (Sensory)	
	L*	*Chroma*	Gloss	Greenness	Surface homogeneity	Crumbliness
Control	77.3 ± 0.8 a	35.6 ± 0.9 a	4.5 ± 0.6 a	1.0 ± 0.4 a	3.5 ± 1.0 a	0.5 ± 0.1 a
30ºC/ 675 MPa	79.5 ± 0.9 a	34.2 ± 1.1 ab	5.2 ± 1.1 ab	1.4 ± 0.7 a	6.2 ± 2.0 a	0.1 ± 0.3 a
70ºC/ 675 MPa	80.9 ± 0.7 a	32.1± 0.9 b	7.0 ± 1.6 ab	3.4 ± 0.9 b	4.6 ± 2.2 a	0.0 ± 0.3 a
90ºC/ 675 MPa	78.7 ± 0.7 a	32.2 ± 0.9 b	8.8 ± 1.6 b	3.6 ± 0.8 b	6.7 ± 2.2 a	0.0 ± 0.3 a

**Different letters indicate significant differences exist between mean values ($p<0.05$) within individual columns.

Texture and syneresis of patty formulation #1

Texture profile of formulation #1 after treatment at 30ºC/675 MPa was similar to control, whereas #1 patties pressure treated at 70ºC and 90ºC rated significantly higher ($p<0.05$) in firmness, density, particle (particulates) size, and mouthfeel roughness (figure 10). TPA analysis (table 8) gave a similar profile comparing the high pressure low temperature treated patty and the control. Changes in hardness values were noticeable at initial chamber temperature of 50ºC and were significantly higher ($p<0.05$) above 70ºC and pressure of 675 MPa.

Even though egg proteins were already coagulated during the cooking process, high pressure conditions at initial temperatures above 50ºC might have induced proteins to further aggregate, providing a firmer structure.

Panelists did not find any difference ($p>0.05$) between the #1 control and the 90ºC/675 MPa sample in terms of astringency, greasy, pasty and mouth coating (data not shown).

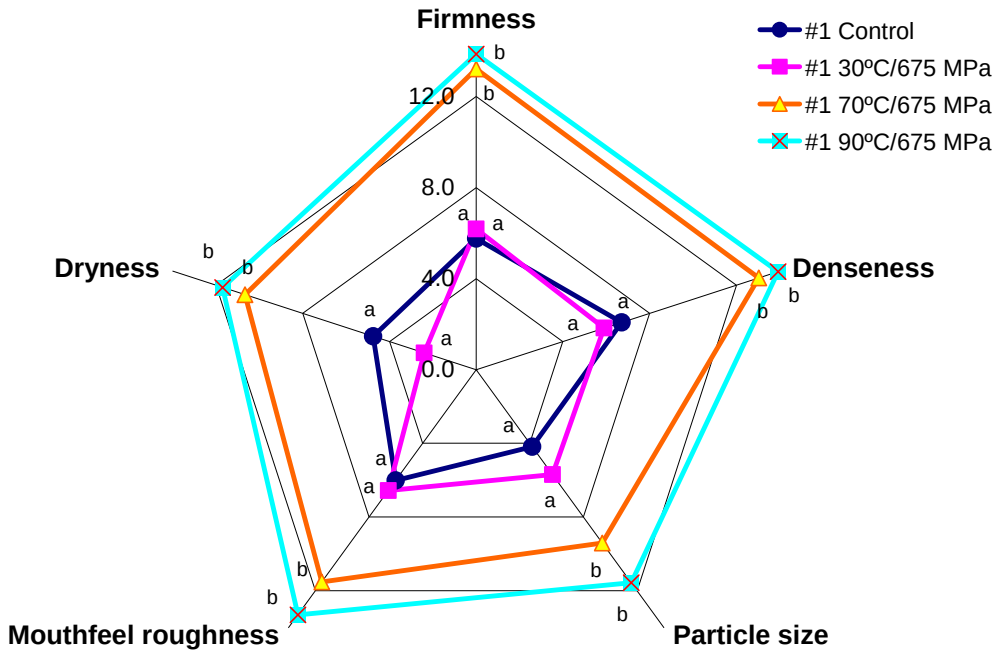

Figure 10. Radar type plot indicating texture profile for egg patty formulation #1. Comparison between control and high pressure treated patties at 675 MPa and initial temperatures 30, 70, and 90ºC. Different letters indicate significant differences between mean values ($p<0.05$, LSD) in the 0 to 14 scale.

Panelists found syneresis in #1 formulation after 70ºC/675 MPa and 90ºC/675 MPa (table 9) treatments to be significantly higher ($p<0.05$) than the control and patty treated at 30ºC/675 MPa. This was supported by results in syneresis, which showed an increase in water loss of six to eight times greater than the control when initial chamber temperatures were higher than 50ºC (table 9). Thus, syneresis is increased by a combination of temperature and pressure. The descriptive panel also found the #1 formulation to be significantly dryer (low moisture release at chewing, $p<0.05$) when treated above protein gelation temperature 70ºC (figure 10).

Table 8. Texture profile analysis of egg patty formulation #1. Comparison between control and high pressure treated patties at 675 MPa and initial temperatures 30, 50, 70, and 90ºC.*

Treatment	Hardness (N)	Cohesiveness (10^2)	Adhesiveness (N.mm, 10^2)	Springiness (10^2)	Resilience (10^2)
Control	22.5 ± 2.2 a	69.5 ± 1.2 a	-43.2 ± 9.1 a	87.9 ± 7.8 a	24.0 ± 1.6 a
30ºC/ 675 MPa	23.0 ± 3.2 a	73.3 ± 1.7 b	-29.7 ± 12.8 a	88.0 ± 11.0 a	27.9 ± 2.3 b
50ºC/675 MPa	39.8 ± 2.6 b	72.2 ± 1.4 b	-44.0 ± 10.4 a	90.2 ± 9.2 a	32.1 ± 1.9 b
70ºC/ 675 MPa	51.6 ± 2.0 c	73.9 ± 1.1 b	-37.1 ± 9.1 a	93.1 ± 7.0 a	29.6 ± 1.5 b
90ºC/ 675 MPa	53.6 ± 1.8 c	74.5 ± 1.0 b	-30.6 ± 7.4 a	96.4 ± 6.4 a	30.4 ± 1.3 b

*Different letters indicate significant differences exist between mean values ($p < 0.05$) within individual columns.

Table 9. Syneresis rated by descriptive panel and percent weight loss after preheat or treatment at 675 MPa and selected temperatures for 5 min.*

Treatment	Syneresis (sensory)	Weight loss (%)
Control	1.0 ± 0.6 a	1.7 ± 1.1 a
30ºC/ 675 MPa	0.5 ± 1.1 a	2.3 ± 1.6 a
50ºC/675 MPa	N/A**	9.8 ± 1.3 b
70ºC/ 675 MPa	3.5 ± 1.2 b	12.5 ± 0.8 b
90ºC/ 675 MPa	3.2 ± 1.2 b	13.3 ± 1.0 b

*Different letters indicate significant differences exist between mean values ($p<0.05$) within individual columns.

**Not available

Flavor of patty formulation #1

HPP of egg patty formulation #1 at low temperature and high temperature conditions did not significantly affect ($p>0.05$) the flavor descriptors: oily, oxidized, scorch, rancid, and foreign (data not shown). Soybean oil present in formulation was equivalently perceived before and after HP/TS treatment. Furthermore, no oxidized or other foreign unexpected tones were noted in formulation #1 by the panel even after treatment at 90ºC/675 MPa. Scorched flavor tones, developed when overcooking or burning the surface of the egg patties during manufacturing steps, were not altered after pressure treatment. All other flavor descriptors (table 10) did not significantly change ($p>0.05$) after egg patty formulation #1 was treated at 30ºC/675 MPa. Thus, flavor profile of formulation #1 remained unchanged after high pressure low temperature

processing. However, samples treated at 90ºC/675 MPa rated higher ($p<0.05$) than control and treated patty ($p<0.05$) at 30ºC/675 MPa in overall flavor, sulfur aroma, retort, unclean flavors, and aftertaste (table 10).

High pressure, in combination with high heat (initial temperature 90ºC), generates new flavor compounds that are perceived similarly to those developed by retort processing and have an unclean lingering flavor (table 10). When egg white is heated at temperatures above 60ºC, there is an increase of –SH groups exposed from protein unfolding, which, through subsequent splitting of disulfide links, results in release hydrogen sulfide (Germs, 1973; Cheftel et al., 1985), thereby increasing sulfur aroma (Yang and Baldwin, 1995).

Table 10. Flavor descriptors found significantly different when comparing egg patty formulation #1 control with high pressure treated patties at 675 MPa and 30, 70, and 90ºC.*

Treatment	Overall flavor	Sulfur aroma	Retort	Unclean flavors	Aftertaste
Control	7.6 ± 0.5 a	3.9 ± 0.4 a	2.2 ± 0.9 a	1.0 ± 0.6 a	4.6 ± 0.6 a
30ºC/ 675 MPa	8.7 ± 1.0 ab	4.0 ± 0.9 a	0.8 ± 1.8 a	0.5 ± 1.2 a	3.0 ± 1.2 a
70ºC/ 675 MPa	8.8 ± 1.0 ab	6.3 ± 1.0 ab	5.2 ± 2.0 ab	5.4 ± 1.3 b	5.7 ± 1.3 ab
90ºC/ 675 MPa	10.8 ± 1.0 b	7.8 ± 1.0 b	7.5 ± 2.0 b	5.3 ± 1.3 b	8.8 ± 1.3 b

*Different letters indicate significant differences exist between mean values ($p<0.05$) within individual columns.

4.2. EFFECT OF PRESSURE LEVEL ON EGG PATTIES' QUALITY

Michael Foods Egg Products Company provided a round commercial scrambled egg patty (#1, code 46025-30020-00). Frozen samples from a single lot were stored frozen at -30ºC. Each patty was cut in half and repackaged in retort pouches (Smurfit-Stone) that measured 6.0 x 10.3 cm. The vacuum packaging machine was a tabletop vacuum chamber (KOCH 15-EasyPack™) used at 400 mbar absolute pressure.

Egg patties were preheated using a water bath at boiling temperature (98ºC) up to 90°C After equilibration egg patties were inserted in a high pressure chamber and pressurized at different pressure levels for five minutes. Times for pressure come up averaged 2.5 ± 0.3 min for 300 MPa, 3.5 ± 0.5 min for 500 MPa, and 4.2 ± 0.5 min for 675 MPa. Following the pressure

treatment, pouches containing egg patties were cooled in an ice bath. Table 11 shows the liner temperatures before, during pressurization, and after pressure release.

Table 11. Temperature profile at different temperature-pressure combinations inside liner.

Pressure	Initial (ºC)	In-Process Initial[a] (ºC)	Final[b] (ºC)
300 MPa	90.6 ± 3.0	106.4 ± 3.3	88.2 ± 2.9
500 MPa	90.9 ± 1.3	114.7 ± 1.6	85.8 ± 1.5
675 MPa	91.0 ± 2.4	121.0 ± 3.5	83.3 ± 0.1

[a]Initial temperature at 675 MPa

[b]Temperature after pressure was released

Product analyses

This section describes the color, texture and weight loss of egg patties formulation #1 after HP/TS treatment at high temperature and different pressure levels.

Color of patty formulation #1

When #1 egg formulation patties were treated at pressure levels 300, 500, and 675 MPa and initial pressure chamber temperature 90ºC, no significant variations ($p > 0.05$) were seen in lightness L* value (table 12) with respect to control. Changes in *Chroma* values were also not significant ($p > 0.05$), when combining high temperature and pressure of at least 300 MPa.

Texture and weight loss of patty formulation #1

TPA descriptors, adhesiveness and springiness, were not affected by pressure processing and remained not significantly different ($p>0.05$) from control (data not shown). On the other hand, TPA hardness, cohesiveness, and resilience (table 12) were still higher ($p<0.05$) than control at lower pressurization conditions of 90ºC/300 MPa. Furthermore, these conditions yielded similar values to the ones obtained by treating #1 egg formulation at 90ºC/675 MPa (table 12). In addition, pressurization at 90ºC/300MPa, or higher pressures, gave significantly higher ($p<0.05$) released serum than egg formulation #1 control in all cases. Hence, even at 300 MPa, the initial high chamber/liner/product temperature of 90ºC significantly affected ($p<0.05$) texture and syneresis of formulation #1.

Table 12. Effect of pressure applied on patties treated at initial temperature 90ºC and pressures 300 MPa, 500 MPa, and 675 MPa for 5 min.**

Treatment	L* (lightness)	*Chroma*	Serum (% weight loss)	Hardness (N)	Cohesiveness (10^2)	Resilience (10^2)
Control	77.3 ± 1.2 a	35.6 ± 1.1 a	1.7 ± 1.3 a	22.5 ± 2.6 a	69.5 ± 1.3 a	24.0 ± 1.5 a
90ºC/ 300 MPa	78.0 ± 1.6 a	31.1 ± 1.4 ab	14.3 ± 1.9 b	50.1 ± 3.0 b	74.7 ± 1.4 b	29.1 ± 1.7 b
90ºC/ 500 MPa	75.8 ± 1.6 a	32.6 ± 1.4 ab	13.2 ± 1.9 b	55.2 ± 3.0 b	74.2 ± 1.4 b	33.0 ± 1.7 b
90ºC/ 675 MPa	78.7 ± 1.3 a	31.2 ± 1.0 b	13.3 ± 1.2 b	53.6 ± 2.1 b	74.5 ± 1.3b	30.4 ± 1.2 b

**Different letters indicate significant differences exist between mean values ($p<0.05$) within individual columns.

Temperatures higher than 70ºC have been reported to affect conformation of the egg proteins livetins, conalbumins, globulins, and ovomacroglobulin (Ma et al., 2001; Feiser and Cotterill, 1982) in both liquid whole eggs and cooked-frozen-thawed-reheated egg products. On the other hand, only pressures higher than 500 MPa and room temperature were shown to affect the conformation of ovomacroglobulin and γ-livetins in liquid eggs (Ma et al., 2001). During the egg patty cooking process, all heat sensitive proteins in the liquid egg mix were denatured, leading to formation of a semisolid coagulum, or gelled foam, with a hardness value similar to the control. Preliminary testing showed that even though initially frozen egg patties were thawed and reheated up to 90ºC, the TPA hardness values did not change. However, when cooked-frozen-thawed-reheated egg patties were treated at pressures of at least 300 MPa, combined with chamber temperature 90ºC, egg matrix densification occurred due to collapse of internal pores, which might have led to further protein gelation and therefore a harder structure than the cooked-frozen-thawed-reheated control. Additional research, including microstructural analysis, is necessary to confirm that additional gelation occurs in preformed frozen egg patties during HP/TS.

4.3. FORMULATION MODIFICATIONS

4.3.1. ADDITION OF XANTHAN GUM, EDTA AND NATURAL AND ARTIFICIAL FLAVORS

In previous sections, it was stated that commercial egg patty formulation #1 provided adequate descriptive characteristics after high pressure treatment at low temperature conditions (30ºC/675

MPa) as a prospective post-packaging pasteurization process. Experimental data on formulation #1 showed that the combination of 675 MPa and initial chamber temperatures above 70ºC, i.e., conditions with potential for egg product sterilization, induced a rougher texture and differences in flavor compared to control. Formulation #1 was modified by adding xanthan gum with the aim of improving water retention, thereby yielding formulation #2. The egg:water ratio was set higher in patty formulation #2 to evaluate if higher water content could reduce hardness and cohesiveness. Furthermore, EDTA was added to improve color retention, and natural and artificial flavors were added to evaluate whether flavor profiles are maintained after HP/TS conditions.

Improved appearance and color

Pressure treated patty formulation #2 scored similarly in gloss values as controls even after sterilization conditions 90ºC/675 MPa, where a process temperature of 121ºC was achieved. This was opposed to pressure treated patty formulation #1 that had an increased gloss score after treatment at these sterilization conditions (table 13). In this case, lightness (L*) did not differ significantly ($p>0.05$) between formulations or after pressure treatment (table 13). Surface appearance was not affected by pressure since no significant differences ($p>0.05$) were found in the descriptors crumbly and surface homogeneity (data not shown).

