Prevention of Foodborne Illness When Keeping Food Hot

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Bacteria can occur in food and produce stable, permanent forms (spores) which are not killed when the food is cooked, roasted or baked. If prepared dishes are kept warm at insufficient temperatures in private and commercial kitchens, any spores that survived the initial preparation may continue to develop viable bacteria that can in turn multiply in the food. Some of these bacteria are able to produce harmful metabolites (toxins). Consuming food contaminated with toxins or high bacterial counts can lead to a foodborne disease with associated diarrhoea or vomiting. For this reason, food must be kept hot enough to prevent the growth of pathogens.

The BfR has studied scientifically the minimum temperatures that must be maintained in order to prevent foodborne diseases. The analysis focused on the spore-forming bacteria of the Bacillus (B.) cereus group and Clostridium (C.) perfringens, which can multiply at high temperatures and are often the cause of diseases associated with heated food.

Previously, the BfR recommended keeping food warm at a temperature of at least 65 °C. A search of the current literature and mathematical simulations showed that growth of B. cereus, B. cytotoxicus or C. perfringens is unlikely at temperatures above 57 °C. However, individual studies indicate that low growth in food is possible even at higher temperatures up to 60 °C. Based on these results, the BfR is now advising to hold heated food so hot that it maintains an overall temperature of at least 60 °C prior to consumption. The EFSA BIOHAZ Panel (2016) also notes that most cases of foodborne illness caused by B. cereus are associated with raw or cooked foods that have not been stored at temperatures below 4 °C or above 60 °C.

1 Subject of the assessment

Sufficiently heating food by cooking, roasting or baking, kills the vegetative cells of bacterial pathogens. The spores of pathogens, such as Bacillus cereus or Clostridium perfringens, however, can survive this type of preparation and, under certain conditions, re-germinate to produce vegetative cells and multiply. For this reason, handling pre-cooked meals is a major challenge for food companies as well as in private households. In order to prevent vegetative bacteria from developing again from spores, which then multiply and produce toxins in the food or intestine, pre-cooked meals must either be cooled quickly or kept sufficiently hot until they are served or consumed.

In 2008, the BfR recommended in an opinion that food should be kept at a temperature of at least 65 °C, or 65 °C should be the limit of the critical temperature range. This recommendation was incorporated into several DIN standards (German industry standard) and hygiene guidelines of economic operators.

In this work, the German Federal Institute for Risk Assessment (BfR) deals with the temperature and time requirements for keeping food hot, as stated by the US food surveillance agency “U.S. Food and Drug Administration” (FDA) in Chapters 3-501.16 and 3-501.19 of its Food Code 2017. The BfR discusses whether these requirements can be seen as an alterna-
tive to its own requirements for keeping food continuously hot, above a specified critical minimum temperature, in the context of retail activities or catering processes by commercial kitchens.

2 Discussion

Introduction

The FDA’s Food Code 2017 describes in Chapter 3-5 “Limitation of growth of organisms of public health concern” specifications that are intended to prevent the growth (= multiplication) of pathogenic microorganisms in food. In its assessment based on existing literature and mathematical modelling, the BfR limits itself to the requirements of Chapter 3-5 of the FDA’s Food Code 2017, which relate to keeping food hot.

Chapter 3-501.16 (A1) stipulates that heated food, in which pathogen growth is possible in principle (“Time / Temperature Control for Safety Food”), must be kept hot at a controlled temperature of at least 57 °C. An exception to this are roasts that have been heated or reheated at a certain temperature-time combination (from 54.4 °C for 112 minutes to 69.4 °C for 14 seconds; see chapters 3-401.11(B) and 3-403.11(E) of the FDA Food Code 2017). Such roasts may be held at a temperature of 54 °C or above. Chapter 3-501.16 does not contain a time limit for the duration of keeping food hot.

Chapter 3-501.19 (B) states that heated foods, in which microbial growth is possible in principle and which are intended for direct consumption (“Ready-To-Eat Time / Temperature Control for Safety Food”), can be stored for a maximum of four hours without temperature control prior to serving, provided that the food was at least 57 °C when removed from hot holding temperature control.

The BfR makes the following assumptions, when assessing the requirements of the FDA’s Food Code 2017:
- Heat treatment of the food before subsequent storage kills the vegetative cells of bacterial pathogens in the food
- No recontamination with pathogens after the food has been heated
- Bacterial spores present in the food are not safely inactivated due to the high heat resistance.

The spore-forming bacteria that are able to trigger foodborne diseases and grow at relatively high temperatures include, in particular, species from the *Bacillus (B.) cereus* group (*B. cereus* sensu lato (s.l.)) and *Clostridium (C.) perfringens*. As a result, these pathogens are often involved in outbreaks related to heated food (EFSA BIOHAZ Panel, 2005a, b, 2016). Bacterial spores present in food can germinate under suitable conditions. The reduced accompanying microbiota favour the growth of vegetative cells resulting from germination.

