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Theoretical and Experimental Investigation of the Thermal Inactivation of *Thermoanaerobacterium Thermosaccharolyticum* and *Geobacillus Stearothermophilus* in Different Canned Food Matrices

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Abstract. In the canning industry, thermal preservation processes typically are designed based on *Clostridium botulinum* thermal destruction kinetics. However, some bacteria can still survive, necessitating implementation of stricter time-temperature regimen for sterilization process. The aim of this study was to compare processing effectiveness at F_0 (sterilization value) 8 ± 1 min from the perspective of the vegetable-based product canning facility, while analyzing the inactivation, viability, and recovery of thermophilic bacteria. Four commercial products [tomato soup and rassolnik soup - acidified food (AF), and mushroom soup and pea porridge - low-acid food (LACF)] with different heat transfer characteristics (convection and conduction) were inoculated with $6.6 \log_{10}$ spores/ml *Geobacillus stearothermophilus* LMKK 244 (reported as DSM 6790 and ATCC 10149 in other collections) and $4.8 \log_{10}$ spores/ml *Thermoanaerobacterium thermosaccharolyticum* DSM 571 spore suspensions. Food samples contaminated with bacterial spores were processed in a steam-air retort at 118°C for 75 min. *G. stearothermophilus* and *T. thermosaccharolyticum* growth was not detected in AF samples ($\text{pH} = 4.4$ and 4.5), but was observed in LACF samples ($\text{pH} = 5.1$ and 5.8). Practical evaluation showed that *T. thermosaccharolyticum* did not survive thermal processing, which was verified using a presence/absence test after incubation at 55°C . *G. stearothermophilus* did not survive thermal processing, but recovered in pea porridge ($\text{pH} = 5.8$) during incubation. Our observations showed that food pH is a crucial factor determining microorganism survival during heat treatment and may be used by the vegetable-based product canning facilities to improve the food sterilization conditions.

Keywords: thermal destruction, thermophilic bacteria, retort sterilization, F_0 , D value.

Introduction

In the canning industry, thermal preservation processes are designed based on the pH of the product, which can be categorized as low-acid food (LACF) with $\text{pH} > 4.6$ (FDA, 2020) (or $\text{pH} > 4.5$ in some European countries) (Bratt, 2013) and acidified food (AF) with $\text{pH} < 4.6$. This threshold has been set to restrain the production of the botulin toxin by the mesophilic spore-forming bacterium, *Clostridium botulinum*, as both growth and toxin production are inhibited at $\text{pH} < 4.6$. The 12D process, which can destroy this bacterial population by a factor of 10^{12} ($12 \log_{10}$ cycles), is used for LACF products. The minimum sterilization value (

$F_{10}^{121.1^\circ\text{C}}$ in this case is 2.8 min, as the $D_{121.1^\circ\text{C}}$ value (the time required for $1 \log_{10}$ reduction of microorganism) of *C. botulinum* is 0.2–0.3 min and the z value (temperature required to achieve $1 \log_{10}$ change in D value) is 10°C . This sterilization value threshold should be considered as the minimum threshold for LACF (Teixeira, 2006).

Nonetheless, although the product is virtually safe for consumption, some microorganisms may withstand this time-temperature regimen. From the perspective of the canning facility where this experiment was carried out, a thermal processing regimen targeting thermophilic spore-forming bacteria that can spoil the products is urgently

required. There are bacterial species that can grow in AF products. One of the most common organisms associated with spoilage of food is the thermophilic *Bacillus coagulans* (André, Vallaëys, & Planchon, 2017), which can grow in LACF products at moderately low pH (4.2–4.3) and can endure high temperatures ($D_{110}^{\circ\text{C}} = 0.27$ min, $z = 8.3^{\circ}\text{C}$) (Peng *et al.*, 2012); furthermore, $F_{121.1}^{\circ\text{C}} = 0.26$ min is required to eliminate it by a factor of 10^{12} (12D concept). Numerous

surveys have reported the cause of microbial spoilage of LACF canned food, which has been discussed in detail by André, Vallaëys and Planchon, 2017. The most common species that cause food spoilage are *Geobacillus stearothermophilus*, *Thermoanaerobacterium thermosaccharolyticum*, *Moorella thermoacetica*, *Bacillus coagulans*, *Bacillus smithii*, and *Bacillus licheniformis*, the D values of which are summarized in Table 1.