Chroma value, representing yellowness, decreased for patty formulation #1 after 70ºC/675 MPa treatment but not for patty formulation #2 at the same conditions, when compared to the controls. Patty formulation #2 control was initially lower in yolk content, or initial concentration of yellow pigments, due to a lower egg:water ratio, thereby giving a lower initial *Chroma* value with respect to formulation #1 (table 13). Even though *Chroma* value of patty formulation #2 was maintained after 70ºC/675 MPa, it decreased after 90ºC/675 MPa. It is possible that xanthophylls contained in patty #2 might have been reduced only at standard sterilization conditions 90ºC/675 MPa.

Table 13. Color rated by descriptive panel and analytical measurements of selected scrambled egg patty formulations untreated and treated at 70ºC/675 MPa and 90ºC/675 MPa.**

Formulation	Treatment	Color (Sensory)		Color (Analytical)	
		Gloss	Greenness	L* (lightness)	*Chroma*
#1	Control	4.5 ± 0.8 a	1.0 ± 0.5 a	77.3 ± 0.7 a	35.6 ± 0.9 a
#2	Control	5.6 ± 1.5 ab	0.7 ± 1.1 a	77.7 ± 0.8 a	32.3 ± 1.0 b
#1	70ºC / 675 MPa	7.0 ± 1.6 ab	3.4 ± 0.9 b	80.8 ± 0.6 a	32.1 ± 0.7 b
#2	70ºC / 675 MPa	6.7 ± 1.4 ab	2.2 ± 1.1 ab	80.4 ± 1.1 a	33.6 ± 1.4 b
#1	90ºC / 675 MPa	8.8 ± 1.6 b	3.6 ± 0.9 b	78.7 ± 0.6 a	31.2 ± 0.8 bc
#2	90ºC / 675 MPa	6.0 ± 1.4 ab	2.6 ± 0.8 ab	79.6 ± 0.9 a	28.8 ± 1.2 c

**Different letters show significant differences between mean values ($p<0.05$) within individual columns.

No significant differences ($p>0.05$) in greening were found between patty #2 and controls after treatment at pressure chamber temperature higher than 70ºC and 675 MPa (table 13). In this case, the chelator EDTA was probably effective in formulation #2 at binding iron, preventing formation of iron green compounds after high pressure thermal treatment.

Improved texture, water retention, and mouthfeel

TPA analysis showed lower hardness values in modified formulation #2 (41 and 25% lower after 675 MPa and initial chamber temperature 70 and 90ºC, respectively) than formulation #1 (table 14). Although firmness and density differences (figure 11) determined by the panel were not significant ($p>0.05$) for formulations #1 and #2 at pressure 675 MPa and initial temperatures above 70ºC, all values were higher than controls ($p<0.05$).

No difference in firmness was found between the controls of formulation #1 and #2, regardless of presence of xanthan gum. However, TPA hardness and cohesiveness were lower in formulation #2 control (table 14). Xanthan gum's helical structure is temperature dependent and it can stretch at high temperatures, therefore it can attain higher viscosity values when exposed to combined HP/TS conditions (≥70ºC/675 MPa). A gum with higher viscosity dispersed within the egg matrix interfered with the egg matrix densification process, thereby providing a less hard structure. Hence, even though the descriptive panel did not find HP/TS treated patties with added xanthan gum significantly less firm than 90ºC/675 MPa #1 formulation, the TPA test showed significantly lower ($p<0.05$) hardness in these patties.

The trained panel gave formulation #2 a lower score ($p<0.05$) in particle size and mouthfeel roughness than formulation #1 when both were treated at 70ºC/675 MPa. Furthermore, formulation #2 treated at 70ºC/675 MPa did not differ from the controls, probably due to the presence of xanthan gum and increased water content. TPA resilience (or elasticity during the first bite) of pressure treated formulation #2, was higher than its control #2. However, TPA adhesiveness and springiness values did not significantly change ($p>0.05$) after pressure treatment (data not shown).

Table 14. Texture profile analysis of different scrambled egg patty formulations treated at 70ºC and 675 MPa.*

Formulation	Treatment	Hardness (N)	Cohesiveness (10^2)	Resilience (10^2)
#1	Control	22.5 ± 2.7 b	69.5 ± 1.6 b	24.0 ± 2.0 a
#2	Control	10.8 ± 3.1 a	52.9 ± 1.8 a	19.7 ± 2.4 a
#1	70ºC / 675 MPa	51.6 ± 2.4 e	73.9 ± 1.4 c	29.6 ± 1.8 b
#2	70ºC / 675 MPa	30.0 ± 2.1 c	73.5 ± 1.2 c	34.2 ± 1.6 b
#1	90ºC / 675 MPa	53.6 ± 1.7 e	74.5 ± 1.3 c	33.0 ± 1.6 b
#2	90ºC / 675 MPa	40.0 ± 1.7 d	75.8 ± 1.2 c	30.4 ± 1.6 b

*Different letters indicate significant differences exist between mean values ($p<0.05$) within individual columns.

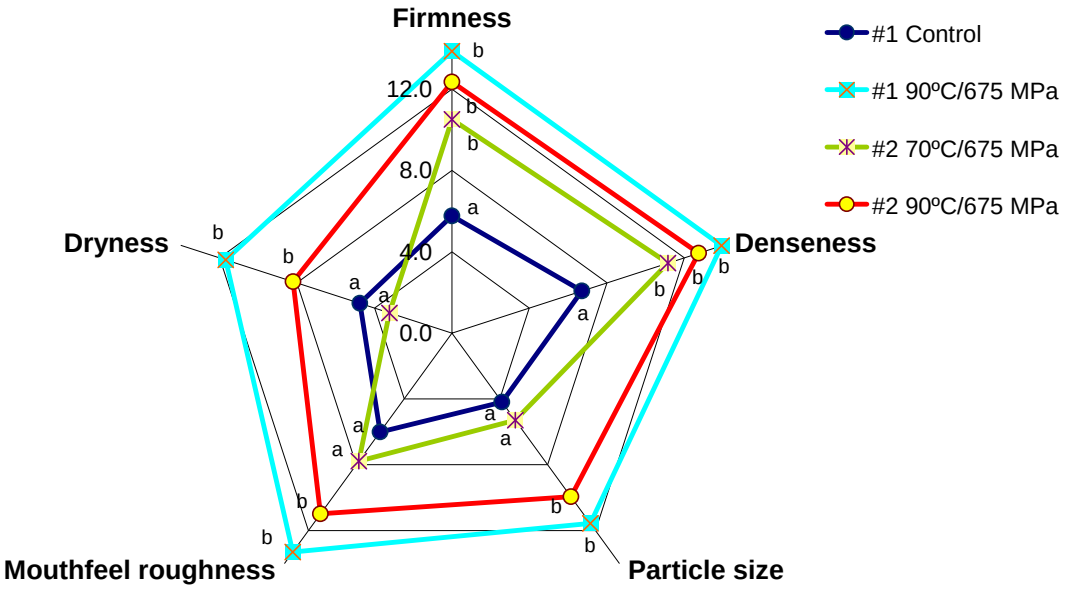

Figure 11. Radar type plot indicating texture and mouthfeel profile as detected by the descriptive panel. Comparison between control #1, formulation #1 after 90ºC/675 MPa, and #2 treated at 70ºC/675 MPa and 90ºC/675 MPa. Significant differences ($p < 0.05$, LSD) are indicated using different letters in the 0 to 14 scale.

Patty formulation #2, treated at pressure chamber temperature higher than 70ºC and 675 MPa had no significantly different ($p>0.05$) scores in greasy and astringent descriptors with respect to controls, as opposed to HP/TS treated formulation #1, which was higher in both descriptors. Xanthan gum and artificial flavors modifying formulation #2 might have masked tangy and tingling sensations present in formulation #1 after high pressure thermal treatment. Slimy, silky, and oily sensations associated with a greasy mouthfeel are also related to addition of xanthan gum and flavors. Mouthfeel descriptors pasty and mouth coating did not change significantly ($p>0.05$) after thermal pressurization with respect to controls (data not shown).

The descriptive panel found syneresis in #2 formulation to be low, although significant differences ($p<0.05$) were detected between pressure treated samples at 90ºC/675 MPa and controls (table 15). When quantified as %weight loss, water released due to HP/TS conditions was significantly decreased ($p<0.05$) in egg patty formulation #2 with respect to formulation #1

by 50 to 55%. High variability observed in % weight loss at 90ºC/675 MPa can be attributed to the higher temperature, which under high pressure conditions, affected water binding components differently, therefore affecting the extent of water retention within the egg matrix. Previous studies on precooked-frozen-reheated omelets (O'Brien et al., 1982) proved xanthan gum to effectively reduce expressible moisture, supporting results shown in Table 10. This significant increase ($p<0.05$) in water retention found in patty formulation #2 after HP/TS treatment coincided with lower dryness compared to formulation #1, mainly observed after 70ºC/675 MPa (figures 7 and 8). Xanthan gum's effect on moisture retention after HP/TS treatment can also be associated with decreased values in hardness.

Table 15. Syneresis rated by descriptive panel and percent weight loss after preheat or high pressure thermal treatment in formulations #1 and #2.*

Formulation	Treatment	Syneresis (sensory)	Weight loss (%)
#1	Control	1.0 ± 0.6 a	1.7 ± 0.8 a
#2	Control	2.0 ± 1.2 a	1.9 ± 0.9 a
#1	70ºC / 675 MPa	3.5 ± 1.2 ab	12.5 ± 0.8 c
#2	70ºC / 675 MPa	3.2 ± 1.2 ab	6.3 ± 0.8 b
#1	90ºC / 675 MPa	3.2 ± 1.2 ab	13.3 ± 1.0 c
#2	90ºC / 675 MPa	3.8 ± 1.2 b	5.8 ± 1.6 b

*Different letters indicate significant differences exist ($p<0.05$) within individual columns.

Improved flavor

As previously mentioned in section 4.1., formulation #1 scores for oily, oxidized, scorched and foreign flavor tones were not significantly ($p>0.05$) affected by HP/TS treatment at temperatures above 70ºC (data not shown). The same was observed in formulation #2 after HP/TS treatment. Moreover, natural and artificial flavors added in formulation #2 changed panelists' perceptions of HP/TS treated egg patties to flavor/aroma profiles similar to control (table 16). It is possible that added flavors masked the retort effects, lingering flavors, and aromas developed at HP/TS conditions, as noted in the case of formulation #1.

Table 16. Flavor and aroma descriptors found significantly different when comparing egg patty formulations #1 and #2 before and after HP/TS treatments.*

Formulation	Treatment	Sulfur aroma	Retort	Unclean flavors	Aftertaste
#1	Control	3.9 ± 0.4 a	2.2 ± 0.9 a	1.0 ± 0.6 a	4.6 ± 0.6 a
#1	90ºC/ 675 MPa	7.8 ± 1.0 b	7.5 ± 2.0 b	5.3 ± 1.3 b	8.8 ± 1.3 b
#2	Control	5.3 ± 1.2 ab	0.3 ± 1.8 a	0.4 ± 1.1 a	4.1 ± 1.3 a
#2	70ºC/ 675 MPa	5.3 ± 1.0 ab	3.1 ± 1.8 ab	1.3 ± 1.1 a	3.9 ± 1.3 a
#2	90ºC/ 675 MPa	6.8 ± 1.2 ab	2.8 ± 1.8 ab	1.1 ± 1.1 a	7.1 ± 1.3 ab

*Different letters indicate significant differences exist between mean values ($p<0.05$) within individual columns.

Modification of formulation with added flavors facilitated obtaining high pressure thermally treated products with profiles similar to control. These results show that adequate product formulation can ensure egg products maintain their sensory characteristics after sterilization treatment using HP/TS processing.

4.3.2. ADDITION OF CHEDDAR CHEESE

Michael Foods Egg Products Company provided three commercial scrambled egg patties: #1, #3 and #4. Each patty was packaged in special flexible pouches of 127 mm x 127 mm (ALCAN, Chicago, IL, USA), and defrosted overnight at 5ºC. Patties were preheated in a water bath using steam injection until 75ºC was reached, indicated by thermocouples (K-type, Omega Engineering Inc., Stamford, CT, USA) located at the patty center. Preheated patties were placed into the high pressure vessel, initially preheated at 75ºC, to reach 105ºC during pressurization at 700 MPa for 5 min. Formulations were tested to gain understanding of their effect on the quality and acceptability of egg patties after HP/TS treatment.

Consumer panel overall acceptability of HP/TS treated egg patties and their controls will be presented in the following paragraphs. Each section includes results from the trained panel and instrumental results in order to provide interpretations of consumers' acceptability for each parameter.

Overall acceptability

Mean scores for consumer overall acceptability of control and treated scrambled egg patties are included in table 17**.**

Table 17. Consumer evaluation of preheated and HP/TS processed egg patties. Different letters indicate significant differences between means within a column (P≤0.05).*

Patty type**	Treatment	Overall	Appearance	Aroma/Flavor	Texture
#1	Control	6.7 ± 1.5 c	6.9 ± 1.6 c	6.5 ± 1.5 c	6.4 ± 1.7 c
#3	Control	6.8 ± 1.6 c	6.8 ± 1.4 bc	6.5 ± 1.7 c	6.8 ± 1.7 c
#4	Control	6.3 ± 1.8 c	6.2 ± 1.7 bc	6.0 ± 2.0 c	6.4 ± 1.8 c
#3	HP/TS	6.0 ± 1.7 bc	5.4 ± 1.8 ab	6.2 ± 1.8 c	5.4 ± 1.9 bc
#4	HP/TS	4.8 ± 1.8 a	5.0 ± 1.8 a	4.6 ± 2.0 a	4.8 ± 1.6 ab

*acceptability scores of 3 = dislike moderately, 4 = dislike slightly, 5 = neither like or dislike, 6 = like slightly, and 7 = like moderately

**#1 (standard egg patty); #3 (added cheese); #4 (added xanthan gum, EDTA and flavors).

In general, overall acceptability of controls was greater than HP/TS treated egg patties. Acceptability of HP/TS treated egg patties #4 (xanthan gum, no cheese) was lower than HP/TS treated egg patties #3 (cheese) and the controls. In particular, controls for egg patties #1 (standard, no cheese) and #3 (cheese) received overall acceptability mean scores close to "moderately liked", whereas control #4 (xanthan gum) was closer to being "liked slightly". When egg patty #3 (cheese) was high pressure treated, it provided similar overall acceptability to control patty #4 (xanthan gum, no cheese). HP/TS treated patty #3 (cheese) was "slightly liked" by consumers whereas HP/TS treated formulation #4 (xanthan gum, no cheese) received significantly lower scores in overall acceptability. When looking at all acceptability parameters (including appearance, aroma/flavor, and texture) for the HP/TS treated patty #2 (cheese), acceptability values were lower in appearance and texture than the controls (table 17). Discussions on the effect of HP/TS on acceptability are shown in the following sections in terms of acceptability of appearance, flavor/aroma, and texture, by comparing with trained panel attributes and analytical descriptors.

Appearance

Regarding appearance, HP/TS treatment affected acceptability, as controls #1 and #3 ("liked moderately") and #4 ("liked slightly") were more acceptable than HP/TS treated patties, which were "neither liked nor disliked" (table 17).

Xanthophylls lutein, zeaxanthin, and crypotaxanthin, that is, carotenoids that provide yellow pigmentation in egg yolk (Yang and Baldwin, 1995), were probably degraded during thermal pressurization. The #3 egg patties maintained their original color better due to higher egg yolk content and possibly due to the presence of cheese in the mix. Indrawati et al. (2004) stated that high pressure treatment slightly affects the carotene content in food products, reporting only 5% losses after a treatment of 75ºC/600 MPa/40 min in carrot homogenates.

Aroma and Flavor

Aroma and flavor of control egg patties and HP/TS treated patty #3 were "slightly liked" by consumers (table 17). HP/TS treated #4 patties were "neither liked nor disliked" and had significantly lower acceptability of aroma/flavor than all other patties. However, the trained panel found no significant differences among controls and HP/TS treated patties in most flavor attributes except for butter flavor intensity in formulation #4 control and salty tones in formulation #3

The trained panel did not identify significant differences in sulfur notes between HP/TS treated egg formulations and the controls (table 18).

Table 18. Significant flavor descriptors* found for controls and HP/TS treated egg patties. Different letters indicate significant differences between means ($P \leq 0.05$) within a column.