When evaluating the temperature / time requirements of the FDA’s Food Code 2017 (3-501.16 (A1), 3-501.19 (B)), the BfR therefore focuses on the growth of these organism groups. A growth in food to high bacterial counts increases the risk of foodborne diseases in humans.
Foodborne diseases caused by *B. cereus* (s.l.) and *C. perfringens*

a) Diseases caused by *B. cereus* (s.l.)

Consuming food contaminated with *B. cereus* (s.l.) can lead to gastrointestinal diseases in humans. It is assumed that in most cases a bacterial content of at least $10^5$ CFU/g (colony-forming units per gram) of food is necessary to trigger disease. Cases of disease have also been described in which lower levels of *B. cereus* (s.l.) have been detected in food (Ceuppens et al., 2013; EFSA BIOHAZ Panel, 2016; Rouzeau-Szynalski et al., 2020). A distinction is made between two types of illness, an emetic disease (vomiting type; intoxication) and diarrhoeal illness (diarrhoea type; toxic infection). Mixed forms of both types of illness can also occur.

In the case of **emetic disease**, the acid, heat and proteolysis-stable toxin cereulide formed in the food by vegetative cells is absorbed. Cereulide causes vomiting and nausea within six hours of uptake, and the symptoms usually disappear within 24 hours. With severe intoxications, cereulide can also cause liver damage and cerebral edema, which have in rare instances resulted in death (Dierick et al., 2005; Naranjo et al., 2011; Shiota et al., 2010). Cereulide intoxication is often associated with the consumption of high starch foods such as rice and pasta. However, cases of emetic disease associated with dairy and meat products have also been mentioned in literature (Messelhäußer et al., 2014). Cereulide production is believed to start during the late exponential growth phase and to continue during the stationary growth phase (Ceuppens et al., 2011; Delbrassinne et al., 2011; Dommel et al., 2011; Häggblom et al., 2002; Lücking et al., 2009; Rouzeau-Szynalski et al., 2020). The number of cells at which the late exponential growth phase is reached depends on ambient conditions. However, it can be assumed that cereulide production only starts from cell counts of at least $10^5$ CFU/g (Agata et al., 2002; Bursova et al., 2018; Delbrassinne et al., 2012; Jääskeläinen et al., 2003; Phat et al., 2017; Rouzeau-Szynalski et al., 2020). Cereulide formed in the food is not destroyed even by heating at 100 °C for 150 min (pH 8.6 to 10.6) or heating at 121 °C for 120 min (pH 7) (Rajkovic et al., 2008).

With the diarrhoea type, (i) spores and/or (ii) vegetative cells and/or (iii) possibly enterotoxins of *B. cereus* (s.l.), already formed in the food, are taken up via food consumption.

Re i) Ingested spores largely survive the gastrointestinal passage and can then germinate close or in direct contact with the small intestine epithelium and form vegetative cells. These can then form enterotoxins (Jessberger et al., 2017; Wijnands et al., 2007).

Re ii) Most of the ingested vegetative cells will be inactivated during the gastrointestinal passage. How high this amount is, however, depends on various factors (e.g. bacteria growth phase, food properties, gastrointestinal environment), meaning that vegetative cells may also be involved in disease incidence (Berthold-Pluta et al., 2015). In a simulated gastrointestinal passage of vegetative *B. cereus* cells, 14% (± 9%) survived in an experiment by Ceuppens et al. (2012).

Re iii) Enterotoxins that may already be formed in the food probably do not play any role in the development of diarrhoea symptoms, since they are largely inactivated during the gastrointestinal passage, because enterotoxins are sensitive to proteinases and low pH values. Enterotoxins are also heat-labile and are deactivated by heating at 55 °C for 20 minutes (Ceuppens et al., 2013; Ceuppens et al., 2011).
The symptoms of diarrhoea usually start within eight to 24 hours after the contaminated food has been consumed and usually include watery diarrhoea and abdominal pain. The disease is generally self-limiting (Messelhäußer and Ehling-Schulz, 2014).

