

DOI 10.17590/20200827-120755

## Prevention of Foodborne Illness When Keeping Food Hot

Updated BfR Opinion No 037/2020 issued 27 August 2020

Bacteria can occur in food and produce stable, resistant forms (spores) that are not killed when the food is cooked, roasted or baked. If prepared dishes are kept warm at insufficiently high temperatures in private and commercial kitchens, any spores that survived the initial preparation may 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 scientifically evaluated 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 hot 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 now advises maintaining heated food at a 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 commercial 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 alternative 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. The BfR assessment, based on existing literature and mathematical modelling, is confined 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 originating from germinated spores.

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<sup>5</sup> 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 illness (vomiting type; intoxication) and a diarrhoeal illness (diarrhoea type; toxicoinfection). Mixed forms of both types of illness can also occur.

In the case of **emetic illness**, the acid-, heat- and proteolysis-stable toxin cereulide formed in the food by vegetative cells is ingested. 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 (Messelhäuser 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) enterotoxins of *B. cereus* (s.l.), possibly already formed in the food, are taken up via food consumption.

Re (i) Ingested spores largely survive the gastric passage and can then germinate close or in direct contact with the epithelium of the small intestine 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 gastric passage. The extent of this inactivation, however, depends on various factors (e.g. bacteria growth phase, food properties, gastric environment), meaning that vegetative cells may also be involved in causing disease (Berthold-Pluta et al., 2015). In a simulated gastric passage of vegetative *B. cereus* cells, 14% ( $\pm$  9%) survived in an experiment by Ceuppens et al. (2012).

Re (iii) Enterotoxins that may have already been formed in the food probably do not play any role in the development of diarrhoea symptoms, since, because enterotoxins are sensitive to proteinases and low pH, they are largely inactivated during the gastric passage. Enterotoxins are also heat-labile and are deactivated by temperatures of 55 °C for 20 minutes (Ceuppens et al., 2013; Ceuppens et al., 2011).

The symptoms usually begin 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 (Messelhäuser and Ehling-Schulz, 2014).

Information about *B. cereus* counts in foodstuffs, which have been associated with diarrhoeal illness varies greatly. In most cases of *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 had been found in the implicated foods. However, there were also outbreaks where *B. cereus* concentrations of only  $10^3$  CFU/g were detected (EFSA BIOHAZ Panel, 2016). However, the *B. cereus* content in the food examined can differ from the *B. cereus* content of the consumed food at the time of consumption. It is difficult to determine a specific bacterial content 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 gastric passage, ii) the ability to attach to enterocytes and germinate and iii) the ability to form relevant amounts of enterotoxins.

### b) *C. perfringens* related disease

A prerequisite for a foodborne disease caused by *C. perfringens* is a high vegetative cell count 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).

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

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 *C. perfringens* is heat-sensitive 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 Skjellkvale, 1977; Naik and Duncan, 1978).

### Spore germination of *B. cereus* (s.l.) and *C. perfringens* in foods at high holding temperatures

Various factors influence the germination of spores, the growth of vegetative cells and the heat resistance of *B. cereus* (s.l.) and *C. perfringens* in food. These include, on the one hand, the properties of the bacterial strain. On the other hand, germination of spores is influenced by 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 limit for growth. For *B. cereus* spores, Knaysi (1964) describes a maximum temperature of 59 °C for the start of germination but without subsequent growth of the cells in the medium. A study by 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 at high holding temperatures**

In both, *B. cereus* (s.l.) and *C. perfringens*, the the upper temperature limits for growth differ greatly between individual strains. The growth rates close to the temperature limits for growth 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 limits for growth of some groups differ. With a view to possible growth in hot food, so-called mesophilic and thermotolerant 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 1). For the strain ATCC 14579, growth was shown 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 upper temperature limit for growth 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 and optimal growth temperature of 46.5 °C and 39.9 °C for strain ATCC 14579, and 46.9 °C and 39.8 °C for strain ATCC 10987, based on the "cardinal temperature model with inflection point" (CTMI) (Rosso et al., 1993).

The temperature limits for growth for the strain ATCC 14579 were again determined in a recent study by Carlin et al. (2013) and growth was observed in the laboratory at up to 46 °C, too. Using the CTMI, a maximum growth temperature of 48 °C (48.4 °C taking into account the 97.5<sup>th</sup> percentile) was calculated. The optimal growth temperature was 37.4 °C. The 97.5<sup>th</sup> percentile of this optimal temperature was 38.2 °C. Very similar values were obtained for another mesophilic strain (F4810; Table 1). 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.) showed that inactivation of mesophilic strains can already be expected at temperatures of 50 °C using (among others) the same strains as in the studies mentioned above (Antolinos et al., 2011; Becker et

al., 2011; den Besten et al., 2006; den Besten et al., 2010; Desai and Varadaraj, 2010) (Table 2).

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 approximately  $10^3$  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.  $10^7$  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 approximately  $10^6$  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 but not after 12 h. Similarly, Bursova et al. (2018) detected cereulide formation in dissolved milk powder inoculated with approximately  $10^3$  CFU/g of a spore suspension after 24 h incubation at 24 °C, but not after 12 h.