Product	Treatment	Butter	Sulfur	Overcooked	Salty
#1	Control	4.2 ± 1.3 ab	3.7 ± 0.8 a	2.3 ± 0.8 ab	0.7 ± 0.3 a
#3	Control	5.8 ± 2.0 ab	3.4 ± 1.1 a	2.9 ± 0.9 ab	4.1 ± 1.9 b
#4	Control	9.0 ± 1.1 b	3.0 ± 0.5 a	0.4 ± 0.2 a	0.8 ± 0.3 a
#3	HP/TS	6.0 ± 2.2 ab	3.4 ± 1.2 a	1.9 ± 1.2 ab	5.2 ± 0.9 b
#4	HP/TS	3.3 ± 1.5 a	7.1 ± 1.1 a	0.1 ± 0.1 a	0.4 ± 0.2 a

*Evaluation by trained sensory panel with 14 cm unstructured line scale

Sulfur containing volatiles, hydrogen sulfide being the major component after heating eggs, contribute significantly to the overall flavor of eggs, mainly originating from egg whites (Warren and Ball, 1991; Chen and Hsu, 1981). Compounds such as dimethyl and trimethyl sulfide, identified for providing "sulfurous, bad egg odor" (MacLeod and Cave, 1975), were probably not present in high amounts in HP/TS treated patties since the average consumer panel scores did not reach the lower "slightly disliked" threshold of 4.0 (table 17). Alternatively, the level found was not offensive for this set of consumers.

The control and HP/TS treated #3 egg patties (cheese), were significantly more salty than egg #3 control, HP/TS, and retort treated patties (table 18). Cheese contained in formulation #3 probably increased the impression of saltiness, and no difference in perceived saltiness was seen in patty #3 (cheese) before or after HP/TS processing. Comments from the consumer panel included that HP/TS treated patty #3 was "a good match of salty and acidic", which probably led to higher flavor acceptability than formulation #4 after HP/TS treatment (table 17).

Texture and mouthfeel

Texture of control patties was "slightly" to "moderately liked" by consumers. Although HP/TS processed patty #3 was neither liked nor disliked, it was not significantly different from control patties (table 17). On the other hand, consumers slightly disliked the texture of HP/TS treated patty #4.

The trained panel supported the decrease in overall acceptability of HP/TS treated patties by describing them as significantly higher in firmness than control patties (table 19).

Table 19. Significant texture and mouthfeel descriptors* found for controls and HP/TS treated egg patties. Different letters indicate significant differences between means ($P \leq 0.05$) within a column.

Product	Treatment	Density	Firmness	Particle size	Oily
#1	Control	8.5 ± 0.8 bc	7.4 ± 0.9 bc	4.8 ± 1.2 ab	4.1 ± 1.4 ab
#3	Control	7.7 ± 1.1 ab	7.6 ± 1.0 bc	4.4 ± 1.7 ab	1.0 ± 1.0 a
#4	Control	4.0 ± 0.8 a	3.2 ± 0.8 a	1.9 ± 0.7 a	1.2 ± 0.7 a
#3	HP/TS	11.9 ± 0.7 c	11.7 ± 0.7 c	7.9 ± 2.1 b	2.5 ± 1.9 a
#4	HP/TS	9.2 ± 1.2 bc	10.5 ± 0.9 c	7.0 ± 1.3 b	8.0 ± 1.4 b

*Evaluation by trained sensory panel with 14 cm unstructured line scale

However, no significant differences were found in firmness between HP/TS treated samples and controls #1 and #3. Juliano et al. (2005, 2006) reported increases in TPA hardness values after treating selected egg patty formulations at initial chamber temperature of 70-75ºC and pressures greater than 300 MPa, due to accelerated protein gelation in egg patties. Furthermore, a higher firmness in HP/TS treated products corresponded to higher product density, as seen when comparing formulations #3 and #4 before and after HP/TS processing (table 19). HP/TS treatment also increased particle size perception of #4 patties compared to the controls when masticated (table 19). Previous research also found high particle size scores in formulation #3 tested in a 1.7 L machine at 675 MPa/98ºC/5 min (Juliano et al., 2006). Furthermore, TPA cohesiveness also increased for formulation #4 after 700 MPa/105ºC/5 min, as reported by Juliano et al. (2005). Even though firmness increased in egg patty #3 after HP/TS treatment, overall and texture acceptability values (table 17) were not significantly changed. Hence, an increase in firmness was not a determinant for the acceptability of formulation #3 after HP/TS treatment, possibly due to the enhanced mouthfeel sensation provided by the added cheese.

In terms of mouthfeel, HP/TS treated #4 patty was significantly oilier than #4 control (table 19). An oily or slimy mouthfeel might be explained by increased oil leached out to the surface during pressurization (and protein gelation at high chamber temperatures). There is evidence that water leaches out of the egg patty matrix after treatment at egg gelation temperatures of 70ºC combined with pressures greater than 300 MPa (Juliano et al., 2006).

Since oily mouthfeel notes remained low in formulation #3, HP/TS conditions might have only had an impact when cheese was not present in the formulation. Alternatively, and more likely, added cheese could have masked the oily mouthfeel. Shelke (2004) mentioned that cheese based ingredients "enhance" viscosity, and thereby creaminess and mouthfeel. In the case of HP/TS treated egg patties, a higher acceptability seen in HP/TS processed formulation #3 (cheese) with respect to #4, is indicated by a lower oily mouthfeel tone as opposed to the scores obtained for HP/TS treated egg patties #4, which are closer to greasy and viscous notes.

4.3.3. Diced egg patties, addition of annatto and carotenoids and liquid egg formulations

The last efforts made to improve the quality of this formulation were focused on appearance and texture improvement. To better match the color of fresh scrambled eggs, it was proposed that annatto and carotenoids be added to counteract the loss in *chroma* values due to HP/TS treatment. Figure 12 shows that addition of annatto and carotenoids can help match scrambled egg patty color.

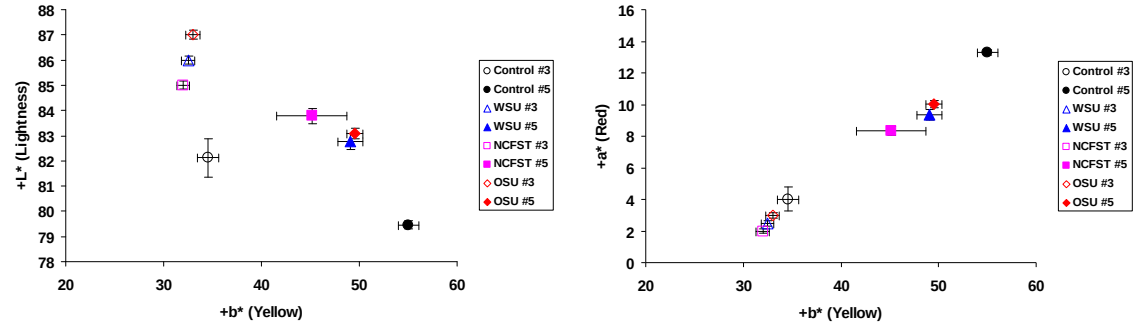

Figure 12. L*-b* and a*-b* chromatic planes for scrambled egg patties formulation #3 and #5 processed at 700 MPa/105°C/5min.

However, it was well established that the main factor controlling overall acceptability was the change in texture due to the gelation of precoagulated egg protein network. Two approaches were tested to improve the texture of scrambled egg patties to obtain commercially desired levels. In the first approach, egg patties were diced to enhance the egg texture and perception of product. In the second approach, a novel liquid formulation (whole egg combined with citric acid, water, soybean oil, salt, modified food starch, whey, skim milk powder, and cheese) was used. Four formulations considered are shown in table 20.

Table 20. Ingredients in Liquid egg-based formulations.

	Ingredients
#1	Whole egg with citric acid, water, soybean oil, dry ingredients (salt, modified food starch, whey protein and skim milk powder), Cheese
#2	Whole egg with citric acid, water, soybean oil, dry ingredients (salt, modified food starch, whey protein and skim milk powder), Cheese + Baking soda + water
#3	Whole egg with citric acid, water, soybean oil, dry ingredients (salt, modified food starch, whey protein and skim milk powder), Cheese + Frozen diced egg patties
#4	Whole egg with citric acid, water, soybean oil, dry ingredients (salt, modified food starch, whey protein and skim milk powder), Cheese + Baking soda + water + Frozen diced egg patties

Among the four formulations evaluated, formulation #3 and 4 appeared to have better sensory evaluation than formulation #1 and 2. An informal panel described formulation #3 as egg samples with a distinct strong egg flavor and some rubbery texture. Formulation #4 was described as having a milder egg flavor and a more acceptable texture.

Sensory evaluation at Natick

Fifteen panelists evaluated the diced egg patties, egg patties with addition of annatto and beta carotene, and liquid egg samples. Overall, the diced egg patties and diced/liquid combination were evaluated as having “good quality” (figure 13).

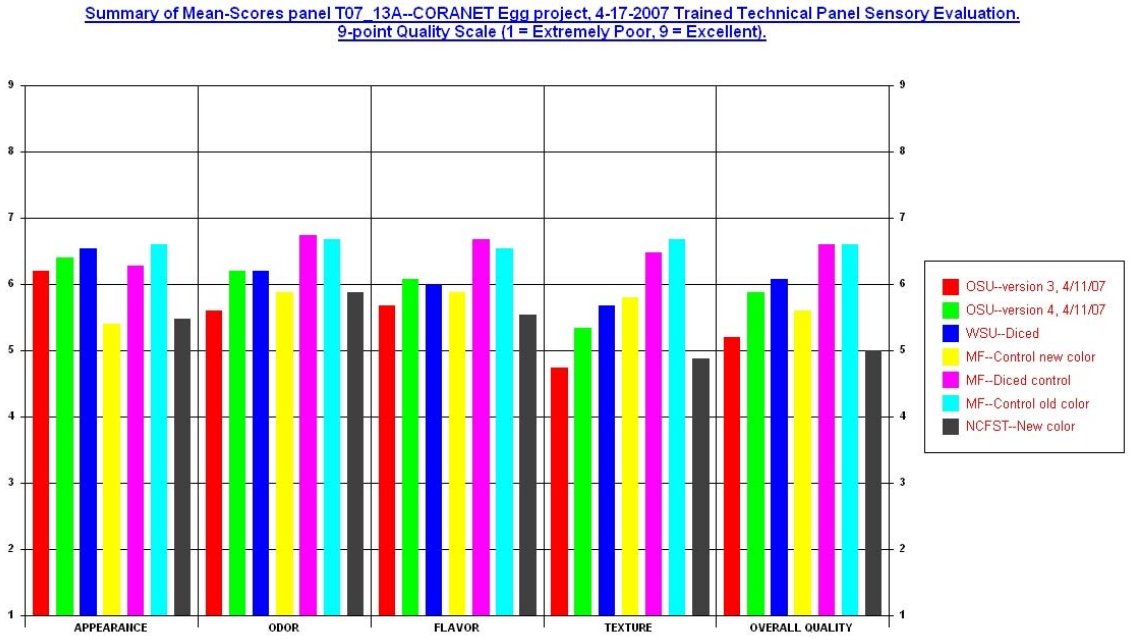

Figure 13: Sensory evaluation of egg-based products at Natick.

4.4. HP/TS VS. IN-POUCH RETORT TREATMENT

For this study, Michael Foods Egg Products Company provided egg patty #4 (added xanthan gum, EDTA and natural and artificial flavors). In order to perform HP/TS treatment, each patty was packaged in special flexible pouches from ALCAN and defrosted overnight at 5ºC.

Egg patties were preheated up to 75 or 90ºC in a water bath using steam injection. Preheated patties were either placed in the high pressure vessel, initially preheated at 75ºC to reach 105ºC, and pressurized at 700 MPa for 5 min (HP/TS1); or initially preheated at 90ºC to reach 121ºC and pressurized at 700 MPa for 3 min (HP/TS2).

HP/TS treated patties #4 were compared with in-pouch retort processed ones, as in-pouch retort offers shorter processing time than conventional retort processing (due to higher heat transfer rates through the thin polymeric films and smaller size of the samples).

For in-pouch retort treatment, egg patties (#4) were packaged in retort pouches (Smurfit-Stone) following the same procedure as high pressure treated patties and thermally processed using a steam rotary retort (Steritort, FMC corporation, San Jose, CA, USA). The in-pouch retort treatment was not conventional since it was performed using small egg product packages of about 45 g, allowing for higher heat transfer rate, and thereby a process time of 16 min. Figure 14 shows the temperature and pressure profile obtained during pressurization (HP/TS), together with the temperature profile for the retort treatment.

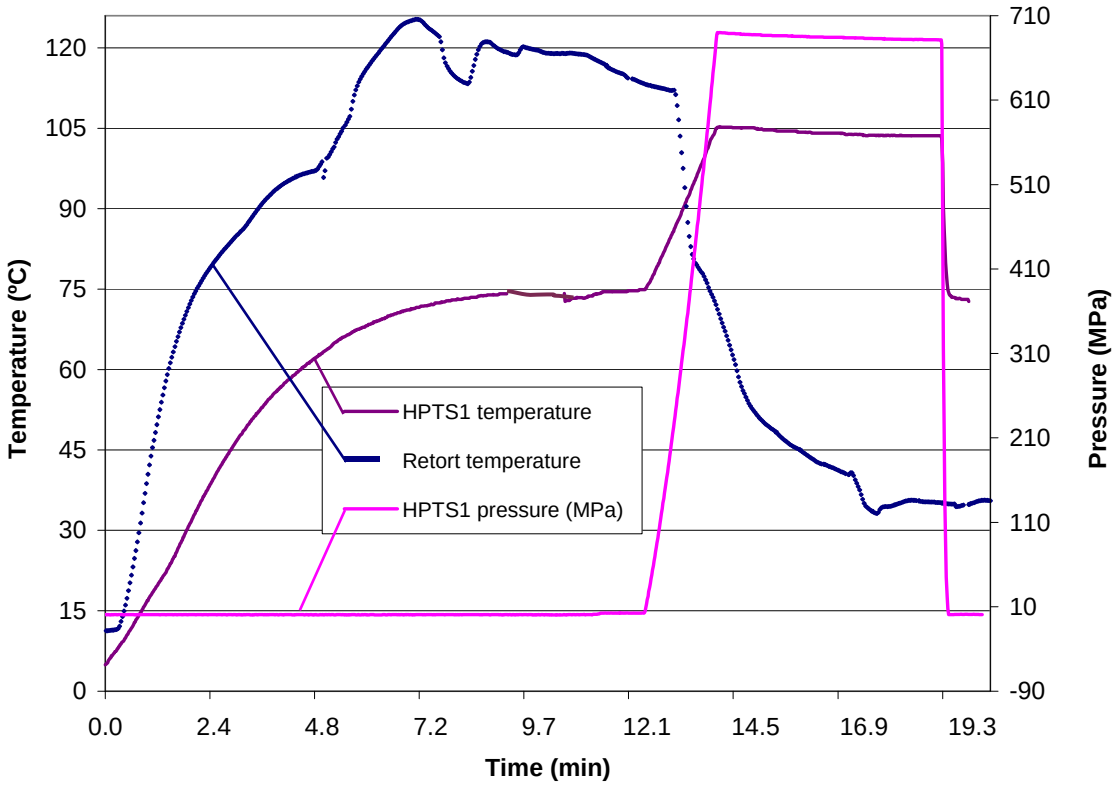

Figure 14. Typical temperature and pressure profiles during pressurization of the transmission in water/egg patty system at 700 MPa/105°C/5 min (HP/TS1). Retort temperature profile is also shown as read from the thermocouples inside the tested scrambled egg patties.

The recorded temperature profiles measured inside the products in both HP/TS2 and retort processes were used to calculate sterilization value F_0 (Eq. 3; Stumbo, 1973):

$$F_0 = \int_0^t 10^{\left[\frac{T-121}{z}\right]} dt \approx \sum_0^t 10^{\left[\frac{T-121}{z}\right]} \cdot \Delta t \qquad (3)$$

where T is the process temperature (°C) inside the egg patty, t is processing time (min), 121 corresponds to the reference temperature (°C), and z, the z value (or thermal sensitivity) (z for *Clostridium botulinum* is 10°C). The integral of Eq. (3) was calculated using the General Method (Holdsworth, 1997). An approximation to the integral value was calculated by using the numerical quadrature formula shown at the right side of Eq. (3) and short time intervals of 1s and 2s for the HP/TS2 and retort processes, respectively.

