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 pH > 4.6 (FDA, 2020) (or pH > 4.5 in some European countries) (Bratt, 2013) and acidified food (AF) with pH < 4.6. This threshold has been set to restrain the growth of the botulin toxin by the mesophilic spore-forming bacterium, Clostridium botulinum, as both growth and toxin production are inhibited at 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 ($F_{121.1{\degree}C}$) in this case is 2.8 min, as the $D_{121.1{\degree}C}$ 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 $^\circ$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 needed.
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* (Andrè, Vallaeys, & Planchon, 2017), which can grow in LACF products at moderately low pH (4.2–4.3) and can endure high temperatures ($D_{121.1^oC} = 0.27$ min, $z = 8.3^oC$) (Peng et al., 2012); furthermore, $F_{121.1^oC} = 0.26$ 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 Andrè, Vallaeys and Planchon, 2017. The most common species that cause food spoilage are *Geobacillus stearothermophilus*, *Thermobacterium thermosaccharolyticum*, *Moorella thermoacetica*, *Bacillus coagulans*, *Bacillus smithii*, and *Bacillus licheniformis*, the $D$ values of which are summarized in Table 1.

### Table 1

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>pH</th>
<th>$D_{105}$</th>
<th>$D_{110}$</th>
<th>$D_{115}$</th>
<th>$D_{120}$</th>
<th>$D_{121}$</th>
<th>$D_{125}$</th>
<th>$z$</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. stearothermophilus</em></td>
<td>6.0</td>
<td>3.14</td>
<td>1.25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>G. stearothermophilus</em></td>
<td>5.0</td>
<td>2.49</td>
<td>0.59</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>G. stearothermophilus</em></td>
<td>4.0</td>
<td>1.39</td>
<td>0.43</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>G. stearothermophilus</em></td>
<td></td>
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<td></td>
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<tr>
<td><em>G. stearothermophilus</em></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><em>G. stearothermophilus</em></td>
<td>7.0</td>
<td>10.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>T. thermosaccharolyticum</em></td>
<td>6.6</td>
<td>12.16</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>T. thermosaccharolyticum</em></td>
<td>6.6</td>
<td>11.80</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>T. thermosaccharolyticum</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Moorella thermoacetica</em></td>
<td>7.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Moorella thermoacetica</em></td>
<td>7.0</td>
<td>83.00-111.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td><em>Moorella thermoacetica</em></td>
<td>7.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Bacillus coagulans</em></td>
<td>7.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td><em>Bacillus licheniformis</em></td>
<td>7.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Bacillus smithii</em></td>
<td>7.0</td>
<td>10.00</td>
<td>10.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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.
**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 $F_v = 8 \pm 1$ 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).

<p>| Canned food products |
|----------------------|----------------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th><strong>Product</strong></th>
<th><strong>Heat transfer</strong></th>
<th><strong>Structure description</strong></th>
<th><strong>Group</strong></th>
<th><strong>pH</strong></th>
<th><strong>Water activity ($a_w$)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Tomato soup</td>
<td>Convection</td>
<td>Thick liquid, ready-to-eat tomato soup puree</td>
<td>AF</td>
<td>4.4</td>
<td>0.99</td>
</tr>
<tr>
<td>Mushroom soup</td>
<td>Convection</td>
<td>Liquid soup concentrate, which consists of shredded mushroom pieces in a broth</td>
<td>LACF</td>
<td>5.1</td>
<td>0.98</td>
</tr>
<tr>
<td>Pea porridge</td>
<td>Conduction</td>
<td>Solid pea porridge without liquid phase, but of soft consistency</td>
<td>LACF</td>
<td>5.8</td>
<td>0.98</td>
</tr>
<tr>
<td>Rassolnik soup</td>
<td>Conduction</td>
<td>Thick soup concentrate, submerged and shredded thick liquid which consists of food pieces in a thick liquid</td>
<td>AF</td>
<td>4.5</td>
<td>0.98</td>
</tr>
</tbody>
</table>

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,HPO₄, 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 MnSO₄ and 10 mg/L CaCl₂, 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 × 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 MnSO₄ and 10 mg/L CaCl₂ 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 × 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 GasPak™EZ, NJ, USA).

**Inoculation of food samples**

The spore concentrates of both bacteria were re-suspended in 40 mL aliquots, resulting in 6.6 log₁₀ spores of *G. stearothermophilus* and 4.8 log₁₀ spores of *T. thermosaccharolyticum* per milliliter. Furthermore, 36 mL suspension (12 mL x 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 log₁₀ and 4.8 log₁₀ respectively) indicates the limit of detection of spore count, as 2 log₁₀ 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.
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 (F₀) 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, \( \log_{10} \);
- F₀ – 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.