Table 1

D values (min) of selected bacteria obtained from literature

Microorganisms	pH ¹²	D ₁₀₅	D ₁₁₀	D ₁₁₅	D ₁₁₆	D ₁₂₀	D ₁₂₁	D ₁₂₅	z
<i>G. stearothermophilus</i> ¹	6.0			3.14		1.25		0.26	8-12
<i>G. stearothermophilus</i> ¹	5.0			2.49		0.59		0.20	8-12
<i>G. stearothermophilus</i> ¹	4.0			1.39		0.43		0.10	8-12
<i>G. stearothermophilus</i> ^{2,3,4}							4.00- 5.00		
<i>G. stearothermophilus</i> ⁵							2.53	0.64	7.42
<i>G. stearothermophilus</i> ⁶	7.0			10.00					
<i>T. thermosaccharolyticum</i> ⁷	6.6		12.16						
<i>T. thermosaccharolyticum</i> ⁸	6.6	11.80							
<i>T. thermosaccharolyticum</i> ^{2,3,4}							3.00 – 4.00		
<i>T. thermosaccharolyticum</i> ⁶	7.0				10.00				
<i>Moorella thermoacetica</i> ⁶	7.0						83.00- 111.00		
<i>Moorella thermoacetica</i> ⁹	7.0							10.00	6.10- 8.60
<i>Bacillus coagulans</i> ¹⁰		1.32	0.16						8.68
<i>Bacillus licheniformis</i> ¹¹						0.18			
<i>Bacillus smithii</i> ⁶	7.0	10.00	10.00						6.10- 7.80

¹ López *et al.*, 1997; ² Jay, Loessner, & Golden, 2005; ³ Ababouch, 2014; ⁴ Warne, 1988; ⁵ Somavat *et al.*, 2012; ⁶ André, Zuber, & Remize, 2013; ⁷ Mtimet *et al.*, 2016; ⁸ Ahn, Balasubramaniam, & Yousef, 2007; ⁹ Byrer, Rainey, & Wiegel, 2000; ¹⁰ Peng *et al.*, 2012; ¹¹ Janštová & Lukášová, 2001; ¹² Given pH values indicates medium pH at which D value was measured.

Previously, several of these bacteria were detected in experimental Kronis Ltd. (Latvia, Bauska) canned ready-to-eat meals. Spoiled products were analyzed at the Latvian National Reference Laboratory (BIOR), and the isolated microorganisms were identified using matrix-assisted laser desorption ionization time-of-flight mass spectrometry. The most common thermophilic bacterial isolates were *B. smithii*, *B. licheniformis*, *G. stearothermophilus*, and *T. thermosaccharolyticum*. Better understanding of heat

penetration into the product core during sterilization and consequent modifications in the time-temperature process has circumvented the problems associated with food spoilage by the *B. smithii* and *B. licheniformis*. However, the latter two are significantly more heat-resistant; for example, to achieve 12 log₁₀ reduction of *G. stearothermophilus* ($D_{121} = 5$ min), it is necessary to achieve $F_{121.1}^{\circ\text{C}} = 60$ min, which is not possible because of considerable reduction in product quality parameters (Kirse-Ozolins *et al.*, 2019).

G. stearothermophilus and *T. thermosaccharolyticum* are thermophilic spore-forming bacteria that grow between 45 °C and 70 °C, with an optimum temperature between 50 °C and 60 °C (Jay; Loessner, & Golden, 2005). The optimal pH and minimal pH for growth of *G. stearothermophilus* are 7.7 (Mtimet *et al.*, 2015) and 4.8-5.0, respectively (Durand *et al.*, 2015), whereas they are 6.5 and 4.1 (Mtimet *et al.*, 2016), respectively, for *T. thermosaccharolyticum*. *G. stearothermophilus* causes “flat sour” (Durand *et al.*, 2015) spoilage of canned food, and *T. thermosaccharolyticum* has been called “the swelling canned food spoiler” (da Silva *et al.*, 2018) because of gas production during growth (Delves-Broughton, 2008).