The relative thermal effect on food quality of the processed scrambled egg patties was also quantified using cook values (C_{100}). The cook values for the retort and HP/TS2 were calculated using the following equation (Lund, 1986):

$$C_{100} = \int_0^t 10^{\left[\frac{T-100}{z}\right]} dt \approx \sum_0^t 10^{\left[\frac{T-100}{z}\right]} \cdot \Delta t \qquad (4)$$

where the chosen z value for the processed egg products is 33°C as it is generally used to calculate the overall quality loss (Lund, 1986; Luechapattanaporn et al., 2005). The reference temperature of 100ºC (instead of 121ºC) used in Eq. (4) has been established for quality degradation studies after thermal processing (Lund, 1986).

Overall acceptability

Mean scores for consumer overall acceptability of control and treated scrambled egg patties are included in table 20.

Table 20. Consumer evaluation of preheated, HP/TS and in-pouch processed egg patties[*]. Different letters indicate significant differences between means within a column (P≤0.05).

Patty	Treatment	Overall	Appearance	Aroma/Flavor	Texture
#4	Control	6.3 ± 1.8 c	6.2 ± 1.7 bc	6.0 ± 2.0 c	6.4 ± 1.8 c
#4	HP/TS1	4.8 ± 1.8 a	5.0 ± 1.8 a	4.6 ± 2.0 a	4.8 ± 1.6 ab
#4	HP/TS2	4.4 ± 2.0 a	4.8 ± 2.1 a	4.8 ± 1.9 ab	3.8 ± 2.2 a
#4	In-pouch retort[♦]	6.0 ± 1.5 bc	5.6 ± 2.1 ab	6.0 ± 1.6 bc	5.9 ± 1.8 bc

[*]acceptability scores of 3 = dislike moderately, 4 = dislike slightly, 5 = neither like or dislike, 6 = like slightly, and 7 = like moderately

[♦]non conventional, due to rapid processing and use of products of thin cross section in small retort pouches

Acceptability was higher after in-pouch retort than after both HP/TS1 and HP/TS2. The fact that the in-pouch retort patty underwent a rapid processing at 121ºC, due to the use of small pouches and low thermal load, explains the acceptable results. Conventional retort treatment of scrambled eggs packaged in larger volumes, such as trays, yields a product of brownish color and rubbery and grainy structure (Luechapattanaporn et al., 2005; Song and Cunningham, 1985; Baliga et al., 1969). The use of smaller packages increased heat transfer rate, thereby providing a shorter process time than conventional treatment in cans. In general, the thinner shape of retort pouches offers less resistance to the transfer of heat with respect to cans, thereby decreasing process time and increasing energy efficiency (Barbosa-Cánovas and Juliano, 2004).

Egg patties treated at HP/TS1 (700 MPa/105ºC/5 min) showed higher acceptability scores, although not significantly different (P>0.05), than when treated at HP/TS2 (700 MPa/121ºC/3 min). A higher pressurization temperature affected the overall acceptability at a standard scenario of 700 MPa/121ºC, by mainly affecting texture, as seen by the lower (though not statistically significant) scores shown. Further sections give special attention to the relation between overall acceptability and the other acceptability parameters.

An F_0 value of 3.3 min was obtained for HP/TS2 using the temperature profile obtained at a standard sterilization scenario of 700 MPa/121ºC/3 min. Processing using rapid retort resulted in a F_0 of 5.6 min. Cook values (C_{100}) for HP/TS2 treatment and retort were 16.4 min and 30.4 min, respectively. The lower value for HP/TS2 shows that the quality damage due to thermal factor should be lower if treated using HP/TS2. However, combined pressure and temperature produced

changes in the overall quality of the scrambled egg patties, some of which affected the acceptability after high pressure high temperature treatment.

Appearance

Regarding appearance, trained panel scores in retort and HP/TS treated, did not significantly differ from controls in green (to yellow) color (table 21). However, scores for in-pouch retort treated patties were four times higher in green color than control patty, which was indicative of the production of green FeS compounds (Song and Cunningham, 1985). *Chroma* (yellow color) values, were lower in retort treated egg patties, these results are in agreement with Luechapattanaporn et al. (2005), who also reported a decrease on +b* value after retorting freshly scrambled eggs in polymeric trays after a F_0 of 4.6 min (about 80 min processing time).

Table 21. Significant appearance descriptors♦ and L* and *chroma* values found for control, HP/TS and in-pouch retort processed egg patties. Different letters indicate significant differences between means within a column (P≤0.05).

Product	Treatment	Gloss intensity	Green color	L*	*chroma*
#4	Control	5.7 ± 0.7 a	0.6 ± 0.3 a	77.6 ± 2.5 a	35.0 ± 1.2 b
#4	HP/TS1	8.8 ± 1.2 ab	1.6 ± 0.5 ab	73.0 ± 2.9 a	26.2 ± 1.4 a
#4	HP/TS2	11.5 ± 0.6 b	2.7 ± 1.1 ab	77.3 ± 3.0 a	27.1 ± 1.5 a
#4	In-pouch retort	7.3 ± 1.4 ab	4.3 ± 1.4 b	76.2 ± 3.4 a	29.3 ± 1.6 a

♦Evaluation by trained sensory panel with 14 cm unstructured line scale

Aroma and flavor

The trained panel detected degradation of flavors after HP/TS and retort due to lower butter notes (table 22), indicative of degradation or volatilization of diacetyl compounds (Andres, 1983). Little is known about the effect of high pressure on butter flavor compounds, and much less has been studied on the effect of high pressure combined with high temperature. Observations in decreased butter notes coincided with those found by Juliano et al. (2006) after treating egg patties at 675 MPa/98ºC/5 min in a 1.7 L machine. Decreased butter flavor likely accounts for the lower aroma/flavor acceptability scores by consumers for HP/TS treated egg patties.

Table 22. Significant flavor descriptors* found for controls, HP/TS and in-pouch retort processed egg patties. Different letters indicate significant differences between means (P≤0.05) within a column.

Product	Treatment	Butter	Sulfur	Overcooked	Salty
#3	Control	9.0 ± 1.1 b	3.0 ± 0.5 a	0.4 ± 0.2 a	0.8 ± 0.3 a
#3	HP/TS1	3.3 ± 1.5 a	7.1 ± 1.1 a	0.1 ± 0.1 a	0.4 ± 0.2 a
#3	HP/TS2	1.5 ± 1.4 a	4.7 ± 1.6 a	1.5 ± 1.1 ab	0.6 ± 0.3 a
#3	In-pouch retort	2.0 ± 2.0 a	3.5 ± 1.0 a	5.0 ± 1.9 b	0.4 ± 0.3 a

*Evaluation by trained sensory panel with 14 cm unstructured line scale

Retort treated egg patties were higher in overcooked flavor/aroma tones than HP/TS treated eggs and control (table 22). Nose burn aroma note is related to the production of ammonia and hydrogen sulfide upon heating the proteins of the egg white (Germs, 1973; Warren and Ball, 1991). Overcooked was enhanced after retort processing, but did not occur during HP/TS processing (table 22).

Texture and mouthfeel

Consumers slightly disliked the texture of HP/TS treated egg patty. Treatment of egg patties at 700 MPa/105ºC/5min yielded higher texture acceptability scores than when treated at 700 MPa/121ºC/3 min. Thus, higher temperature, in combination with 700 MPa, decreased texture acceptability. In fact, trends in both texture and overall acceptability scores of HP/TS treated egg patties suggest texture was the controlling factor.

Lower texture acceptability scores found by consumers in HP/TS2 treated patty with respect to in-pouch retorted patty, were also reflected in the trained panel. Higher firmness and density values were reported for the HP/TS2 treated patty than retort one (table 23). Hence, the sterilization scenario of 121ºC combined with 700 MPa, induced further gelation and hardening in comparison to a 121ºC treatment only.

Table 23. Significant texture and mouthfeel descriptors* found for controls, HP/TS and in-pouch retort processed egg patties. Different letters indicate significant differences between means (P≤0.05) within a column.

Product	Treatment	Density	Firmness	Particle size	Oily
#4	Control	4.0 ± 0.8 a	3.2 ± 0.8 a	1.9 ± 0.7 a	1.2 ± 0.7 a
#4	HP/TS1	9.2 ± 1.2 bc	10.5 ± 0.9 c	7.0 ± 1.3 b	8.0 ± 1.4 b
#4	HP/TS2	12.5 ± 0.6 c	10.4 ± 1.4 c	8.1 ± 1.7 b	4.1 ± 1.8 ab
#4	In-pouch retort	8.3 ± 2.2 b	6.3 ± 1.6 ab	5.4 ± 1.4 ab	4.7 ± 1.4 ab

*Evaluation by trained sensory panel with 14 cm unstructured line scale

5. MICROBIAL CHALLENGE STUDIES

Two studies were carried out to evaluate the safety status of PATP-treated egg patties:
In the first study, egg patties were inoculated with *Bacillus stearothermophilus* spores (10^6 spores/g). Test samples were preheated and then PATP-treated at 105°C at various pressures and pressure holding times. These pressure assisted treatments were compared with thermal inactivation of spores at 121°C.

The objectives of the study were:

1. To compare the efficacy of PATP and thermal processing against spores, inoculated in egg patties or suspended in deionized water
2. To evaluate the applicability of selected linear and nonlinear models to describe PATP spore inactivation in egg products.

In the second study, Bacillus amyloliquefaciens spores were used as a potential surrogate for Clostridium botulinum in validation studies involving bacterial spore inactivation by pressure assisted thermal processing. Bacillus amyloliquefaciens spores were inoculated (~1.4×10^8 spores per g) into egg patty mince. The product was treated with combinations of pressure (0.1 to 700 MPa) and heat (95 to 121°C).

The objectives of the study were:

1. To determine the linear and nonlinear PATP kinetics parameters for inactivation of *Bacillus amyloliquefaciens* spores in egg patty mince at different pressures and temperatures
2. To evaluate the pressure and temperature coefficients (z_P and z_T, respectively) of *Bacillus amyloliquefaciens* spores.

5.1. INACTIVATION OF BACILLUS STEAROTHERMOPHILUS SPORES IN EGG PATTIES BY PRESSURE ASSISTED THERMAL PROCESSING

Spores of the nonpathogenic flat-sour thermophilic organism have been used as one of the surrogate organisms for *Clostridium botulinum* in thermal processing (IFT, 2000).

Preparation of *Bacillus stearothermophilus* spore suspensions

Spore suspensions of *B. stearothermophilus* ATCC 7953 were prepared as described by Sala et al., (1995). Stock cultures of *B. stearothermophilus* were spread-plated onto a nutrient agar (BD Difco; Becton, Dickinson and Co., Sparks, MD, USA) that was supplemented with 500 mg/kg dextrose (BD Difco) and 3 mg/kg manganese sulfate (Fisher Scientific, Pittsburgh, PA, USA). The inoculated plates were incubated for 10 d at 55°C before harvesting the spores. The plates were packaged during incubation to prevent drying. The level of sporulation was verified by inspection of the sample colony using a phase contrast microscope. At > 97% sporulation, the plates were flooded with water and the growth was scraped, washed 6–7 times by centrifugation (8000*g* for 20 min at 4°C), and resuspended in deionized water. The suspension was treated with lysozyme (100 μg/ml for 30 min) and trypsin (200 μg/ml for 2 h) to minimize interfering cell debris. After enzyme treatment the spore suspension was again washed 4–5 times, and then the spore pellet was diluted using deionized water to approximately 10^8 spores/ml. The spore suspension was stored at 4°C until use.

Inoculation of egg patties for thermal inactivation studies

Frozen commercial egg patties from Michael Foods #2 (whole eggs, water, soybean oil, modified food starch, whey solids, salt, natural and artificial flavors, nonfat dry milk, xanthan gum, citric acid and EDTA) were thawed in a refrigerator (4°C) one day prior to use, after mashed manually

in sterile stomacher bags (Fisher Scientific) and then 10.0 g of mashed patty were transferred to another stomacher bag. Spore suspension (1 ml; ~10^8 spores/ml) was added to the mashed patties and thoroughly mixed to get a final concentration of approximately 10^6 spores/g egg patty.

Thermal inactivation of spores

The thermal resistance of *B. stearothermophilus* spores, suspended in deionized water, was determined at 121°C using custom fabricated aluminum tubes (Luechapattanaporn et al., 2004). Spore suspension (1 ml in deionized water) was transferred into each of the six aluminum tubes. The tubes were then submerged simultaneously into a 28-l circulating oil bath (Fisher Scientific), which was maintained at 121±0.1°C. The come-up time required to reach the desired process temperature of 121°C was monitored using a K-type thermocouple (Omega Engineering, Stamford, CT, USA). The temperature was recorded using a data logger (IOtech, Cleveland, OH, USA). The heating time was recorded when the temperature reached 121°C. The first aluminum tube was removed from the oil bath at the end of come-up time (3.33 min). The other aluminum tubes were subjected to five different heating times, from 2.5 to 12.5 min. After the thermal treatments, the samples were immersed promptly into an ice bath to avoid further inactivation. Spores surviving thermal treatment were enumerated as described later. The untreated control samples were heated at 80°C for 30 min to activate the spores before enumeration. For the thermal inactivation experiments using egg patties, 1 g of the mashed egg patties was inoculated with the spore suspension, and transferred into one of the six aluminum tubes. The thermal inactivation of spores in egg patties was conducted using the same procedure as described earlier for deionized water.

PATP of spores

Preparation of samples containing aqueous spore suspensions

Aliquots (5 ml) of *B. stearothermophilus* spore suspensions (10^8 spores/ml) in deionized water were transferred into stomacher bags (10×15 cm; Fisher Scientific), which were sealed using an Impulse heat sealer (American International Electric, Whittier, CA, USA). Each stomacher bag was then placed inside a larger high-barrier plastic pouch and vacuum packaged. The sealed pouches were kept under refrigerated conditions until PATP-treated.

Preparation of spore-inoculated egg patties

Frozen egg patties were thawed under refrigeration (4°C). Each egg patty was inoculated with one ml of spore suspension, which was uniformly spread on the patty using a sterile glass spreader. The inoculated egg patties had a final spore concentration of $\sim 10^6$ spores/g. The egg patties were then transferred into stomacher bags (18×30 cm; Fisher Scientific), which were then sealed under a vacuum setting of 20 kPa and sealing time of 0.1 s (Sipromac Vacuum Sealer, St-Germain, Que., Canada). The bags were further packaged and refrigerated until PATP treatment, as described earlier.

PATP treatment

Pouches containing spore suspensions or spore-inoculated egg patties were preheated to the desired initial temperature using a water bath (Isotemp 928; Fisher Scientific). The preheating time for the spore suspension to achieve the desired initial temperature was 4.5 min, as compared with 27 min for egg patties. Sets of preheated spore samples were enumerated for the spore count (N_0') to determine the effects of preheating on the initial spore population (N_0). No significant difference was observed between (N_0) and (N_0') (data not shown).

In order to achieve a maximum final process temperature of 105°C, the initial temperatures of the spore suspension or egg patties were adjusted as a function of final target pressure. The initial temperature was estimated based on the heat of compression of glycol and egg patties (Balasubramaniam, Ting, Stewart, & Robbins, 2004). Preheated samples were then immediately loaded into a high pressure food processor (QFP-6, Flow Autoclave Systems, Columbus, OH, USA) and subjected to the desired pressure level (400–700 MPa) over different holding times (0–300 s). PATP come-up times were 1.9, 2.2 and 2.4 min for 400, 600 and 700 MPa, respectively. The process holding time did not include the pressure come-up or the depressurization times. The initial temperature of the pressure-transmitting fluid (equal volumes of water and glycol) (Houghton Safe-620TY, Houghton International Inc., Valley Forge, PA, USA) was also adjusted to a temperature close to that of the samples, taking the heat of compression of glycol–water mix into account. The pressure and temperature data were recorded at regular intervals during the pressure come-up, holding and depressurization times (figure 15).

After depressurization, the samples were cooled immediately in an ice bath. The untreated control samples (nonpressure treated spore suspension or inoculated egg patties) were heated at 80°C for 30 min, to activate the spores for enumeration purposes. The contents of the pouches containing spore suspension (control and pressure treated) were directly used for enumeration. Pouches containing inoculated egg patties (control and pressure treated) were opened aseptically, their contents were mixed with an equal volume of peptone water (1 g/l) and homogenized for 2 min in a stomacher (Seward Lab Stomacher, Norfolk, UK) to release the surviving spores in the aqueous solution. The aqueous solution was then used for the enumeration of survivors.