Information about \textit{B. cereus} concentration in foods which have been associated with diarrhoeal illness varies greatly. In most cases of \textit{B. cereus}-related outbreaks with diarrhoea symptoms, which were reported to the EFSA in the period between 2007 and 2014, concentrations above \(10^5\) CFU/g have been found in the implicated foods. However, there were also outbreaks where \textit{B. cereus} concentrations of only \(10^3\) CFU/g were detected (EFSA BIOHAZ Panel, 2016). However, the \textit{B. cereus} content in the food examined can differ from the \textit{B. cereus} content of the food consumed at the time of consumption. It is difficult to determine a specific bacterial concentration in the food, which poses a health risk, since the pathogenic potential strongly depends on the properties of the strain. The main factors involved in the development of the pathogenic potential are: i) the ability of the spores/cells to survive the gastrointestinal passage, ii) the ability to attach to enterocytes and germinate and iii) the ability to form relevant amounts of enterotoxins.

b) \textit{C. perfringens} related disease

A prerequisite for a foodborne disease caused by \textit{C. perfringens} is high vegetative cell counts of \(10^6-10^7\) CFU/g in food. Some of the vegetative cells survive the acidic gastric environment and reach the small intestine. The bacterial cells sporulate in the small intestine and form an enterotoxin (CPE), which is released during lysis of the vegetative cells. After proteolytic activation of the toxin, pores form in the cell membrane of the enterocytes. As a result, symptoms such as diarrhoea and abdominal cramps that last for about a day appear after an incubation period of eight to 24 hours. The disease is usually mild and is self-limiting (EFSA BIOHAZ Panel, 2005b; Labbe and Juneja, 2017; Taormina and Dorsa, 2004).

\textit{C. perfringens} grows particularly well in protein-rich foods. Correspondingly, foodborne diseases caused by \textit{C. perfringens} are often associated with heated meat dishes (roasts, sauces, soups and stews) or pea soup, which after being prepared are not stored at either sufficiently hot or cold temperatures (EFSA BIOHAZ Panel, 2005b).

Enterotoxin formation in food is probably not a factor for diseases, since the time required for sporulation and toxin release would lead to significant sensory changes of the food (Labbe and Juneja, 2017). The enterotoxin from \textit{C. perfringens} is heat-labile and is inactivated at temperatures of 60 °C, whereby the necessary heating times vary between one minute and more than 20 minutes depending on the medium in which the toxin is located (Bradshaw et al., 1982; Granum and Skjelkvale, 1977; Naik and Duncan, 1978).

Spore germination of \textit{B. cereus} (s.l.) and \textit{C. perfringens} in foods kept hot

There are various factors that influence the germination of spores, the growth of vegetative cells and the heat resistance of \textit{B. cereus} (s.l.) and \textit{C. perfringens} in food. These include, on the one hand, the properties of the bacterial strain and, on the other hand, the conditions in the food, such as water activity (\(a_w\)), salinity, pH, oxygen content, available nutrients and the temperatures that prevail during preparation and subsequent storage (Doyle, 2002; Wells-Bennik et al., 2016). The heat resistance of the spores differs greatly between different strains (van Asselt and Zwietering, 2006). Depending on the temperature and the duration of heat treatment of the food, existing spores can be inactivated, damaged or activated. Damage to spores can delay germination. In contrast, the activation of spores, for example by heat treatment at 70 °C to 80 °C for 10 min, accelerates spore germination (Laurent et al.,
1999; Samapundo et al., 2014; Doyle, 2002). Under suitable conditions, spores can germinate in less than 30 minutes (Doyle, 2002; Tegiffel et al., 1995; Warda et al., 2015).

In general, temperatures at which vegetative cells can multiply (see 4.4) also allow the spores to germinate. However, spores can also germinate at temperatures above the upper temperature growth limit. For *B. cereus* spores Johnson et al. (1983) describe a low germination in rice at 55 °C, but without subsequent growth of the resulting vegetative cells. Similarly, Knaysi (1964) describes a maximum temperature of 59 °C for the beginning of germination, but again without subsequent growth of the cells in the medium. A study from Ellerbroek (2008) reported spore germination in rice at 60 °C. In inactivation experiments, Wei et al. (2009) showed for *B. cereus* that if moderate pressure is used as a germination promoter, spore germination can still take place at 65 °C. For *C. perfringens*, Akhtar et al. (2009) reported spore germination up to 65 °C in the presence of germ-promoting nutrients.

Growth of vegetative cells of *B. cereus* (s.l.) and *C. perfringens* in foods kept hot

In both *B. cereus* (s.l.) and *C. perfringens*, the temperature growth limits differ greatly between individual strains. The growth rates close to the temperature growth limits are significantly lower than at the optimal temperature.

In a comprehensive study by Guinebretiere et al. (2008), 75 *B. cereus* (s.l.) strains from seven different phylogenetic groups (panC groups I to VII) were characterised with regard to their growth at different temperatures. It was shown that the temperature growth limits of some groups differ. With a view to possible growth in hot food, so-called mesophilic and thermo-tolerant representatives are particularly relevant and are therefore discussed in more detail below.

a) Growth of mesophilic *B. cereus* (s.l.)