As the D values for *G. stearothermophilus* and *T.*

thermosaccharolyticum vary in literature (Table 1), in this study, we decided to compare processing effectiveness at $F_0 = 8 \pm 1$ min from the perspective of the canning industry, while analyzing the inactivation, viability, and recovery of selected thermophilic bacteria and heat transfer in AF/LACF products. This study will enhance our understanding regarding the thermal sterilization process targeting thermophilic bacteria, which will be useful for generating safer canned food products.

Materials and Methods

Tested products

Four commercial products with different pH and heat transfer types were selected for this study (Table 2).

Table 2

Canned food products

Product	Heat transfer type	Structure description	Group	pH	Water activity (a_w)
Tomato soup	Convection	Thick liquid, ready-to-eat tomato soup puree	AF	4.4	0.99
Mushroom soup	Convection	Liquid soup concentrate, which consists of shredded mushroom pieces in a broth	LACF	5.1	0.98
Pea porridge	Conduction	Solid pea porridge without liquid phase, but of soft consistency	LACF	5.8	0.98
Rassolnik soup	Conduction	Thick soup concentrate, which consists of submerged and shredded food pieces in a thick liquid	AF	4.5	0.98

Tomato soup and rassolnik soup concentrate are both AFs, but with different heat transfer behaviors. The same is true for the LACF, mushroom soup and pea porridge (Kronis Ltd., Bauska, Latvia). Products were packaged in 500 mL glass jars, 12 units each, and pre-treated at 116 °C for 60 min (holding phase) to achieve “commercial sterilization”.

Bacterial strain

G. stearothermophilus LMKK 244 active culture was obtained from the Microbial Strain Collection of Latvia (University of Latvia, Riga). This strain has been reported in other collections under the numbers DSM 6790 and ATCC 10149. Freeze-dried *T. thermosaccharolyticum* DSM 571 was obtained from the German Collection of Microorganisms and Cell Cultures (Leibniz Institute DSMZ, Braunschweig). This strain has been reported in other collections under the accession number ATCC 7956.

Media

G. stearothermophilus LMKK 244 was grown in tryptic soy broth (TSB) (pH 7.4, Biolife, Milan, Italy) and tryptic soy agar (TSA) (pH 7.4, Biolife, Milan, Italy). Spore concentration and viable cell counts were determined by performing serial dilutions on TSA using tryptone salt broth (pH 7.0 Biolife, Milan, Italy).

T. thermosaccharolyticum DSM 571 was grown in chopped liver broth [500 g/L fresh beef liver (from local

market), 10 g/L peptone (Biolife, Milan, Italy), 1 g/L K_2HPO_4 , and 1 g/L soluble starch; pH adjusted to 7.0 before sterilization via autoclaving] and sporulation medium, which was chopped liver broth supplemented with 10 mg/L of $MnSO_4$ and 10 mg/L $CaCl_2$, pH 6.6. All chemicals were purchased from Chempur (Karlsruhe, Germany). Spore concentration and viable cell counts were determined by performing serial dilutions on reinforced Clostridial agar (RCA, pH 6.8, Sigma-Aldrich, MO, USA) using tryptone salt broth.

Spore preparation

G. stearothermophilus was firstly pre-cultured in a 100 mL glass bottle containing 50 mL TSB and incubated for 48 h at 55 °C under aerobic conditions. Then, 200 µL of the 48-h culture was spread onto 135 identical TSA plates and incubated at 55 °C for 8 days, under aerobic conditions. During the incubation, the plates were placed inside plastic bags containing wet tissue to minimize dehydration of the agar. The presence of spores was visually assessed using a light microscope (100 × 4 Oil ph3, Nikon, Shanghai, China) after staining with Malachite Green (Sigma-Aldrich, MO, USA). After incubation, *G. stearothermophilus* spores were harvested from the plates by adding 4 mL of sterile distilled water to the surface of each plate and scraping of the bacterial lawn with a sterile plastic spreader. The spore suspension was transferred to 50 mL centrifuge tubes. The samples were then washed four times with distilled water, separated via

centrifugation ($4,700 \times g$, 20 min), and stored in distilled water at 4 °C until experimental use. Prior to inoculation of the food samples, spores of *G. stearothermophilus* were re-suspended in distilled water and heat-treated at 90 °C for 30 min in a water bath to eliminate vegetative cells.