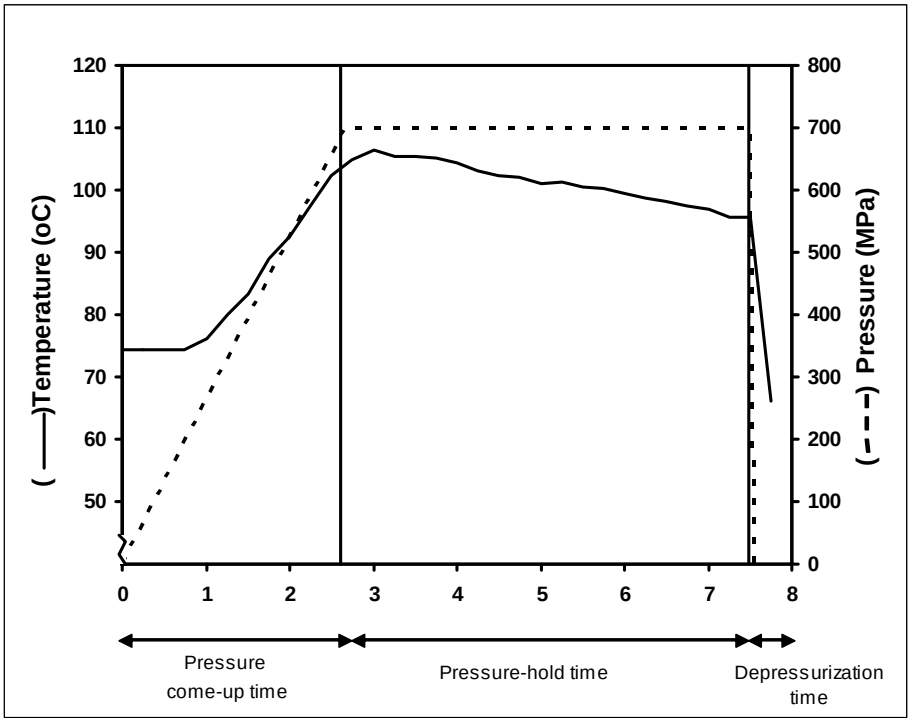

Figure 15. Sample pressure–temperature profile observed during PATP. Data presented are mean values of two independent trails.

Enumeration of survivors

Heat and pressure treated samples were serially diluted in peptone water (1 g/l). Dilutions were pour plated using plate count agar (PCA; BD Difco), and the plates were incubated at 55°C for 48 h before enumeration. The enumeration of colonies was done using a dark-field Quebec colony counter (Leica Microsystems, Ont., Canada)

Data analysis

Multiple analyses of variance using the general linear model (GLM) procedure of Statistical Analysis Software (SAS, release 9.2.1, SAS Institute Inc., Cary, NC, USA) was done to analyze the effect of pressure level, holding time, and pressure–time interactions on spore inactivation. A similar analysis was done using thermal treatments data. Significant mean differences among pressure treatments and holding times were calculated by Fisher's least significant difference at 5% of significance level (P<0.05). All the pressure experiments were replicated on separate dates while thermal inactivation studies were done in triplicate.

Modeling of inactivation curves

Modeling parameters were calculated using spore count (N_0'') after process (thermal or pressure) come-up times as the initial point of the survivor plots.

First order kinetics

The decimal reduction time (D value) was calculated based on the reduction in microbial population at a constant pressure (or temperature) from the linear portion of the survivor curve using the equation.

$$Log\frac{N}{N_0^{''}} = \frac{-t}{D} \tag{5}$$

where N_0'' is the spore count after process (thermal or pressure) come-up time, and N is the number of survivors after being exposed to a lethal treatment for a specific time (t).

Log-logistic model

Cole et al. (1993) described nonlinear thermal inactivation of microorganism using the following equation:

$$LogN = \alpha + \frac{\omega - \alpha}{1 + e^{4\sigma(\tau - \log t)/(\omega - \alpha)}} \tag{6}$$

where α is the upper asymptote (log cfu/ml), ω is the lower asymptote (log cfu/ml), σ is the maximum rate of inactivation (log (cfu/ml)/log min), and τ is the log time to the maximum rate of inactivation (log min).

Since log t at 0 min is not defined, a small value of t (t=10^{-6}) was used to approximate t=0. Log N_0'' was calculated from Eq. (6) and substituted back into Eq. (6) to avoid the direct use of different initial numbers (Chen & Hoover, 2003):

$$Log\frac{N}{N_0''} = LogN - LogN_0'' = \frac{\omega-\alpha}{1+e^{4\sigma(\tau-Logt)/(\omega-\alpha)}} - \frac{\omega-\alpha}{1+e^{4\sigma(\tau+6)/(\omega-\alpha)}} \quad (7)$$

By taking A=ω-α, the number of parameters were reduced from 4 to 3:

$$Log\frac{N}{N_0''} = \frac{A}{1+e^{4\sigma(\tau-Logt)/A}} - \frac{A}{1+e^{4\sigma(\tau+6)/A}} \quad (8)$$

Weibull model

The following equation is a cumulative form of the Weibull distribution and it was used by Peleg and Cole (1998) to describe microbial survival.

$$Log\frac{N}{N_0''} = -bt^n \quad (9)$$

where b and n are the scale and shape factors (Peleg & Cole, 1998).

Model comparison and curve fitting

Mean square error (MSE), R^2 and accuracy factor (A_f) values were used to compare the above models. Better fitting models produce smaller MSE values (Neter, Kutner, Nachtsheim, & Wasserman, 1996). Higher R^2 values mean that the model is adequate to describe the data (Neter et al., 1996) and an A_f value closer to 1 indicates that the model produces a perfect fit to the data. The curve fitting was done using the nonlinear (PROG NLIN) procedure of the statistical

program (SAS) and the MSE and R^2 values were obtained from the analysis. The A_f values were calculated using the following equation:

$$A_f = 10^{\frac{\sum\left|Log\left(predicted/observed\right)\right|}{n}} \quad (10)$$

5.1.1. Inactivation of *B. stearothermophilus* spores

B. stearothermophilus spores exhibited different inactivation patterns during thermal and PATP treatment. While thermal inactivation of spores follows first order kinetics, PATP-treated spores clearly showed divergence from this trend and exhibited a nonlinear behavior beyond 100 s pressure holding time (figure 16). This nonlinear survivor curve may indicate that HPP has multiple targets of action in bacterial spores. The observed tailing phenomenon could also be attributed to cell clumping, stress adaptation, or the genetic heterogeneity of the microbial population (Tay, Shellhammer, Yousef, & Chism, 2003). At the initial stages of the pressure treatment (pressure holding time of less than 100 s), there was rapid inactivation, but for pressure holding time beyond 100 s, microbial inactivation rates decreased at all the pressure levels tested. Similar trends in inactivation have been observed in other works involving HPP treatments of *Listeria monocytogenes, Escherichia coli* and spores of *B. stearothermophilus* suspended in mashed broccoli (Hoover, Metrick, Papineau, Farkas, & Knorr, 1989; Ananta et al., 2001).

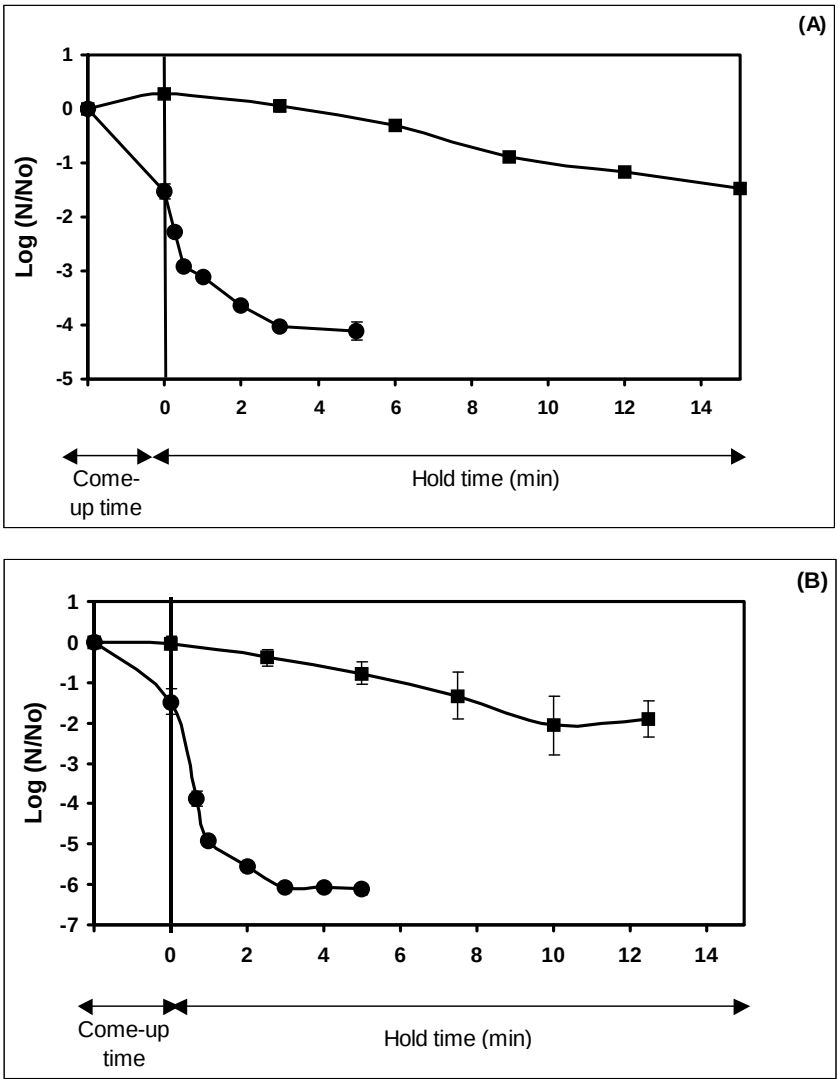

Figure 16. Log survivor fraction of *B. stearothermophilus* spores, in different media, during thermal treatment and PATP. Come-up time for the thermal treatment was 3.33 min and that for PATP was 2.4 min. Media: (A) egg patties and (B) deionized water. Process: (■) heating at 121°C, and (●) PATP at 700 MPa and 105°C.

Decrease in inactivation rate seems to coincide with the decline in process temperature during the PATP treatment (figures 15 and 16). While the temperature increase (due to heat of compression) in polar compounds such as water readily follow pressurization rate, nonpolar compounds such as fats and oils exhibit a shouldering effect, and they reach maximum process temperature 30–60 s after the targeted pressure is attained (Rasanayagam et al., 2003). Due to differences in heat of compression between test sample, pressure-transmitting fluid and pressure vessel, a temperature gradient develops during PATP of food samples. In the absence of

insulation in the pressure vessel, cooling of the sample medium by conventional heat transfer occurs. During the first 100 s of pressure holding time, the heat of compression generated within the food sample and pressure-transmitting medium negate the convectional loss of heat to the surroundings. However, beyond the 100 s pressure holding time, heat loss to the surroundings becomes more dominant than the temperature increase due to heat of compression, causing the temperature of the sample to decline steadily from the process temperature of 105 to 95±1°C at the end of the 5 min holding time (table 24). The survivor plots also showed deviation from linearity beyond the 100 s holding period (figure 16). Statistical analysis of the data also revealed that microbial inactivation during longer pressure holding times (beyond 3 min) were not significantly different (P<0.05), highlighting a tailing effect.

Table 24. Typical pressure and temperature conditions during PATP

Sample	**Preheating time (min)**	**Target pressure (MPa)**	**Conditions within the high pressure processor**				
			Water Jacket temperature (°C)	**Pressure transmitting fluid temperatures (°C)**			
				Initial	**Maximum[a]**	**Final[b]**	**After depressurization**
Egg patty	27	700	80	71.6±1.2	105.5±1.1	95.5±0.5	64.9±1.4
Egg patty	27	600	80	74.5±1.2	105.1±0.9	93.8±0.8	67.4±0.8
Egg patty	27	400	80	84.9±0.7	105.3±0.7	96.2±0.1	69.8±0.6
Deionized water	4.5	700	80	73.5±0.6	105.8±0.6	96.9±0.8	63.5±1.1

[a] Maximum temperature at target pressure (at the end of come-up time).

[b] Temperature just before depressurization.

With the maximum processing temperature at 105°C, an increase in the pressure level from 400 to 600 MPa decreased spore viability (figure 17) considerably. However, an increase in pressure from 600 to 700 MPa (keeping processing temperature constant at 105°C) did not affect the spore viability significantly (P<0.05). Similar observations have been made by Gola et al. (1996). Further, higher inactivation was achieved during pressure come-up time at 600 and 700

MPa than at 400 MPa. Though the final process temperature was kept at 105°C, depending upon the target pressure, the initial temperature of the test samples was different (table 24). The microbial lethality attributed to nonisothermal effects during combined pressure–thermal treatment is not well characterized and this is a topic of current research interest (Ting, Balasubramaniam, & Raghubeer, 2002).

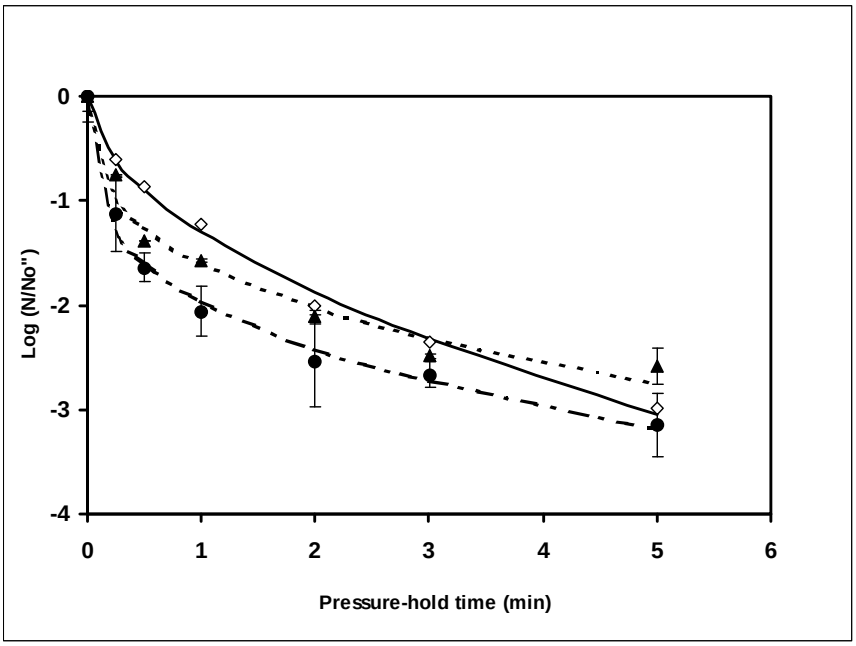

Figure 17. Observed and predicted (as fitted by Weibull model) survivor fraction of *B. stearothermophilus* spores in egg patty by PATP at different pressure levels. Observed: (◆) 400 MPa, (●) 600 MPa and (▲) 700 MPa. Predicted: (— –) 400 MPa, (– - –) 600 MPa and (- - - -) 700 MPa.

Thermal treatment of *B. stearothermophilus* spores in egg patties at 105°C and atmospheric pressure, for up to 20 min, did not yield any significant lethality (data not shown). Therefore, a thermal process with measurable spore lethality (i.e., 121°C for up to 15 min) was compared with the investigated PATP. When the process resistance was compared using *D* values (calculated from the linear segments of survivor plots), *B. stearothermophilus* spores exhibited significantly greater resistance ($P<0.05$) to the thermal treatment than to PATP (table 25). Accordingly, the combined pressure–thermal treatment, compared to temperature alone, accelerated the inactivation of *B. stearothermophilus* spores in egg patties. Gola et al. (1996) found that the application of high pressures caused morphological changes in the outer structure of bacterial spores. The visible changes in spore coats may serve as evidence that other sub-lethal

changes in the inner structures may have occurred due to the pressure treatment. Pressure-induced changes in the inner membrane, for example, may sensitize spores to the concurrent thermal treatment. Resistance of spores to PATP is greater in *B. stearothermophilus* than in many other *Bacillus* spp. such as *B. polymyxa* and *B. subtilis* (data not shown), but it is smaller than that in *C. botulinum* type A and B (Reddy et al., 2003). As mentioned before, *B. amyloliquefaciens* spores have been suggested as a possible surrogate for *C. botulinum* spores in PATP treatments.