The mesophilic *B. cereus* (s.l.) strains belong to the phylogenetic groups III and IV, which in the study of Guinebretiere et al. (2008) demonstrated growth at a temperature of 45 °C, but not at 50 °C. Within group III there are also the strains with the ability to form cereulide (so-called emetic strains).

Auger et al. (2008) have characterised growth for the mesophilic *B. cereus* strains ATCC 14579 (group IV) and ATCC 10987 (group III) (Table 2). For the strain ATCC 14579, growth was proven in the laboratory up to 46 °C and for ATCC 10987 up to 47 °C. The relationship between growth rate and temperature was used to calculate a theoretical temperature growth limit of 51 °C and 53 °C for the strains using the Ratkowsky model (Ratkowsky et al., 1983). Based on the data from Auger et al. (2008), Afchain et al. (2008) calculated a maximum or optimal growth temperature of 46.5 °C or 39.9 °C for strain ATCC 14579, and 46.9 °C or 39.8 °C for strain ATCC 10987, based on the "cardinal temperature model with inflection point" (CTMI) (Rosso et al., 1993).

The temperature growth limits for the strain ATCC 14579 were again determined in a recent study by Carlin et al. (2013) and growth was observed in the laboratory up to 46 °C, too. Using CTMI, a maximum growth temperature of 48 °C (48.4 °C taking into account the 97.5th percentile) was calculated. The optimal growth temperature was 37.4 °C (38.2 °C taking into account the 97.5th percentile). Very similar values were obtained (Table 2) for another mesophilic strain (F4810). This information coincides with previous work by Carlin et al. (2006) in which a maximum growth temperature of 48 °C was determined for emetic strains.
In contrast, various studies on the inactivation of vegetative *B. cereus* (s.l.), in part using the same strains as in the studies mentioned above, showed that inactivation of mesophilic strains can also be expected at temperatures above 50 °C (Antolinos et al., 2011; Becker et al., 2011; den Besten et al., 2006; den Besten et al., 2010; Desai and Varadaraj, 2010) (Table 3).

Some mesophilic strains of *B. cereus* are able to produce the heat-stable emetic toxin cereulide in certain foods. An overview of factors and foods that favour the formation of cereulide can be found in Messelhäußer et al. (2014). The temperature range in which these strains can, in principle, form cereulide is probably between 10 °C and 48 °C (Carlin et al., 2006; Finlay et al., 2000; Guinebretiere et al., 2010, Wang et al., 2014), with the optimum being between 20 °C and 40 °C (Agata et al., 2002; Apetroaie-Constantin et al., 2008; Häggblom et al., 2002; Kranzler et al., 2016; Rajkovic et al., 2006).

In a study by Agata et al. (2002), rice was inoculated with approx. $10^3$ CFU/g of an overnight culture and an increase in the number of cells to over $10^6$ CFU/g and cereulide formation were observed within 4 h at a temperature of 35 °C (temperatures higher than 35 °C were not tested). In a study by Wang et al. (2014), rice was inoculated with approx. $10^3$ CFU/g vegetative *B. cereus* cells and at a temperature of 45 °C an increase in the number of cells to approx. $10^5$ CFU/g and cereulide formation were detected within 6 h (temperatures higher than 45 °C were not tested). Phat et al. (2017) observed cereulide formation after 12 h at 30 °C (overnight culture in LB medium). Rajkovic et al. (2006) found cereulide formation in mashed potatoes, milk and rice, which were inoculated with approx. $10^6$ CFU/g (24 h culture), within 12 h at 28 °C. With an inoculum of 150 CFU/g of an overnight culture in cooked rice, Bauer et al. (2010) found cereulide formation only after 24 h incubation at 24 °C (has previously been tested after 12 h). Similarly, Bursova et al. (2018) detected cereulide formation in dissolved milk powder inoculated with approx. $10^3$ CFU/g of a spore suspension after 24 h incubation at 24 °C, but not after 12 h.

b) Growth of thermo-tolerant *B. cereus* (s.l.) (*B. cytotoxicus*)

The thermo-tolerant strains of the *B. cereus* group belong to phylogenetic group VII, which only contains the species *B. cytotoxicus*. *B. cytotoxicus* was first described as an independent species in 2013 (Guinebretiere et al., 2013). The type strain of the species (NVH391-98) was isolated in a disease outbreak with diarrhoea symptoms in 44 patients (including three deaths and six cases with bloody diarrhoea) in a retirement home in France. The isolate was obtained from vegetable puree which was contaminated with $3 \times 10^5$ CFU/g of *B. cereus* (s.l.) (Lund et al., 2000). The species *B. cytotoxicus* forms the unusual CytK-1 variant of the enterotoxin cytotoxin K. CytK-1 exhibits a significantly higher cytotoxicity than the widely found CytK-2 variant in the *B. cereus* group (Fagerlund et al., 2004; Guinebretiere et al., 2013). However, due to the different levels of toxin formation, not all *B. cytotoxicus* strains are highly cytotoxic (Fagerlund et al., 2007; Heini et al., 2018).