The first anaerobic pre-culture of *T.*

thermosaccharolyticum DSM 571, obtained from 1 mL frozen aliquots, was performed in 100 mL liver broth in a glass jar at 55 °C for 24 h with horizontal shaking (100 rpm). Ten milliliter inoculate in eight 200 mL glass jars containing 100 mL liver broth supplemented with 10 mg/L $MnSO_4$ and 10 mg/L $CaCl_2$ was used for sporulation. Sporulation was then conducted at 55 °C for at least a month under anaerobic conditions. The presence of spores was visually assessed using a light microscope (Nikon, 100 × 4 Oil condenser annulus ph3) after staining with Malachite Green. The spore suspension was transferred to 50 mL centrifuge tubes and harvested via centrifugation ($8,000 \times g$, 20 min), washed four times with distilled water, and stored at 4 °C until experimental use. Anaerobic conditions were created in plastic 7 L containers (GENbox, bioMerieux, Lyon, France) with anaerobe paper sachets (BD GasPakTM EZ, NJ, USA).

Inoculation of food samples

The spore concentrates of both bacteria were re-suspended in 40 mL aliquots, resulting in $6.6 \log_{10}$ spores of *G. stearothermophilus* and $4.8 \log_{10}$ spores of *T. thermosaccharolyticum* per milliliter. Furthermore, 36 mL suspension (12 mL × 3) was inoculated in four types of commercial products as follows: (1) 12 mL spore suspension for determining the number of spores in 1 g product; (2) 12 mL for determining the number of spores 24 h after sterilization; (3) 12 mL for determining the number and presence of spores 2 weeks after sterilization with incubation at 55 °C. The remaining 4 mL spore suspension was used to quantify spores in 1 mL heat-shocked cultures and to

inoculate triplicates of the target products with the aim of (4) determining the growth rate of spores in the products under optimal conditions (see below) when 12 mL decimal dilution of the spore suspension was used.

To determine the growth rate of *G. stearothermophilus*, 1 mL spore suspension was added to 500 g of each product in glass jars and incubated for 48 h at 55 °C under aerobic conditions. To determine the growth rate of *T.*

thermosaccharolyticum, 1 mL spore suspension was added to 100 g of each product in open sterile bags instead of glass jars due to technical limitations. Samples were placed in plastic 7 L containers with anaerobe paper sachets to create anaerobic conditions during the 72 h incubation at 55 °C.

Cell count and presence/absence detection

After sterilization, the contaminated and heat-treated tomato soup, rassolnik soup, mushroom soup, and pea porridge jars in triplicate were incubated at 21 ± 2 °C for 24 h before cell count analysis, and in thermostats at 55 °C for two weeks to allow germination of the bacteria that were able to survive processing temperatures. Prior to cell counting and presence/absence detection, all samples were homogenized using a dispersing system T18 Ultra-Turrax with S18N-19G headpiece (IKA, Staufen, Germany). For cell count analysis, 10 g of each sample was diluted with 90 mL tryptone salt broth. Subsequently, the serial dilutions were transferred to the plates. For presence/absence analysis, 1 g of the product was transferred to 10 mL broth. Bacterial count analysis is performed after serial dilutions and represents the microorganism count in 1 g product; hence, inoculation of 500 g sample with 1 mL spore suspension ($6.6 \log_{10}$ and $4.8 \log_{10}$ respectively) indicates the limit of detection of spore count, as $2 \log_{10}$ spores are lost during the number conversions (from spores per 500 g of sample to spores per 1 g of sample). Analysed bacteria were detected during presence/absence analysis.

Figure 1. Study design.