Table 25. Model parameters of linear, Weibull and log-logistic models under various treatment conditions

Process	**Media**	**Temperature (°C)**	**Pressure (MPa)**	**Linear**					
				***D* (min)**	**MSE**[a]	R^2[b]	A_f[c]	***b***	***n***
PATP	Egg patty	105	400	0.72±0.17	0.04	0.95	1.55	1.30	0.53
PATP	Egg patty	105	600	0.41±0.12	0.17	0.93	1.97	1.96	0.30
PATP	Egg patty	105	700	0.53±0.17	0.12	0.92	1.86	1.60	0.34
PATP	Deionized water	105	700	0.41±0.12	0.66	0.93	1.67	3.19	0.27
Thermal	Egg patty	121	0.1	8.50±1.39	0.01	0.98	1.15		
Thermal	Deionized water	121	0.1	6.95±1.66	0.03	0.95	1.09		
Process	**Media**	**Temperature (°C)**	**Pressure (MPa)**	**Weibull**					
				MSE[a]	R^2[b]	A_f[c]	***A***	***σ***	
PATP	Egg patty	105	400	0.01	0.99	1.04	−7.90	−1.36	
PATP	Egg patty	105	600	0.02	0.99	1.05	−4.08	−0.70	
PATP	Egg patty	105	700	0.05	0.99	1.10	−3.27	−0.68	
PATP	Deionized water	105	700	0.10	0.99	1.08	−4.73	−2.20	
Process	**Media**	**Temperature (°C)**	**Pressure (MPa)**	**Log-logistic**					
				τ	**MSE**[a]	R^2[b]	A_f[c]		
PATP	Egg patty	105	400	2.31	0.01	0.99	1.33		
PATP	Egg patty	105	600	−0.13	0.01	0.99	1.17		
PATP	Egg patty	105	700	−0.13	0.03	0.99	1.22		
PATP	Deionized water	105	700	−0.45	0.02	0.99	1.11		

[a] The smaller the MSE values, the better the model can fit the data.

[b] The higher the R^2 values, the better the adequacy of the model to fit the data.

[c] A_f value closer to 1 indicates that the model produces the closest fit.

5.1.2. Influence of egg matrix on *B. stearothermophilus* inactivation

The *D* values for PATP inactivation of *B. stearothermophilus* in egg patties and deionized water were not significantly different (table 25). The $D_{p(105°C,\ 700\ MPa)}$ value for spore inactivation in egg patties was 0.53±0.17 min as compared to 0.41±0.12 min in deionized water. This is consistent with the observations of Reddy et al. (2003), who reported that a crabmeat blend (pH 7.2–7.4) did not protect *C. botulinum* BS-A and 62-A spores against inactivation by high pressure processing. The decimal reduction values for thermal inactivation (121°C) in egg patties and deionized water also showed no significant difference ($P<0.05$) (table 25). Despite the fact that the egg patties tested had a fat content of approximately 11 g/100 g of patty, the product does not seem to provide any protective effect against PATP treatments. Ananta et al. (2001) suggested that the protective effect in a fat rich medium is not due to the high fat content itself, but to the lower water activity in a fat-rich system. The egg patties tested had a high water activity ($\sim$0.99). This could explain why the solid constituents of egg patties (fats, proteins and minerals molecules) did not reduce the lethality of the combined pressure–heat treatment.

5.1.3. Modeling of inactivation curves

For the modeling of inactivation data, N_0'' (spore count after pressure come-up time) was chosen as the initial point of the log survivor fraction curve. Thus the inactivation achieved during pressure come-up time (0.75, 1.35 and 1.50 log reductions for 400, 600 and 700 MPa curves, respectively) was not considered in modeling. Among the nonlinear models tested, the Weibull model described best the PATP inactivation curves of *B. stearothermophilus* in egg patties (figure 17) followed by the log-logistic model (table 25). Though the Weibull and log-logistic model had similar MSE and R^2 values, the A_f (1.04 for 400 MPa, 1.05 for 600 MPa and 1.10 for 700 MPa) values for the Weibull model were considerably closer to 1, when compared to the A_f (1.33 for 400 MPa, 1.17 for 600 MPa and 1.22 for 700 MPa) values for the log-logistic model. This indicates that the Weibull model produces a better fit to the data obtained within the range of experimental conditions of the present study. The value of the parameters (*b* and *n*) of the Weibull model for different pressure levels is shown in table 25. For all the pressure levels tested, it was observed that *n* is less than 1, indicating that log survivor fraction curve has an upward concavity (Peleg & Cole, 1998).

It is evident that the *D* value and the *n* value of the Weibull model (table 25) change in a similar manner when the pressure level is changed. Accordingly, the pressure levels that yielded higher *D* values also resulted in higher *n* values. For example, at a pressure level of 600 MPa, the *D* value was 0.41 and the *n* value in the fitted Weibull model was 0.30. A decrease in the pressure level to 400 MPa resulted in a higher *D* value (0.72) and also a higher *n* value (0.53) in the Weibull model. The parameter b of the Weibull model increased for a treatment that yielded a greater log reduction. For example, treating egg with PATP for 5 min at 700 MPa inactivated 4.1 log *B. stearothermophilus* spores but a similar treatment for the spores suspended in water caused 6.1 log reductions. The *b* values for the curves fitted to these two sets of data were 1.6 and 3.2, respectively. Therefore, the Weibull model not only fit the PATP data closely, but also the parameters of this model are good indicators of inactivation kinetics.

5.2. INACTIVATION OF BACILLUS AMYLOLIQUEFACIENS SPORES IN EGG PATTY MINCE BY PRESSURE ASSISTED THERMAL PROCESSING

B. amyloliquefaciens spore preparation

B amyloliquefaciens Fas 82, a strain initially isolated from ropy bread, was kindly provided by M. Gänzle (Lehrstuhl für Technische Mikrobiologie, Technische Universität München, Freising, Germany). The bacterium was grown aerobically at 32 C for 24 h in Trypticase soy broth supplemented with 0.1% yeast extract (Difco, Becton Dickinson, Sparks, MD, USA). Preparation of spore suspension was adapted from the method of Margosch et al. (Margosh et al., 2004). Freshly prepared cultures (100 µl) of *B. amyloliquefaciens* were spread plated onto Trypticase soy agar (TSA: Difco, Becton Dickinson) supplemented with 10 ppm MnSO4 (Fisher Scientific). The inoculated plates were incubated at 32°C until more than 95% sporulation was observed (at least 10 days) by microscopic examination. Spores were then collected by flooding the surface of the plates with 10 ml of sterile distilled water and scraping the colonies with a sterile glass spreader. The collected spore suspension was washed five times by differential centrifugation (from 2000 to 8000 x g for 20 min each at 4°C), sonicated for 10 min with a sonicator (SM275HT, Crest, ETL Testing Laboratory, Cortland, NY, USA) having a peak power of 270 W, and then heated at 80°C for 10 min to destroy any remaining vegetative cells. The spore pellet was resuspended in deionized water to obtain approximately 10^9 spores per ml and

stored at 4°C until used. The spore concentration was determined by pour plating 1 ml of the spore suspension on TSA, and counting the number of colonies after incubation at 32°C for 48h.

Thermal inactivation of spores

The thermal inactivation of B. amyloliquefaciens spores in the egg patty mince was determined at 95, 105, 110 and 121°C in custom-fabricated aluminum tubes (12 mm inside diameter, 42 mm high, and 3 mm wall thickness). A sample of egg patty mince (0.9 g) was transferred into each aluminum tube, and 0.1 ml of the spore suspension (~10^9 spores per ml) was added to obtain a final spore concentration of approximately 1.4×10^8 spores per g of egg mince. Six tubes were then submerged simultaneously into a 28-liter circulating oil bath (Fisher Scientific), which was maintained at the desired target temperature. The sample temperature was monitored and recorded by inserting a K-type thermocouple attached to a data logger into a representative dummy aluminum cell containing the egg patty mince without spores. The heating time (come-up time) was recorded when the target temperature of the sample was reached. The process come-up times were approximately 3.58 min for 95°C, 3.33 min for 105°C, 3.25 min for 110°C, and 3.3 for 121°C. The first aluminum tube was removed from the oil bath at the end of the come-up time. The other aluminum tubes were subjected to five different holding times (up to 180 min), and the holding time intervals were different for each target temperature. After the thermal treatments, the tubes containing samples were immersed promptly into an ice water bath to avoid further inactivation. Spores surviving the thermal treatment were then counted.

High pressure microbial kinetic tester

A custom made high pressure kinetic tester (pressure test unit PT-1, Avure Technologies Inc., Kent, WA, USA) was used in the study (figure 18). The unit is rated to 700 MPa pressure and 130°C process temperature. It has a 54 ml stainless steel pressure chamber immersed in a temperature controlled bath, and the system is pressurized by an intensifier. The bath surrounding the pressure chamber was maintained at a suitable temperature (table 26) so that isothermal process conditions could be maintained throughout the pressure holding time. Propylene glycol was used as the heating medium in the bath.

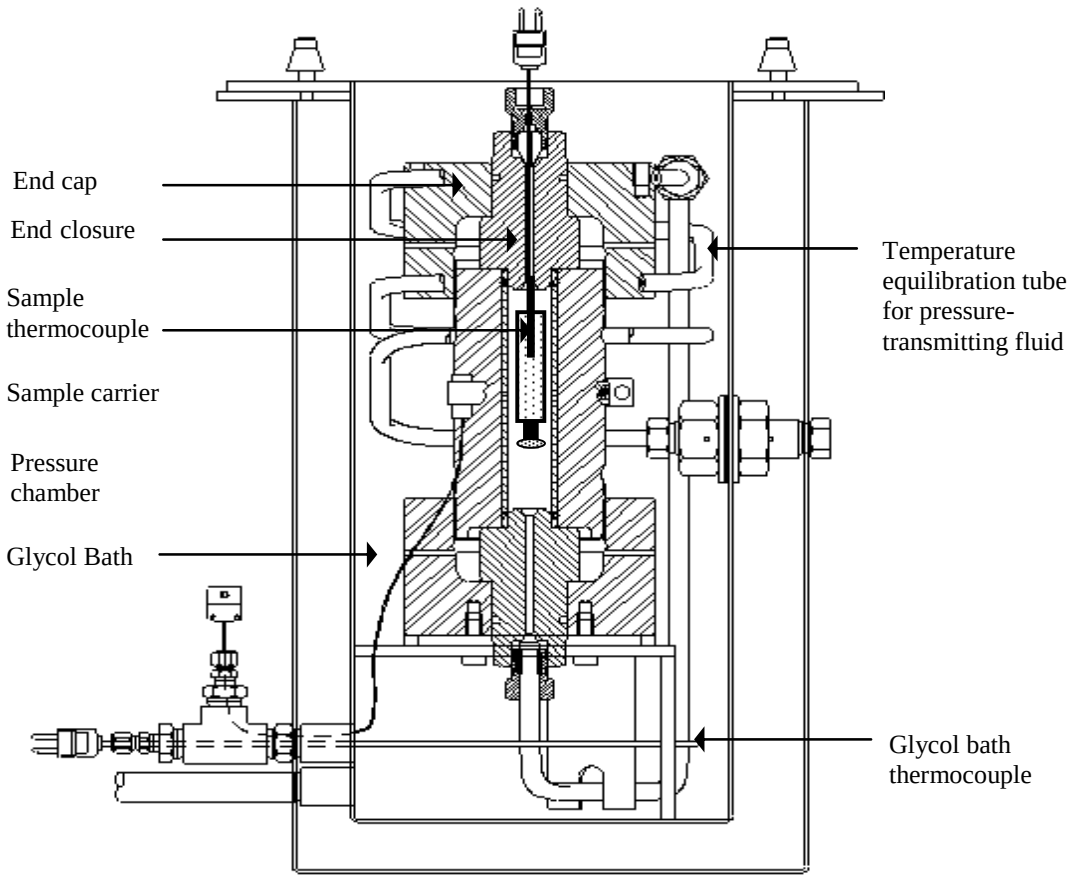

Figure 18. Cross section of high pressure kinetic tester (Adapted from the pressure test unit (PT-1) operation manual, Avure technologies, Kent, WA, USA).

Figure 19 shows sample pressure and temperature profiles at a combination of 700 MPa and 105°C. Although the pressure come-up time depended on the target pressure (table 26), the depressurization occurred in less than 4 s, regardless of the pressurization level. The sample temperature and chamber pressure were recorded every 2s during the entire treatment cycle with K-type thermocouple sensors and pressure transducers, respectively. A data acquisition computer equipped with relevant hardware and software was used to record the data.

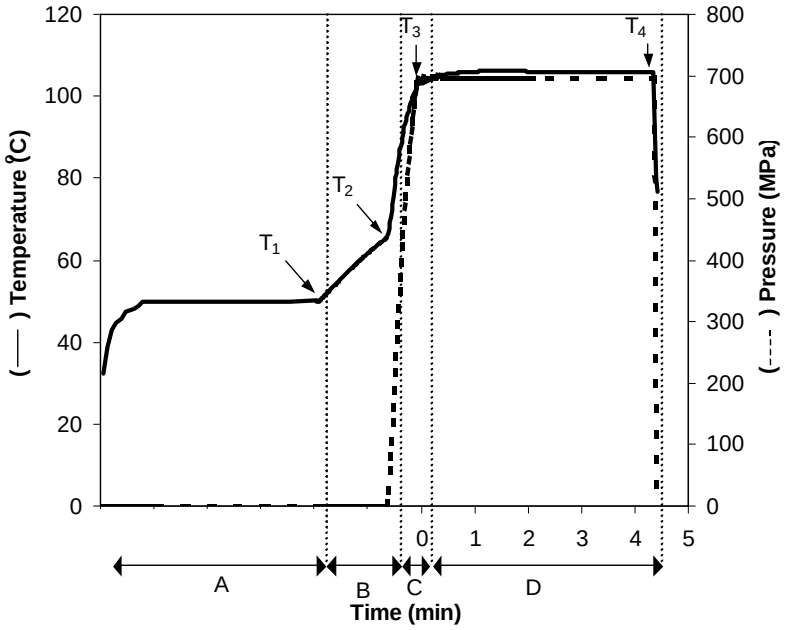

Figure 19. Pressure and temperature history experienced by the spore sample during preheating and pressure assisted thermal treatment at 700 MPa and 105°C. Data presented are mean values of two independent trials. Time scale includes: A. spore sample preheating time (min) in water bath prior to loading into pressure machine; B. time (min) for the sample in the pressure chamber to reach desired initial temperature before the commencement of pressurization; C. pressure come-up time (min); D. pressure holding time (min). Depressurization time (< 2 s) is not shown. Glycol bath was maintained at 105°C.

Preparing microbial test samples for PATP

Pouches (5 by 2.5 cm) made from sterile filter bags were used as sample holders. A sample of egg patty mince (0.9 g) and 0.1 ml of the spore suspension (~10^9 spores per ml) were placed inside the pouches to obtain a final spore concentration of approximately 10^8 spores per g of egg patty mince. The pouches were then heat sealed and the contents of the pouch were mixed thoroughly. The packaged samples were then placed in a sample carrier consisting of a 10 ml polypropylene syringe covered with two layers of insulating material. Water was used as the pressure-transmitting fluid within the syringe. Prior to pressurization experiments, the sample carrier containing the spore-inoculated egg patty mince was preheated in a water bath to a suitable preprocessing temperature, T_1. (table 26 and figure 19).