In the study of Guinebretiere et al. (2008), *B. cytotoxicus* strains showed growth at 50 °C, but not at 55 °C. Auger et al. (2008) have examined the type strain of the species *B. cytotoxicus* (NVH391-98) in more detail and demonstrated growth up to 53 °C in the laboratory. Based on calculations using the Ratkowsky model, a theoretical growth limit of 58 °C was determined in this study (Table 2). Based on the data from Auger et al. (2008), Afchain et al. (2008) were able to calculate a maximum and optimal growth temperature of 56.5 °C and 41.4 °C for *B. cytotoxicus* using the CTMI. The strain NVH391-98 was also examined in the study by Carlin et al. (2013) and growth was demonstrated in the laboratory up to 52 °C. A maximum growth temperature of 55 °C
was calculated using the CTMI (55.9 °C taking into account the 97.5th percentile). The optimal growth temperature was 43.1 °C (44.3 °C taking into account the 97.5th percentile). Very similar values were obtained (Table 2) for another *B. cytotoxicus* strain (NVH883/00). Taking into account the 97.5th percentile, the theoretical maximum growth temperature of this strain was even slightly higher at 56.8 °C.

Contrary to these theoretical, upper temperature growth limits, Guerin et al. (2017) have previously reported a slight inactivation of strain NVH391-98 at 53 °C (Table 3). At 55 °C, however, a clear decrease in the number of cells was measured. In this study, a slight reduction of another *B. cytotoxicus* strain at 53 °C and 54 °C and a significant reduction at 55 °C was also observed.

This contradiction between the theoretical, upper temperature growth limits and inactivation temperatures may arise for various reasons. First, the estimation of the model parameters - especially the growth limits - is associated with a higher degree of uncertainty due to the comparatively smaller number of relevant measured values. Second, differences in the experimental setup of the studies to determine microbial growth and inactivation, as well as differences in cell history, can lead to different results. In addition, it is known that even within a population of the same strain there can be significant heterogeneity in terms of heat resistance or the specific growth rate, so that the different experimental data can also be explained by biological variability (Aryani et al., 2015; Wells-Bennik et al., 2016).

It is important to consider that the temperature growth limits and optima mentioned above were generated under experimental laboratory conditions. However, the properties of a food include many more parameters than can hardly be tested under controlled conditions. Therefore, it cannot be ruled out that growth parameters in food differ from those determined in the laboratory. For example, with similar starting conditions, the lag phases and growth rates can vary greatly depending on the food (Carlin et al., 2000; Warda et al., 2015; Ziane et al., 2014).

With a view to the possible growth of *B. cereus* (s.l.) in food which is kept hot, Gilbert et al. (1974) detected growth of three different *B. cereus* strains in rice up to 43 °C, while at 55 °C the cells were already inactivated. In a study by Kim et al. (2018), growth of *B. cereus* in rice was detected at 45 °C, however, inactivation of cells was observed at the next highest test temperature of 60 °C. Johnson et al. (1983) detected growth in rice of four different strains (including an emetic strain) up to 50 °C. In a study by Ellerbroek (2008) the *B. cereus* concentration in cooling rice increased already over a temperature range of 60.2 °C to 58.8 °C (natural *B. cereus* contamination in rice).

This table lists the maximum and optimal growth temperatures and growth rates of mesophilic and thermo-tolerant strains of the *B. cereus* group (panC groups III, IV and VII).

<table>
<thead>
<tr>
<th>Strain (panC group)</th>
<th>$T_{\text{opt}}$ (°C)</th>
<th>$\mu_{\text{opt}}$ (h$^{-1}$)</th>
<th>Theoretical $T_{\text{max}}$ (°C) at which growth was proven in the medium</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. cytotoxicus</em> a NVH391-98 (VII)</td>
<td>46</td>
<td>n.d.</td>
<td>58$^b$</td>
<td>Auger et al., 2008</td>
</tr>
<tr>
<td><em>B. cereus</em> ATCC14579 (IV)</td>
<td>35 - 40</td>
<td>n.d.</td>
<td>51$^b$</td>
<td>46</td>
</tr>
<tr>
<td><em>B. cereus</em> ATCC10987 (III)</td>
<td>35 - 40</td>
<td>n.d.</td>
<td>53$^b$</td>
<td>47</td>
</tr>
<tr>
<td><em>B. cytotoxicus</em> a NVH391-98 (VII)</td>
<td>41.4</td>
<td>n.d.</td>
<td>56.5$^c$</td>
<td>n.d.</td>
</tr>
</tbody>
</table>
Table 3: Inactivation of vegetative cells from mesophilic and thermo-tolerant strains of the B. cereus group (time in minutes for a 3 log reduction at different inactivation temperatures)