Sterilization

Sterilization of inoculated samples was performed in a SN4280 steam-air retort (Panini Ltd., Modena, Italy) at the Kronis Ltd. (Bauska, Latvia) production facilities. The process holding time was set to 75 min at 118 °C. The product core temperatures were measured using wireless thermocouple (Tecnosoft, Peschiera Borromeo, Italy). The sterilization value (F_0) was calculated by the general method based on the time-temperature data (Bigelow *et al.*, 1920).

Theoretical evaluation of spore count after heat treatment

Theoretical evaluation of spore count in the product after heat treatment was performed as follows:

$$A = B - \left(\frac{F_0}{D} \right),$$

where:

- A – theoretical evaluation of spores in the sample after heat treatment, $\log_{10}$;
- B – spore concentration in inoculant, $\log_{10}$;
- F_0 – sterilization value calculated based on time-temperature data obtained from the core of the sample during the heat treatment, min;
- D – D value of chosen bacteria, min.

For theoretical evaluation, it was decided to select the highest D values found in literature, i.e. D = 5 min for *G. stearothermophilus* and D = 4 min for *T. thermosaccharolyticum*.

Data processing

Theoretical evaluation of spore count was carried out in Microsoft Excel v16.0. Represented data of microbiological analysis results are the means of three replicates. Means with p-values less than 0.05 were considered statistically significant. Descriptive statistics were carried out in IBM SPSS Statistics v26.

Results

Bacterial growth behavior in different food matrices

Bacterial growth at optimal temperature was analyzed to study bacterial behavior in the non-heat-treated samples. The results (Table 3) showed that the *G. stearothermophilus* count did not change significantly ($p < 0.05$) after incubation for AF products, but increased in LACF products. *T. thermosaccharolyticum* was not detected in AF products, but were detected in LACF products ($p < 0.05$). Among the tested products, pea porridge appeared to be the best medium for the growth of thermophilic bacteria.

Table 3

Bacterial growth in selected food matrices

Product	pH	<i>Geobacillus stearothermophilus</i>		<i>Thermoanaerobacterium thermosaccharolyticum</i>	
		Spore count in the product	Cell count after 48 h at 55 °C	Spore count in the product	Cell count after 72 h at 55 °C
		$\log_{10}$ CFU/g	$\log_{10}$ CFU/g	$\log_{10}$ CFU/g	$\log_{10}$ CFU/g
Ts ¹	4.4	2.9 ± 0.01	2.5 ± 0.01	1.1 ± 0.01	<1
Ms ²	5.1	2.9 ± 0.07	5.8 ± 0.02	1.1 ± 0.07	4.5
Pp ³	5.8	2.9 ± 0.17	7.1 ± 0.04	1.1 ± 0.18	7.2
Rs ⁴	4.5	2.9 ± 0.09	2.7 ± 0.02	1.1 ± 0.08	<1

¹ Tomato soup; ² Mushroom soup; ³ Pea porridge; ⁴ Rassolnik soup.

Theoretical evaluation of spore count in the product

The products showed various F_0 values, which were achieved during the sterilization process (Table 4). Owing to the differences in the structures of the product, which affected the heat penetration characteristics, the most liquid and homogenous product had the largest F_0 values, whereas the thickest and heterogeneous product (rassolnik soup) had the lowest F_0 value. This sterilization value is expected to destroy 1.4–1.6 CFU $\log_{10}$ /g of *G. stearothermophilus* with $D_{121} = 5$ min (Table 1); therefore, the number of surviving *G. stearothermophilus* should be 2.3–2.5 CFU $\log_{10}$ /g (Table 4). For *T. saccharolyticum* with $D_{121} = 4$ min (Table 1), the

expected destruction rate is 1.8–2 CFU $\log_{10}$ /g; therefore, 0.1–0.3 CFU $\log_{10}$ /g can be detected via counting.