PATP of egg patty mince

Each preheated sample carrier was immediately loaded into the chamber of the pressure kinetic tester, and pressurization began when the sample temperature reached a predetermined value, T_2 (table 26 and figure 19). This temperature was estimated based on the following equation:

$$T_2 = T_3 - \{[(CH) \times \Delta P] + \Delta T_H\} \quad (11)$$

where T_3 is the desired process temperature, CH is the compression heating value of the test sample, ΔP is the pressure applied (MPa), and ΔT_H is the heat absorbed from the surrounding glycol bath during pressurization. Depending upon the target process temperature (T_3) and process pressure, ΔT_H was estimated on a trial and error basis. Compression heating values for egg samples were experimentally determined (data not shown) and were similar to the published value for water. The test samples were subjected to a combination of process temperatures (95, 105, 110 and 121°C) and pressures (500, 600, and 700 MPa) for a maximum of 15 min. The process holding times were adjusted for each combination of process pressure and temperature so that enough data were collected for subsequent analysis. The process holding times did not include the pressure come-up time or the depressurization time. After depressurization the samples were cooled immediately in an ice water bath. The untreated control samples (nonpressure treated inoculated egg patty mince) were heated at 80°C for 30 min to activate the spores for enumeration. Pouches containing the inoculated egg product (control and pressure treated) were opened aseptically, and their contents were used for determining the total viable spore count.

Table 26. Typical pressure assisted thermal processing settings used in this study

			Sample temp (˚C) at different stages of processing[a]		
Process pressure (MPa)	Pressure chamber glycol bath temp (˚C)	Come-up time (min)	Preheating (T_1)	Immediately before pressurization (T_2)	During pressure holding (T_3-T_4)
500	95	0.5 ± 0.1	60 ± 1.0	67.7 ± 0.6	95 ± 1.0
	105	0.5 ± 0.1	60 ± 1.0	75.7 ± 1.0	105 ± 1.0
	105	0.5 ± 0.1	60 ± 1.0	81.9 ± 0.3	110 ± 1.0
	105	0.5 ± 0.1	60 ± 1.0	92.6 ± 0.5	121 ± 1.0
600	95	0.6 ± 0.1	55 ± 1.0	61.0 ± 0.7	95 ± 1.0
	105	0.6 ± 0.1	55 ± 1.0	70.4 ± 1.2	105 ± 1.0
	105	0.6 ± 0.1	55 ± 1.0	75.9 ± 0.6	110 ± 1.0
	105	0.6 ± 0.1	55 ± 1.0	87.4 ± 0.8	121 ± 1.0
700	95	0.7 ± 0.1	50 ± 1.0	57.7 ± 1.1	95 ± 1.0
	105	0.7 ± 0.1	50 ± 1.0	67.1 ± 1.1	105 ± 1.0
	105	0.7 ± 0.1	50 ± 1.0	72.9 ± 0.4	110 ± 1.0
	105	0.7 ± 0.1	50 ± 1.0	84.1 ± 0.1	121 ± 1.0

[a] Figure 19 illustrates processing stages and the points at which T_1 through T_4 were measured. Data presented are mean ± standard deviation of two independent trials of various combinations of pressure, temperature, and holding time.

Enumeration of survivors

Heat or PATP treated samples (1g) were mixed with 9 ml of 0.1% peptone water and homogenized for 2 min in a stomacher. The homogenized sample was further serially diluted in peptone water and the dilutions (1 ml) were pour-plated on duplicate TSA plates. The plates were then incubated at 32°C for 48 h before enumeration. Colonies were counted with a dark field Quebec colony counter.

Determination of kinetic inactivation parameters

The decimal reduction time (D values) for different pressure and temperature combinations was calculated from the linear portion of the survivor curve, which occurred immediately after the come-up time, using the equation:

$$\log \frac{N}{N_0''} = \frac{-t}{D} \quad (12)$$

where N_0'' is the initial spore count measured immediately after the process (thermal or pressure) come-up time and N is the spores count after exposure to the lethal treatment for a specific time (t). The pressure coefficient, z_p (MPa), at constant temperature (i.e., the pressure required at constant temperature to achieve a 10-fold change in the D value) was estimated as a negative reciprocal of the slope resulting from plotting log D against pressure. Similarly, the temperature coefficient, z_T (°C) at constant pressure (i.e., the temperature change required at constant pressure to achieve a 10-fold change in the D value) was estimated as a negative reciprocal of the slope resulting from plotting log D against temperature. The reaction rate constant, k (min^{-1}), is inversely related to the D value and was determined using the relationship:

$$k = \frac{2.303}{D} \quad (13)$$

The volume change of activation ΔV (m^3 $mole^{-1}$), which is a measure of the net effect of pressure reactions causing physiological changes at constant temperature, was estimated using the following equation:

$$\Delta V = -RT\left(\frac{\Delta \ln k}{\Delta P}\right)_T \quad (14)$$

where P is the pressure (MPa), T is the absolute temperature (K), and R is the universal gas constant (8.314 10-6 m^3 mole $^{-1}$ MPa K^{-1}). The energy of activation Ea (J $mole^{-1}$), which describes the effect of temperature changes on the reaction rate (at constant pressure), was obtained from the following equation:

$$Ea = -R\left(\frac{\Delta \ln k}{\frac{1}{\Delta T}}\right)_P \quad (15)$$

where k is the rate constant (min^{-1}), T is the absolute temperature (K), and R is the universal gas constant 8.314 J mole $^{-1}$ K^{-1}.

Weibull model parameter estimation and curve fitting

The Weibull model was initially proposed by Peleg and Cole (1998) to describe microbial inactivation curves. The Weibull model is described by the following equation:

$$\log \frac{N}{N_0^{"}} = -bt^n \qquad (16)$$

where b and n are the scale and shape factors, respectively.

The curve fitting and model parameter estimation were achieved with the nonlinear procedure of the Statistical Analysis system software. Mean square error (MSE), regression coefficient (R^2), and accuracy factor (A_f) were used to evaluate the goodness of fit. The A_f values were calculated with the following equation:

$$A_f = 10^{[\Sigma|\log(predicted/observed)|]/n} \qquad (17)$$

5.2.1. Inactivation of *B. amyloliquefaciens* spores

Figure 20 is a comparison of survivor curves for B amyloliquefaciens during treatment of spores in egg patty mince by a combination of pressure (0.1 to 700 MPa) and heat (95 to 121°C). In general, PATP inactivation of B. amyloliquefaciens spores was biphasic, with rapid initial inactivation immediately after the pressure come-up time followed by a characteristic tailing during the extended pressure holding times.

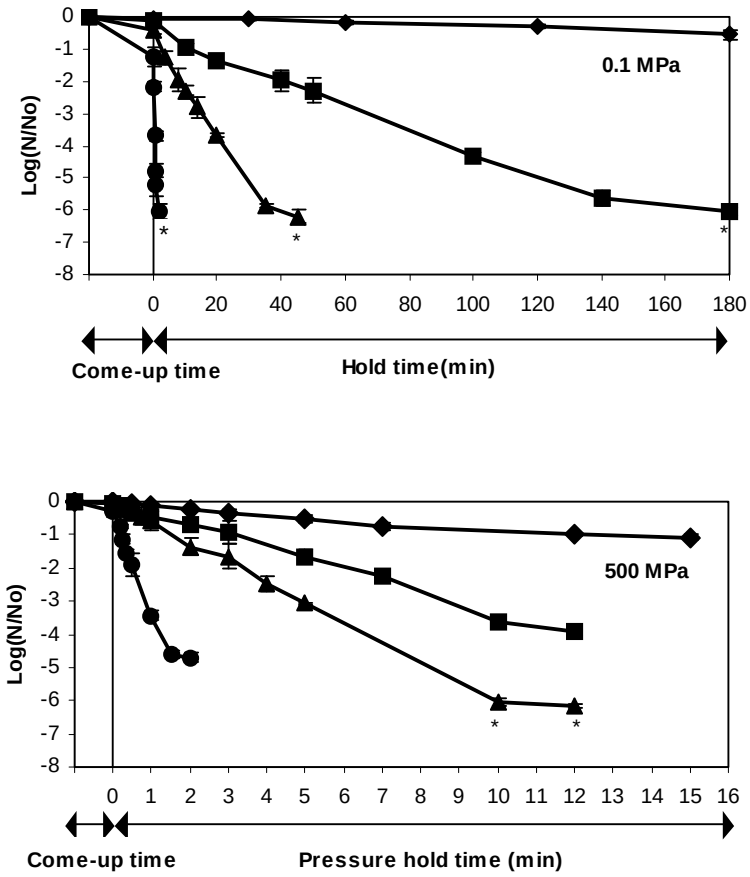

Figure 20. Log survivor fraction of *Bacillus amyloliquefaciens* spores in egg patty mince subjected to pressures of 0.1-700 MPa at temperatures of 95-121°C. (♦) 95°C, (■) 105°C, (▲) 110°C and (●) 121°C, during come-up time (hold time and pressure hold time).* Number of survivors was estimated (<20 spores/g).

The reduction in number of spores during the pressure come-up time varied between 0.1 and 1.2 log spores per g of egg product, depending on the treatment. Within the range of conditions tested in the present study, lower pressure-heat combinations (e.g., 500 MPa at 95 to 105°C) resulted in no or limited reduction in the spore count during the pressure come-up time, but application of higher pressure (700 MPa) and temperature (121°C) inactivated up to 1.2 log spores per g during that period (figure 20).

The lethality of PATP against B amyloliquefaciens spores increased with increases in process pressure at a given temperature (figure 20). For example, the D value for PATP at 105°C decreased with a pressure increase from 500 to 700 MPa (table 27).

Table 27. Linear and Weibull model kinetic parameters of Bacillus amyloliquefaciens spores during thermal or pressure assisted thermal processing

Pressure (MPa)	Temp (°C)	Linear, D (min)	Weibull[a]				
			b (95% CI)	n (95% CI)	MSE	R^2	A_f
500	95	11.58 ± 0.39	0.15 (0.12-0.18)	0.75 (0.66-0.83)	0.01	0.99	1.14
	105	2.9 ± 0.16	0.31 (0.23-0.38)	1.03 (0.85-1.02)	0.03	0.99	1.10
	110	1.66 ± 0.01	0.66 (0.43-0.71)	0.94 (0.86-1.21)	0.08	0.99	1.09
	121	0.32 ± 0.03	2.83 (2.60-3.07)	0.76 (0.63-0.89)	0.11	0.98	1.16
600	95	1.79 ± 0.10	0.91 (0.79-1.04)	0.66 (0.59-0.73)	0.03	0.99	1.12
	105	0.72 ± 0.01	1.60 (1.24-1.97)	0.56 (0.42-0.71)	0.23	0.97	1.40
	110	0.46 ± 0.01	2.30 (1.76-2.84)	0.59 (0.41-0.77)	0.44	0.97	1.36
	121	0.11 ± 0.02	4.92 (4.33-5.52)	0.51 (0.34-0.68)	0.52	0.97	1.23
700	95	0.80 ± 0.01	1.63 (1.35-1.92)	0.60 (0.46-0.74)	0.12	0.98	1.19
	105	0.31 ± 0.01	2.83 (2.49-3.17)	0.55 (0.41-0.69)	0.20	0.98	1.21
	110	0.21 ± 0.02	3.94 (3.51-4.36)	0.53 (0.40-0.67)	0.38	0.98	1.21
	121	0.08 ± 0.01	5.83 (5.01-6.65)	0.47 (0.32-0.61)	0.37	0.97	1.22
0.1	95	349 ± 49					
	105	24 ± 3.4					
	110	5.90 ± 0.87					
	121	0.25 ± 0.01					

a The smaller the MSE (mean square error) values and the higher the R^2 values, the better the model fits the data. A_f values closer to 1 indicate that the model produces a closer fit to the data.

Comparison of survivor curves (figure 20) illustrated as expected that increases in process temperature (at constant pressure) decreases the spore viability considerably. A temperature rise from 95 to 121°C at all pressure tested decreased the D-value significantly ($P < 0.05$) (table 27). Different combinations of pressure and temperature can bring about a similar level of B amyloliquefaciens spore inactivation. For example, PATP at 600 MPa and 105°C produced lethality similar to that of PATP at 700 MPa and 95°C.

Researchers have hypothesized that the nonisothermal conditions inside the pressure vessel can influence spore inactivation (De Heij et al., 2003; Rajan et al., 2005; Ting et al., 2002). Figure 21 shows the effect of temperature decline (nonisothermal conditions) during PATP of the survivor curves of B amyloliquefaciens spores.

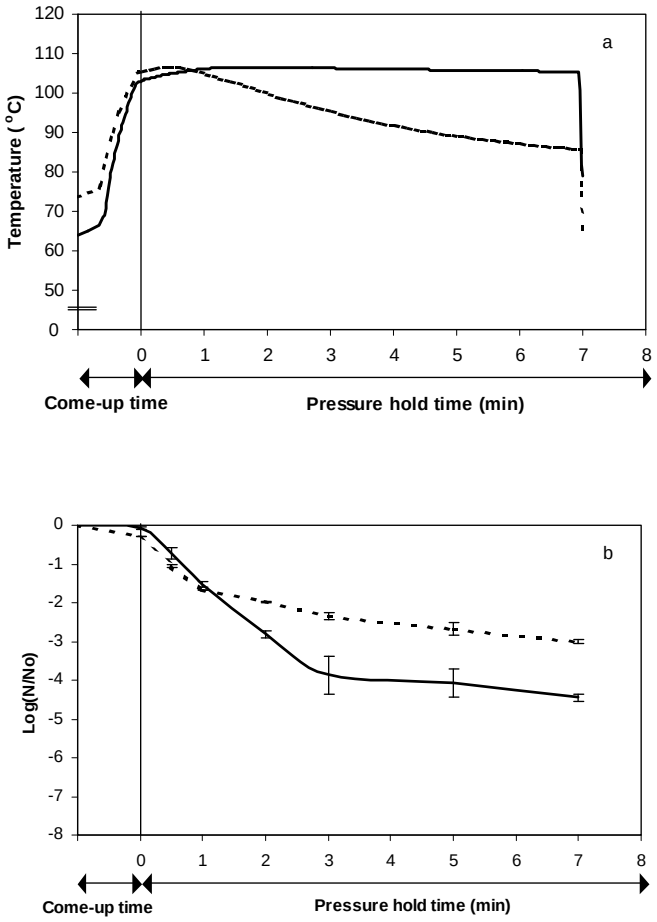

Figure 21. Time-temperature profile and the corresponding inactivation of *Bacillus amyloliquefaciens* spores in egg patty mince, when subjected to a pressure assisted thermal treatment at 600 MPa and 105^{o}C. (a) time-temperature profile. (b) survivor plot. (——) isothermal treatment at 105^{o}C. (------) nonisothermal treatment in the range of 105 to 85^{o}C.

The decline in temperature was simulated in the pressure kinetic tester by maintaining the temperature of the glycol bath below that achieved in the pressure chamber from compression heating. When spore survivor curves obtained under nonisothermal and isothermal conditions were compared, the nonisothermal conditions were less lethal overall (figure 21), which illustrates the importance of maintaining uniform conditions during PATP.

The interval between the beginning of the isobaric thermal process (i.e., when the targeted pressure and temperature are achieved) and the tailing of the spore population appeared to be a

function of processing conditions. Visual examination of figure 20 suggests that at a high process pressure (700 MPa) and temperature (121°C), tailing occurs easily, and these processing conditions were effective in decreasing spore populations by up to 4.6 to 5.8 log spores per g of egg product. Extended treatment appears to be ineffective for increasing lethality. More research is needed to understand bacterial tailing and to develop procedures that can minimize it and enhance process lethality.

5.2.2. Spore resistance during thermal processing or PATP

Resistance of B. amyloliquefaciens spores to PATP (as measured by the kinetic parameter, *D* value) was significantly less than that to thermal processing at an equivalent process temperature (table 27). However, synergy between heat and pressure diminishes at higher temperatures, and heat becomes the dominant contributor to lethality at 121°C. In figure 22, thermal treatment at 121°C (6.04-log reduction after 3.3 min come-up time and 2 min process holding time) appears to be more lethal than PATP treatment at 500 MPa and 121°C (4.38-log reduction after 0.5 min come-up time and 2 min process holding time). However a considerable part of heat treatment lethality (1.23-log reduction in spore numbers) occurred during the longer come-up time (3.3 min), whereas the shorter PATP come-up time (0.5 min) resulted in only a 0.32-log reduction. The come-up time in the pressure kinetic tester used in this study cannot be changed; therefore, no attempts were made to match, the come-up times of PATP and thermal treatments. In the absence of such comparable come-up times, it may not be possible to draw meaningful conclusions about the presumed protective effect of pressure at higher temperatures.