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 \( G. \) steatornerophilus 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.

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.

Table 3

<table>
<thead>
<tr>
<th>Product</th>
<th>pH</th>
<th>( \log_{10} ) CFU/g (Spore count in the product)</th>
<th>( \log_{10} ) CFU/g (Cell count after 48 h at 55 °C)</th>
<th>( \log_{10} ) CFU/g (Spore count in the product)</th>
<th>( \log_{10} ) CFU/g (Cell count after 72 h at 55 °C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ts¹</td>
<td>4.4</td>
<td>2.9 ± 0.01</td>
<td>2.5 ± 0.01</td>
<td>1.1 ± 0.01</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Ms²</td>
<td>5.1</td>
<td>2.9 ± 0.07</td>
<td>5.8 ± 0.02</td>
<td>1.1 ± 0.07</td>
<td>4.5</td>
</tr>
<tr>
<td>Pp³</td>
<td>5.8</td>
<td>2.9 ± 0.17</td>
<td>7.1 ± 0.04</td>
<td>1.1 ± 0.18</td>
<td>7.2</td>
</tr>
<tr>
<td>Rs⁴</td>
<td>4.5</td>
<td>2.9 ± 0.09</td>
<td>2.7 ± 0.02</td>
<td>1.1 ± 0.08</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

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

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

Expected destruction rate is 1.8–2 CFU log₁₀/g; therefore, 0.1–0.3 CFU log₁₀/g can be detected via counting.
Growth and post-heat treatment recovery

Growth and post-heat treatment recovery

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

\begin{table}
\centering
\begin{tabular}{|l|c|c|c|}
\hline
\textbf{Product} & \textbf{F$_0$} & \textbf{log$_{10}$ CFU/g} & \textbf{log$_{10}$ CFU/g} \\
\hline
\textit{G. stearothermophilus} & \textit{T. thermosaccharolyticum} \\
\hline
\text{Ts$^1$} & 8.2 & 1.6 & 2.3 \\
\text{Ms$^2$} & 7.6 & 1.5 & 2.5 \\
\text{Pp$^3$} & 7.3 & 1.5 & 2.4 \\
\text{Rs$^4$} & 7.1 & 1.4 & 2.5 \\
\hline
\end{tabular}
\caption{Growth and recovery of \textit{Geobacillus stearothermophilus} and \textit{Thermoanaerobacterium thermosaccharolyticum} in the product after heat treatment}
\end{table}

\begin{table}
\centering
\begin{tabular}{|l|c|c|c|c|}
\hline
\textbf{Product} & \textbf{Spore count in the inoculant} & \textbf{Spore count in the product} & \textbf{Cell count} & \textbf{Cell presence} \\
\hline
\textit{G. stearothermophilus} & \textit{T. thermosaccharolyticum} \\
\hline
\text{Ts$^1$} & 6.6 & 3.9 & 1.6 & 1.8 & 2.3 \\
\text{Ms$^2$} & 6.6 & 3.9 & 1.5 & 1.8 & 2.5 \\
\text{Pp$^3$} & 6.6 & 3.9 & 1.4 & 2.4 & 2.5 \\
\text{Rs$^4$} & 6.6 & 3.9 & 1.4 & 2.5 & 0.3 \\
\hline
\end{tabular}
\caption{Theoretical evaluation of spore count in the product}
\end{table}

\begin{table}
\centering
\begin{tabular}{|l|c|c|c|}
\hline
\textbf{Product} & \textbf{Spore count after 22 h} & \textbf{Cell count} & \textbf{Cell presence} \\
\hline
\textit{G. stearothermophilus} & \textit{T. thermosaccharolyticum} \\
\hline
\text{Ts$^1$} & <1 & 4.8 & 2.1 \\
\text{Ms$^2$} & <1 & 4.8 & 2.1 \\
\text{Pp$^3$} & 4.7 & 4.8 & 2.1 \\
\text{Rs$^4$} & <1 & 4.8 & 2.1 \\
\hline
\end{tabular}
\caption{Growth and recovery of \textit{Geobacillus stearothermophilus} and \textit{Thermoanaerobacterium saccharolyticum} in the product after heat treatment}
\end{table}

Considering that the count analysis does not reveal the 2 CFU log$_{10}$ 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. \textit{G. stearothermophilus} was detected only in pea porridge (pH = 5.8), while \textit{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 log_{10} /g and 0.1 – 0.3 CFU log_{10} /g, respectively. Practical evaluation showed 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 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 log_{10} 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 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_{121} 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_{121} value of *C. sporogenes* at pH 5 is 7 min; however, it gradually increases with pH, and D_{121} = 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, pH7.4). The *T. saccharolyticum* 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 log_{10} 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 log_{10} 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 log_{10} 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 log_{10} 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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