Table 4

Theoretical evaluation of spore count in the product

<i>Geobacillus stearothermophilus</i>					<i>Thermoanaerobacterium thermosaccharolyticum</i>			
Product	F ₀	Spore reduction on reference = 5 min	log ₁₀ based D ₁₂₁	Theoretical cell count in the product*	F ₀	Spore reduction on reference = 4 min	log ₁₀ based D ₁₂₁	Theoretical cell count in the product*
	min	log ₁₀ CFU/g		log ₁₀ CFU/g	min	log ₁₀ CFU/g		log ₁₀ CFU/g
Ts ¹	8.2	1.6		2.3	8.0	2.0		0.1
Ms ²	7.6	1.5		2.5	7.8	1.9		0.1
Pp ³	7.3	1.5		2.4	7.3	1.8		0.2
Rs ⁴	7.1	1.4		2.5	7.2	1.8		0.3

¹ Tomato soup; ² Mushroom soup; ³ Pea porridge; ⁴ Rassolnik soup;

* Theoretical spore count is based on spore count in the product (Table 5).

Growth and post-heat treatment recovery

Spore count analysis after sterilization (Table 5) did not reveal *G. stearothermophilus* and *T. thermosaccharolyticum* growth, which does not agree with the theoretical calculations (see 3.2 section).

Table 5

Growth and recovery of *Geobacillus stearothermophilus* and *Thermoanaerobacterium saccharolyticum* in the product after heat treatment

<i>Geobacillus stearothermophilus</i>						<i>Thermoanaerobacterium thermosaccharolyticum</i>				
Pro-duct	Spore count in the inoculant	Spore count in the product	Cell count after 22 ± 2 h	Cell count*	Cell pre-sence*	Spore count in the inoculant	Spore count in the product	Cell count after 22 ± 2 h	Cell count*	Cell pre-sence*
	log ₁₀ CFU/g	log ₁₀ CFU/g	log ₁₀ CFU/g	log ₁₀ CFU/g	g ⁻¹	log ₁₀ CFU/g	log ₁₀ CFU/g	log ₁₀ CFU/g	log ₁₀ CFU/g	g ⁻¹
Ts ¹	6.6	3.9 ±0.01	<1	<1	-	4.8	2.1 ±0.01	<1	<1	-
Ms ²	6.6	3.9 ±0.07	<1	<1	-	4.8	2.1 ±0.07	<1	<1	-
Pp ³	6.6	3.9 ±0.17	<1	4.7 ±0.32	+	4.8	2.1 ±0.18	<1	<1	-
Rs ⁴	6.6	3.9 ±0.09	<1	<1	-	4.8	2.1 ±0.08	<1	<1	-

¹ Tomato soup; ² Mushroom soup; ³ Pea porridge; ⁴ Rassolnik soup; *after 2 weeks at 55 °C

Considering that the count analysis does not reveal the 2 CFU log₁₀ that are “lost” because of number conversions from 1 mL of inoculant to 1 g of product (considering inoculation of 500 g of product), samples were stored at 55 °C to create the most favorable environment for bacterial growth, followed

by spore counting and presence/absence analyses. *G. stearothermophilus* was detected only in pea porridge (pH = 5.8), while *T. thermosaccharolyticum* was not detected in any sample.

Discussion

Growth of either *G. stearothermophilus* and *T. thermosaccharolyticum* was not detected in AF samples (pH = 4.4 and 4.5), but was observed in LACF samples (pH = 5.1 and 5.8). Theoretical calculations showed that the expected detectable number of *G. stearothermophilus* and *T. thermosaccharolyticum* after sterilization were 2.3–2.5 CFU log₁₀/g and 0.1–0.3 CFU log₁₀/g, respectively. Practical evaluation showed (Table 5) that *T. thermosaccharolyticum* does not survive thermal processing, which was verified using a presence/absence test after incubation (2 weeks). *G. stearothermophilus* did not grow after processing, but recovered in pea porridge (pH = 5.8) during incubation (2 weeks).

Considering that the difference in F_0 values between the pea porridge and mushroom soup was not outstanding, and that both bacteria can grow in LACF products (Table 3), we expected them to grow in mushroom soup and pea porridge after 2 weeks at 55 °C. Surprisingly, spore count analysis of samples incubated in a thermostat for 2 weeks showed that *G. stearothermophilus* grew only in the pea porridge, whereas *T. thermosaccharolyticum* growth was not detected after product processing. Subsequent presence/absence analysis did not show growth of either bacterium in the products, except for that of *G. stearothermophilus* in the pea porridge.