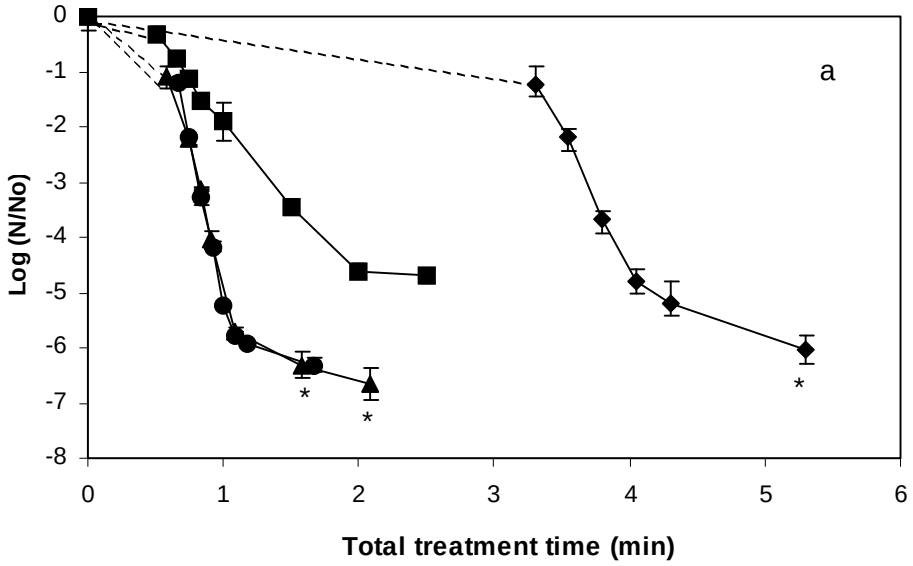

Figure 22. Log survivor fraction of *Bacillus amyloliquefaciens* spores in egg patty mince subjected to a process temperature of 121°C at pressures 0.1-700 MPa. (♦) 0.1 MPa, (■) 500 MPa, (▲) 600 MPa and (●) 700 MPa. Total treatment time (come-up time ------ and process holding time ——) and process holding time only. * Number of survivors was estimated (<20 spores/g).

5.2.3. Pressure and Thermal coefficients of B. amyloliquefaciens spores

The z_P values of *B. amyloliquefaciens* spores subjected to PATP increased from 171 MPa at 95°C to 332 MPa at 121°C (table 28). At 121°C, a similar z_P value (370MPa) was reported for *B. stearothermophilus* spores (Reddy et al., 1999). Our results suggest that the spores became increasingly less sensitive to pressure changes as the processing temperature increased. Therefore, contribution of pressure to spore lethality was less pronounced at higher (121°C) than at lower (95, 105 or 110°C) process temperatures. Values for activation volume (ΔV) at all temperatures (table 28) were negative, indicating that pressure has a lethal effect on *B. amyloliquefaciens* spores; this result is consistent with Le Chatelier's principle. In general, a less negative ΔV value signifies less sensitivity of spores to changes in pressure. In the current study, the ΔV value became less negative with an increase in process temperature; therefore the activation volume concept further confirms that the spores become less sensitive to pressure changes when the processing temperature increases.

B. amyloliquefaciens spores were more sensitive to temperature changes at atmospheric pressure (z_T = 8.2°C) than at higher pressure (z_T = 26.8°C at 700 MPa) (table 29). Activation energy decreased with increasing pressure (from 3.4×10^5 J/mole at 0.1 MPa to 1.1×10^5 J/mole at 700 MPa), suggesting that pressure synergistically contributed to spore lethality within the range of conditions studied.

Table 28. Pressure coefficients (z_P and ΔV) for *Bacillus amyloliquefaciens* spores suspended in egg patty mince at different temperatures during pressure assisted thermal processing

Process T (°C)	z_P (MPa)[a]	ΔV ($\times 10^{-5}$ m^3/mole)[b]
95	170 ± 1	-4.4
105	206 ± 2	-3.7
110	220 ± 6	-3.3
121	332 ± 42	-2.2

[a] higher z_P value implies less sensitivity to pressure change

[b] less negative ΔV value implies less sensitivity to pressure change

Table 29. Temperature coefficients (z_T and Ea) for *Bacillus amyloliquefaciens* spores suspended in egg patty mince at different pressures during pressure assisted thermal processing

Process pressure (MPa)	z_T(°C)[a]	Ea ($\times 10^{-5}$ J/mole)[b]
0.1	8.2 ± 0.2	3.4
500	16.7 ± 0.4	1.7
600	21.5 ± 1.5	1.3
700	26.8 ± 1.2	1.1

[a] Higher z_T value implies less sensitivity to temperature change.

[b] Lower Ea value implies less sensitivity to temperature change.

5.2.4. Influence of pressure and temperature on inactivation parameters of the Weibull model

For the model parameter estimation and curve fitting, spore lethality during the pressure come-up time was not considered. Preliminary model fitting was done using the Weibull and log-logistic models; however, the Weibull model was chosen to describe the inactivation curves because it resulted in a better fit to the raw data.

The Weibull model parameter b increased with increases in pressure and temperature (table 27). A higher b value corresponds to a steeper slope of the log survivor curve, which in turn implies that spore inactivation occurred at a faster rate. Consequently, there is an inverse relationship between b and D values (table 27), which can be described by the following equation:

$$b = \frac{t^{1-n}}{D} \qquad (18)$$

Thus, when n = 1 (indicating a linear inactivation), *b* and *D* are inversely proportional to each other. A value of *n* less than 1 indicates that the survivor curve in concave upward with the presence of tailing. In our study, most inactivation curves (fitted with Weibull models) produced *n* values less than 1 (table 27). An increase in pressure at a constant process temperature resulted in curves with a distinct linear slope region (i.e., a sharp tailing) (figure 20). Correspondingly, the value of the parameter *n* also decreased. Thus, curves in which tailing occurs suddenly have a lower *n* value than curves where tailing is gradual.

Different combinations of pressure and temperature bring about similar lethality for *B. amyloliquefaciens* Fad 82 spores during PATP. D values decreased with increases in pressure or temperature and were inversely related to the values of the Weibull parameter *b*. The processing resistance parameter, the D value for *B. amyloliquefaciens* spores in egg patty mince, was lower for PATP than for thermal processing at an equivalent temperature; however *B. amyloliquefaciens* spores were less sensitive to pressure changes at 121°C (indicated by the z_P and less negative ΔV values) than at lower temperatures. The sensitivity of *B. amyloliquefaciens* spores to temperature changes was less at 700 MPa (indicated by the higher z_T and lower Ea values) than at lower pressures.

5.3. INCUBATION STUDIES AND SHELF LIFE TESTS

Shelf stability of formulations #3 and #4 after HP/TS processing was evaluated using the end-point method, which consists of incubating samples at 37ºC and evaluating gas formation or

package bulging at selected times (Guan et al., 2003). Ten pouches of each formulation, control and processed at HP/TS1, HP/TS2, and retort (table 30), were incubated at 37°C for three months. Half of the pouches were removed for testing and the other half continued in incubation for three additional months. The pouches were checked for bulging every 2 to 3 d during incubation. Bulged pouches were indicative of presence of gas forming bacteria. At the end of the incubation period, all pouches were opened, and those without signs of gas formation or putrefaction were tested for TPA hardness, lightness L*, and *chroma* to evaluate texture and color degradation during the incubation period.

HP/TS processed products did not produce gas or decompose for at least six months (table 30). Control patties degraded after at least one week of incubation, some of them producing gas and some others undergoing proteolytic reactions; probably due to spoilage bacteria (Lake et al., 1985) that survived the milder heat treatment. In-pouch retort treated patties #4 had 10% positive samples after three months of incubation. It is possible that some spore-forming spoilage bacteria survived the in-pouch retort treatment.

Table 30. Percentage of packages showing gas formation and/or product decomposition, after three- and six-month incubation at 37ºC, of egg patty formulations #3 (cheese) and #4 (xanthan gum) treated at 700 MPa/105°C (HP/TS1), and formulation #4 treated at 700 MPa/121°C (HP/TS2) and in-pouch retorted.

Patty	Treatment	Storage time at 37ºC	Gas formation / decomposition
#4	Control	3 mo	Positive (100%)
#4	Control	6 mo	Positive (100%)
#4	HP/TS1	3 mo	Negative
#4	HP/TS1	6 mo	Negative
#4	HP/TS2 (F_0 = 3.3 min)	3 mo	Negative
#4	HP/TS2 (F_0 = 3.3 min)	6 mo	Negative
#4	In-pouch retort (F_0 = 5.6 min)	3 mo	Positive (10%)*
#4	In-pouch retort (F_0 = 5.6 min)	6 mo	Positive (10%)*
#3	HP/TS1	3 mo	Negative
#3	HP/TS1	6 mo	Negative

*Complete product degradation without gas formation

After three months, a darker surface was observed in all patties due to browning, which was reflected by lower L* values, indicating that 37ºC is a harsh condition for storage of egg-based products for long periods of time.

Egg patty #4, retort and HP/TS treated, did not change significantly in hardness and *chroma* during incubation at 37ºC for up to six months (table 31), whereas HP/TS1 treated patty #3 showed increasingly higher hardness values and lower yellow color, indicated by L* and *chroma* values.

Table 31. Accelerated shelf life test of formulations #2 (cheese) and #3 (xanthan gum) treated at 700 MPa/105°C (HP/TS1), and formulation #3 treated at 700 MPa/121°C (HP/TS2) and in-pouch retorted. TPA hardness and color values after 0, 3, and 6 mo storage at 37ºC. Different letters within the same treatment indicate significant differences between means ($P \leq 0.05$) within a column.

Patty	Treatment	Storage time at 37ºC	TPA Hardness (N)	L*	*chroma*
#4	HP/TS1	0 mo	35.5 ± 7.0 a	80.3 ± 0.8 c	26.2 ± 0.7 a
		3 mo	35.6 ± 5.4 a	73.3 ± 0.7 b	29.2 ± 0.6 a
		6 mo	46.1 ± 6.1 a	67.6 ± 0.9 a	29.7 ± 0.8 a
#4	HP/TS2	0 mo	36.5 ± 5.5 a	78.7 ± 0.8 c	27.1 ± 0.7 a
	(F_0 = 3.3 min)	3 mo	49.9 ± 5.5 a	71.8 ± 0.8 b	27.7 ± 0.7 a
		6 mo	49.7 ± 5.5 a	65.5 ± 0.8 a	28.4 ± 0.7 a
#4	In-pouch retort	0 mo	24.3 ± 5.7 a	76.2 ± 0.9 b	29.3 ± 0.8 a
	(F_0 = 5.6 min)	3 mo	24.7 ± 5.7 a	72.3 ± 0.9 a	29.1 ± 0.8 a
		6 mo	29.5 ± 5.5 a	71.9 ± 0.8 a	28.5 ± 0.7 a
#3	HP/TS1	0 mo	35.1 ± 4.5 a	77.1 ± 0.6 c	33.8 ± 0.5 a
		3 mo	73.8 ± 6.1 b	70.9 ± 0.8 b	32.6 ± 0.7 a
		6 mo	120.7 ± 5.5 c	53.2 ± 0.8 a	35.0 ± 0.7 b

Changes in *chroma* can also be directly related to the browning occurring during storage; however, there are other reasons that could explain changes in texture in egg patty #3: (a) continuing gelation during storage at 37ºC, (b) low serum remaining in the package after thermal pressurization, and (c) permeation of water outside the package.

Gelation did not occur to a great extent in formulation number #4, as shown by hardness values, which remained practically unchanged throughout the storage time (table 31). A factor that contributed to maintaining or increasing texture could be the water released inside the package after thermal pressurization. Previous work on egg patty formulations #3 and #4 showed that #3 patties gave 2-3% free liquid inside the package immediately after HP/TS1, whereas HP/TS1 treated formulation #4 gave 8-10% (Juliano et al., 2006). Therefore, a higher serum surrounding the surface of patty #4 could have contributed to maintaining the softness of the patty #4, as opposed to patty #3.

Permeation of water out of the package could be another factor that may have increased the hardness in patty #3 during storage, besides the low residual serum yielded. Little is known about the vapor permeability of packages after HP/TS treatment, which might be increased. In fact, after incubation, both formulations were observed to have reduced free liquid remaining in the package (data not shown). The surface of the HP/TS1 treated patty #3 looked drier, and no residual fluid was left on it; this is indicative of slow water diffusion during storage at 37ºC, which thereby created a harder structure. This phenomenon depends not only on the type of packaging film used, but also on the thickness of the sample, which sets its ability to retain water.

6. CONCLUSIONS

This short term project aimed to establish the feasibility of HP/TS processing for the production of shelf stable egg-based scrambled egg patty formulations. Special attention was given to identifying minimal process requirements for bacterial spores inactivation based on the current knowledge in the literature, egg product selection and characterization, product/process modification for texture improvement, and consumer acceptability of the end product.

The CORANET STP 2015 project proved the feasibility of high pressure thermal sterilization processing for the production of shelf stable egg-based scrambled egg patty formulations; however attainment of optimal commercial products requires more research.

The concept of combining high hydrostatic pressure and heat to commercially sterilize low acid foods is advancing from the laboratory bench to the pilot plant, as four pilot 35 L high pressure sterilization vessels are being used in industrial/government consortia projects. Patents have been published proposing different approaches, among which the application of a single pressure pulse of 600 MPa or greater, combined with temperature between 90 and 130°C, seems most appropriate from a food safety and economic point of view.

The preheating step makes HP/TS processing feasible from an economic point of view. Selection of preheating methods that provide minimal preheating time may contribute to minimizing quality damage after thermal pressure treatment. The HP/TS process parameters—preheating rate, pressure come up time, temperature at the beginning and end of holding/pressurization time, and at the end of pressure release—can be used as indicators of process performance in terms of heat retention. These parameters, or heat transfer models accounting for time-temperature (pressure) profiles, will ultimately allow determination of microbial inactivation and quality factors for process design and control.

From a quality standpoint, packaging material development and optimization can help meet the desired quality. Some polymeric plastic and aluminum foil laminated packaging materials have been identified that provide protection to the egg-based product during processing, shipping and handling.

Among the tested commercial egg patty formulations, all survived the high pressure/low temperature treatment, while maintaining overall quality. Thus, HP at low temperatures showed potential to produce stabilized prepackaged egg products intended for refrigerated storage. On the other hand, high temperature ≥ 70°C in combination with applied pressure enhanced further gelation of precoagulated egg protein network, increasing egg product hardness.

Various approaches tested, to improve texture and increase water retention of scrambled egg patties to commercially desired levels after HP/TS treatment, were identified. Product reformulation, egg patty porosity reduction, and use of low vacuum pressure helped lower egg product hardness.

Adequate product formulation can ensure that egg products maintain their sensory characteristics after HP/TS treatments. In fact, consumer acceptability of a HP/TS-treated egg patty formulation with added processed cheese shows that HP/TS processing may be a potential alternative for the production of novel shelf stable egg products for an increasing ready-to-eat market.

PATP not only accelerated the inactivation of *B. stearothermophilus* spores but also reduced the temperature required for inactivation. The egg patties did not offer any protective effect to *B. stearothermophilus* spores. Although PATP treatments at 600 and 700 MPa produced similar inactivation, higher pressure is preferred, since the desired final temperature can be achieved starting at a lower initial temperature. Among the nonlinear models tested, the Weibull model described the best PATP inactivation of *B. stearothermophilus* spores to be in egg. The use of such mathematical models to predict PATP inactivation of bacterial spores could allow processors and manufacturers to predict and control the safety and shelf life of foods at a design stage.

Different combinations of pressure and temperature bring about similar lethality for *B. amyloliquefaciens* spores during PATP. D values decreased with increases in pressure or temperature and were inversely related to the values of the Weibull parameter *b*, which is an indicator of the inactivation rate. The processing resistance parameter, the D value for *B. amyloliquefaciens* spores in egg patty mince, was lower for PATP than for thermal processing at an equivalent temperature; however, *B. amyloliquefaciens* spores were less sensitive to pressure changes at 121°C than at lower temperatures. The sensitivity of *B. amyloliquefaciens* spores to temperature changes was less at 700 MPa than at lower pressures.

Based on microbial spore surrogate inactivation data, a process of 700 MPa/105°C/5 min would suffice for commercial sterility. However, lower processing temperatures than 121°C can not yet assure sterilization. For this purpose, additional microbial inactivation data on many *C. botulinum* strains as well as surrogate spore-forming microorganisms of higher resistance are greatly needed. Hence, based on the current knowledge, regulatory approval can only be obtained by filing this technology as a thermal process.

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