<table>
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<tr>
<th>Strain (panC group)</th>
<th>48 °C</th>
<th>50 °C</th>
<th>53 °C</th>
<th>54 °C</th>
<th>55 °C</th>
<th>Reference</th>
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<tr>
<td>B. cytotoxicus NVH391-98 (VII)</td>
<td>n.d.</td>
<td>n.d.</td>
<td>44.1 ± 7.0</td>
<td>n.d.</td>
<td>9.1 ± 1.1</td>
<td>Guerin et al., 2017</td>
</tr>
<tr>
<td>B. cytotoxicus AFSSA 08CEB 44BAC (VII)</td>
<td>n.d.</td>
<td>n.d.</td>
<td>25.5 ± 5.1</td>
<td>15.5 ± 3.0</td>
<td>12.9 ± 1.1</td>
<td></td>
</tr>
<tr>
<td>B. cereus ATCC14579 (IV)</td>
<td>n.d.</td>
<td>ca. 55</td>
<td>n.d.</td>
<td>n.d.</td>
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<td>den Besten et al., 2010</td>
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n.d.: not determined

c) Growth of vegetative cells of C. perfringens

Based on reviews (Doyle, 2002; Labbe and Juneja, 2017; Taormina and Dorsa, 2004), it can be assumed that C. perfringens can multiply at temperatures up to 50 °C, with the optimal growth temperature being between 43 °C and 45 °C. In this optimal temperature range, the generation times can be less than 10 minutes. In Annex 3 of the FDA Food Code 2017 it is assumed that C. perfringens can even grow at temperatures up to 52 °C, but only under anaerobic conditions and following long lag phases. Taormina and Dorsa (2004) have evaluated a large number of studies dealing with the growth of C. perfringens in heated, meat-based foods in the cooling phase. Starting from spores, depending on the cooling condition, growth of more than one log level is only expected after six hours in most cases. However, some studies also show significant growth of 1.5 to 4 log levels within six hours (Blankenship et al., 1988; Kalinowski et al., 2003; Shigehisa et al., 1985) or even within just four hours (Shigehisa et al., 1985). Naik and Duncan (1977) found considerably stronger growth based on vegetative cells and a storage temperature of 37 °C. The number of cells in the artificially contaminated minced meat samples increased from 5.5 x 10⁴ to 2.2 x 10⁷ CFU/g within four hours under aerobic conditions and from 2 x 10⁵ to 3 x 10⁷ CFU/g under anaerobic conditions.
Heat resistance may vary greatly depending on the bacterial strain, its growth conditions and the food, which contains the cells. Inactivation of vegetative cells is possible from temperatures of 55 °C upwards (Doyle, 2002; Jaloustre et al., 2012; Roy et al., 1981; Smith et al., 1981).

**Modelling the growth of** *B. cereus* (s.l.), *B. cytotoxicus* and *C. perfringens* **in food**

In order to illustrate the change in the number of bacteria under certain temperature conditions, the microbial growth of selected pathogens was simulated with various model-based programs. The following conservative (worst case) assumptions were made:

- Activation of spore germination by prior heat treatment of the food
- Optimal pH, $a_w$ and nutrient conditions for spore germination and cell growth
- The lag phase is very short and is not taken into account in the modelling

Based on the requirements of the FDA Food Code 2017, different scenarios for keeping food hot were considered during modelling.

a) Modelling according to the requirements of Chapter 3-501.16 (A1) of the FDA Food Code 2017

With a view to the requirements of Chapter 3-501.16 (A1) of the FDA Food Code 2017 (controlled hot holding at 57 °C or 54 °C for roasts), the possible growth of the thermo-tolerant *B. cytotoxicus* strain NVH-883-00 was predicted when holding hot at constant temperatures of 50 °C, 54 °C, 55 °C, 56 °C and 57 °C over a period of 10 hours (Figure 1).

For this purpose, the CTMI model and the 97.5$^{th}$ percentiles of the maximum growth temperature $T_{\text{max}}$ and the maximum growth rate $\mu_{\text{opt}}$ for NVH-883-00 determined in the study by Carlin et al. (2013) were used (see Table 2).
Figure 1: Prediction of the growth of *B. cytotoxicus* strain NVH-883-00 while holding hot over a period of 10 hours at 50 °C, 54 °C, 55 °C, 56 °C and 57 °C under conservative model assumptions

For each of the two relevant model parameters $T_{\text{max}}$ and $\mu_{\text{opt}}$ we used as parameter values in the predictive model the upper bound of the parameter's 95% confidence interval from the Carlin et al. 2013 publication. It can therefore be assumed that the predicted growth of *B. cytotoxicus* NVH-883-00 cells under the assumed temperature conditions is only achieved in practice in less than 2.5% of cases (otherwise the growth is less).