This contradiction in theoretical and the empirical data (Table 4) can be explained in two ways:

a) The D values of the strains used in this study were lower than those described earlier (Table 1). For example, based on the calculation, $F_0 = 7.64$ is expected to destroy 6.6 log₁₀ spores inoculated species with heat resistance parameter $D_{121} = 1.2$ min. The D values used for the calculations are approximate for the genus in general, whereas in reality, the values may be dispersed. Kotzekidou (2014) reported variability in D_{120} values between various strains of *G. stearothermophilus* (1.0–5.8 min). Rigaux, Denis, Albert, & Carlin (2013) performed a meta-analysis on *G. stearothermophilus* spore heat resistance by analyzing D values in published studies and arrived at a mean D value of 3.3 min.

Various investigators have observed correlations between spore heat resistance and medium pH, as well as sporulation conditions (temperature, pH). Palop *et al.* (1999) found that the D_{111} values of *B. coagulans* spores varied from 0.49 min at pH 4 to 1.7 min at pH 7. Cameron, Leonard and Barrett (1980) showed that the $D_{112.8}$ value of *C. sporogenes* at pH 5 is 7 min; however, it gradually increases with pH, and $D_{112.8} = 15$ min at pH 6.5.

Mtimet *et al.* (2016, 2015) studied *G. stearothermophilus* ATCC 12980 and *T. saccharolyticum* DSM 571 spore heat resistance as a function of sporulation parameters and observed that spore heat resistance is generally higher in spores that are produced under conditions most favorable for the growth of a particular strain. In this study, spores of the *G. stearothermophilus* LMKK 244 strain, were produced under optimal conditions (55 °C, pH 7.4). The *T. thermosaccharolyticum* DSM 571 spores used in this study were produced under the same conditions that were considered to be most favorable for developing maximum heat resistance of spores by Mtimet *et al.* (2016). The D values for the strains used in this study are not available; however, we assumed that the pH of the product medium affected spore

heat resistance, which led to higher spore destruction rate than expected.

b) Bacteria damaged during processing were not able to recover. André *et al.* (2021) and Mtimet *et al.* (2016) showed that the rate of post-heat-treatment reduction in bacterial count depends on the pH-temperature combination; for example, for *G. stearothermophilus* strain 2804 138, time of first decimal reduction (δ) was 97 h at pH 5.0, 77 h at pH 4.6, and 65 h at pH 4.2. This allows us to assume that if < 2 log₁₀ CFU/g bacteria have survived, which cannot be detected using plate count analysis, *G. stearothermophilus* spores cannot recover in mushroom soup, and *T. thermosaccharolyticum* cannot recover in either mushroom soup or pea porridge after storage at 55 °C for 2 weeks.

This study has certain limitations. Serial dilutions have to be performed to determine the bacterial count in 1 g of the product; as the inoculation was performed in 500 g of the product, we could not detect 2 log₁₀ CFU/g via counting after sterilization. However, consequent incubation (2 weeks at 55 °C) allowed survived microorganisms to germinate and subsequent analysis of samples allowed us to obtain and extrapolate the results.

Conclusions

While designing the sterilization process based on the heat destruction characteristics of the target microorganism, the retention of the D value with respect to the pH of the product should be considered. The closer the product pH (4.4, 4.5, 5.1, 5.8) is to the optimum pH required for the growth of the target organism, which is pH 7.7 for *G. stearothermophilus* and pH 6.5 for *T.*

thermoanaerobacterium, the larger should be the F_0 setpoint. To destroy 6.6 log₁₀ spores of *G. stearothermophilus*, F_0 value of 8±1 min is sufficiently high for products of pH 4.4, 4.5 and 5.1, but should be increased for products with pH 5.8 if the product is to be subjected to tropical conditions. To destroy 4.8 log₁₀ spores/ml of *T. thermoanaerobacterium*, F_0 value of 8±1 min is sufficiently high for all studied products (pH 4.4, 4.5, 5.1, 5.8). This study provides in-depth knowledge regarding heat resistance of thermophilic bacteria in commercial products and expands our understanding regarding industrial sterilization process designing.

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