In case of model-based predictions for temperatures above 55 °C, it is important to bear in mind that these are purely mathematical calculations, since the range of applicability of the model is limited to the temperature range (up to and including 55 °C) that was actually investigated experimentally. This does not affect the key message of this simulation, that no growth of *B. cytotoxicus* in food is expected above 57 °C. Also, none of the other established...
software tools from the field of predictive microbiology offer growth models for temperatures above 55 °C (see https://foodrisklabs.bfr.bund.de/openfsmr). Nor are there any data in the world's largest publicly available data collection on microbial growth and inactivation experiments (ComBase) that show growth at 57 °C or above.

Overall, based on the available models and the generated simulation results it cannot completely ruled out that, in individual cases, low growth of B. cytotoxicus (e.g. B. cytotoxicus NVH-883-00) is possible even at 56 °C. According to the currently available data, however, growth should no longer be expected at 57 °C.

b) Modelling according to the requirements of Chapter 3-501.19 (B) of the FDA Food Code 2017

With regard to the requirements of Chapter 3-501.19 (B) of the FDA Food Code 2017 (storage without temperature control for a maximum of four hours), the possible growth of a mesophilic, emetic B. cereus strain, a thermo-tolerant B. cytotoxicus strain and C. perfringens was considered. Two different cooling scenarios were considered as examples:
- A decrease in temperature by 6 °C per hour from 57 °C (see Figure 2a)
- A decrease in temperature to 37 °C within 30 minutes with a subsequent constant temperature of 37 °C (worst case, see Figure 2b)

Figure 2a: Predicted growth of mesophilic, emetic B. cereus (strain F4810/72), thermo-tolerant B. cytotoxicus (strain NVH-883-00) and C. perfringens cells subject to cooling from 57 °C by 6 °C/h for 4 h with an assumed initial microbial content of 1 CFU/g (0 log CFU/g). CFU: colony forming units
The model-based forecasts shown were created using a modelling tool. In the case of the simulations described above for the mesophilic, emetic *B. cereus* strain F4810/72 and for the thermo-tolerant *B. cytotoxicus* strain NVH-883-00, new models were created and used based on the delineated model parameters from the Carlin et al. 2013 publication (mean estimate, the standard deviations were calculated from the specified 97.5th percentile). All simulation calculations are conservative because they assume no lag phase and optimal pH and aw values (worst case). In the case of the model described for *C. perfringens*, the model parameters used are based on information provided in Microorganisms in Food 5 (ICMSF, 1996) and Willardsen et al. (1979).

The key message of this analysis is that if food is cooled down below 57 °C in an uncontrolled manner, significant microbial growth could occur already within four hours. This is also supported by experimental data in the ComBase as well as model-based forecasts with other prediction tools (e.g. “Perfringens Predictor” https://www.combase.cc/, “Pathogen Modelling Program (PMP) Online” https://pmp.errc.ars.usda.gov/PMPOnline.aspx).

3 Conclusions from the literature data and the modelling

With regard to the requirements of Chapter 3-501.16 (A1) of the FDA Food Code 2017 (controlled hot holding at 57 °C or 54 °C of roasts), growth of *C. perfringens* and mesophilic *B. cereus* is not to be expected, and therefore the formation of cereulide is excluded.

Based on the calculations of Carlin et al. (2013) and the associated simulation results of the BfR growth of thermo-tolerant *B. cytotoxicus* at 56 °C is theoretically possible. At temperatures of 57 °C and above, growth is no longer expected based on data from Carlin et al. (2013) and the simulation results of the BfR. However, individual study results indicate that growth is still possible in foods even at higher temperatures (Ellerbroek 2008).
With regard to the requirements of Chapter 3-501.19 (B) of the FDA Food Code 2017 (storage without temperature control for a maximum of four hours), growth of thermo-tolerant *B. cytotoxicus* (≤ 56 °C), *C. perfringens* (≤ 50 °C) and mesophilic *B. cereus* (≤ 48 °C) is possible. Depending on the spore and vegetative cell concentration at the start of the hot holding period (initial microbial concentration) and the temperatures actually prevail in the food over the four hours, microbial concentrations can be reached that can cause foodborne illnesses (Figures 2a and 2b).

### 4 Conclusions

The BfR comments below whether the temperature/time requirements of Chapters 3-501.16 (A1) and 3-501.19 (B) of the FDA Food Code 2017 can be seen as an alternative to the requirements of the BfR from 2008 for continuous hot holding of heated food in the context of retail or catering operations by commercial kitchens.

**Specifications of Chapter 3-501.16 (A1) of the FDA Food Code 2017 (controlled hot holding at 57 °C or 54 °C for roasts)**

As the risk of foodborne illnesses increases with an increase in the numbers of *B. cereus* (s.l.) and *C. perfringens* in food, in the opinion of the BfR, the selected storage conditions for heated food should prevent the growth of these organisms. As stated, growth of *B. cereus* (s.l.), *B. cytotoxicus* or *C. perfringens* is not expected at 57 °C. This result largely coincides with information from the literature. However, individual study results indicate that low growth is still possible in food even at higher temperatures (Ellerbroek 2008). Therefore, in the opinion of the BfR, temperatures of heated food should not fall below 60 °C in any part of the product during hot holding. For the same reason, the requirements of Chapter 3-501.16 (A1) of the FDA Food Code 2017 do not constitute, in general, a suitable alternative in the opinion of the BfR.

The EFSA BIOHAZ Panel (2016) also notes that most cases of foodborne illness caused by *B. cereus* (s.l.) are associated with raw or cooked foods that have not been stored at temperatures below 4 °C or above 60 °C (the range between 4 °C and 55 °C is stated as growth temperature for *B. cereus* (s.l.) in this case). In its previous Scientific Opinion, the EFSA BIOHAZ Panel (2005a) recommended hot holding temperatures above 63 °C. A recommended hot holding temperature of at least 60 °C is published also in Labbe and Juneja (2017) and above 60 °C in Kramer and Gilbert (1989).

Basis of the requirements of the FDA Food Code 2017 is the assumption that controlling the growth of *C. perfringens* also controls the growth of *B. cereus* (3-501.16, Annex 3, FDA Food Code 2017). However, this assumption only applies to mesophilic *B. cereus*. With respect to new findings concerning the temperature growth limits of thermo-tolerant representatives of the *B. cereus* group (*B. cytotoxicus*), the assumption should be: Temperatures that control (i.e. prevent) the growth of *B. cytotoxicus* also control growth of other species of the *B. cereus* group, as well as *C. perfringens*.

**Specifications of Chapter 3-501.19 (B) of the FDA Food Code 2017 (storage without temperature control for a maximum of four hours)**

As stated, thermo-tolerant *B. cytotoxicus*, *C. perfringens* and mesophile *B. cereus* (s.l.) can multiply within four hours if the temperatures in the food drop below certain values (≤ 56 °C, ≤ 50 °C or ≤ 48 °C). How fast the growth is and whether critical microbial levels are reached...
depends on the initial microbial concentration of the food and the temperatures that actually reveal in all parts of the food during the four hours, among other things.

The assumptions based on the requirements of Chapter 3-501.19 (B) are specified in a position paper under 3-501.19, Annex 3 of the FDA Food Code 2017. These assumptions are: i) the initial concentration of \textit{B. cereus} or \textit{C. perfringens} in food is max. $10^3$ CFU/g, ii) one log growth is therefore tolerable and iii) the food cools quickly enough when stored at room temperature so as to limit the growth of \textit{B. cereus} or \textit{C. perfringens}. This assumption for cooling is limited to roasts, rolled meat products, products that are stirred and products that cool faster than roasts. According to the position paper, this assumption is based on an assessment of the cooling behaviour of roasts from an initial temperature of 54 °C based on published studies and data collected at the FDA.

According to the BfR, Chapter 3-501.19 (B) does not just refer to the foods mentioned above. It is questionable to what extent the assumption of sufficient cooling can be applied to all heated dishes. The results of the literature evaluation and modelling by the BfR suggest that, depending on the temperature conditions, growth of \textit{B. cereus}, \textit{B. cytotoxicus} and \textit{C. perfringens} of considerably more than one log-level is possible within four hours. Assuming a worst-case scenario, it can be suspected that cereulide formation could even be possible within four hours (see Figure 2b and Agata et al., 2002). Therefore, the requirements of Chapter 3-501.19 (B) of the FDA Food Code 2017 do not represent an acceptable alternative, in the opinion of the BfR, with regard to holding heated food hot at a temperature of at least 60 °C in all parts of the product.

Further information on the topic of food hygiene is available from the BfR website:

Summary page for all publications on food hygiene:
https://www.bfr.bund.de/en/a-z_index/food_hygiene-129858.html

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BfR "Opinions app"
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About the BfR

The German Federal Institute for Risk Assessment (BfR) is a scientifically independent institution within the portfolio of the Federal Ministry of Food and Agriculture (BMEL) in Germany. It advises the German federal government and German federal states ("Laender") on questions of food, chemical and product safety. The BfR conducts its own research on topics that are closely linked to its assessment tasks.
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