#### **b) Growth of thermotolerant *B. cereus* (s.l.) (*B. cytotoxicus*)**

The thermotolerant 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 1). 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 at up to 52 °C. A maximum growth temperature of 55 °C was calculated using the CTMI (55.9 °C taking into account the 97.5<sup>th</sup> percentile). The optimal growth temperature was 43.1 °C. The 97.5<sup>th</sup> percentile of this optimal temperature was 44.3 °C. Very similar values were obtained (Table 1) for another *B. cytotoxicus* strain

(NVH883/00). Taking into account the 97.5<sup>th</sup> percentile, the theoretical maximum growth temperature of this strain was even slightly higher at 56.8 °C.

Contrary to these theoretical, upper temperature limits for growth, Guerin et al. (2017) have previously reported a slight inactivation of strain NVH391-98 at 53 °C (Table 2). 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 were also observed.

This contradiction between the theoretical, upper temperature limits for growth 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. Therefore, 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 maximum and optimal growth temperatures mentioned above were generated under experimental laboratory conditions. However, the properties of a food include many more parameters than can 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. In a study by Ellerbroek (2008) the *B. cereus* count in cooling rice increased already at a temperature range of 60.2 °C to 58.8 °C (natural *B. cereus* contamination in rice).

*Table 1. Maximum and optimal growth temperatures and growth rates of mesophilic and thermotolerant strains of the B. cereus group (panC groups III, IV and VII)*

Strain ( <i>panC</i> group)	T <sub>opt</sub> (°C)	μ <sub>opt</sub> (h <sup>-1</sup> )	Theoretical T <sub>max</sub> (°C)	Maximum T (°C) at which growth was proven in the medium	Reference
<i>B. cytotoxicus</i> <sup>a</sup> NVH391-98 (VII)	46	n.d.	58 <sup>b</sup>	53	Auger et al., 2008
<i>B. cereus</i> ATCC14579 (IV)	35 - 40	n.d.	51 <sup>b</sup>	46	
<i>B. cereus</i> ATCC10987 (III)	35 - 40	n.d.	53 <sup>b</sup>	47	
<i>B. cytotoxicus</i> <sup>a</sup> NVH391-98 (VII)	41.4	n.d.	56.5 <sup>c</sup>	n.d.	Afchain et al., 2008 (based on data from Auger et al., 2008)
<i>B. cereus</i> ATCC14579 (IV)	39.9	n.d.	46.5 <sup>c</sup>	n.d.	
<i>B. cereus</i> ATCC10987 (III)	39.8	n.d.	46.9 <sup>c</sup>	n.d.	
<i>B. cytotoxicus</i> <sup>a</sup> NVH391-98 (VII)	43.1 (44.3)	3.89 (4.29)	55 (55.9) <sup>c</sup>	52	Carlin et al., 2013

<i>B. cytotoxicus</i> <sup>a</sup> NVH883/00 (VII)	37.6 (38.6)	2.31 (2.54)	55 (56,8) <sup>c</sup>	52	
<i>B. cereus</i> ATCC14579 (IV)	37.4 (38.2)	2.76 (2.91)	48 (48,4) <sup>c</sup>	46	
<i>B. cereus</i> F4810 (III)	38.7 (39.3)	3.12 (3.30)	48 (48,4) <sup>c</sup>	46	

<sup>a</sup>The species *B. cytotoxicus* was first described in 2013. The strains NVH391-98 and NVH883/00 are referred to as strains of the *B. cereus* group in the publications mentioned.

T<sub>opt</sub>: optimal growth temperature

μ<sub>opt</sub>: growth rate under optimal growth conditions

T<sub>max</sub>: theoretical maximum growth temperature, <sup>b</sup>calculated using the Ratkowsky model;

<sup>c</sup>calculated using the cardinal temperature model with inflection point (CTMI)

n.d.: not determined

Figures in brackets are values for the 97.5<sup>th</sup> percentile.

*Table 2: Inactivation of vegetative cells from mesophilic and thermotolerant strains of the B. cereus group (time in minutes for a 3 log reduction at different inactivation temperatures)*

Strain ( <i>panC</i> group)	48 °C	50 °C	53 °C	54 °C	55 °C	Reference
<i>B. cytotoxicus</i> NVH391-98 (VII)	n.d.	n.d.	44.1 ± 7.0	n.d.	9.1 ± 1.1	Guerin et al., 2017
<i>B. cytotoxicus</i> AFSSA 08CEB 44BAC (VII)	n.d.	n.d.	25.5 ± 5.1	15.5 ± 3.0	12.9 ± 1.1	
<i>B. cereus</i> ATCC14579 (IV)	n.d.	ca. 55	n.d.	n.d.	n.d.	den Besten et al., 2010
<i>B. cereus</i> ATCC10987 (III)	ca. 160	n.d.	n.d.	n.d.	n.d.	

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 time 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 only 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 samples of minced meat increased from 5.5 x 10<sup>4</sup> to 2.2 x 10<sup>7</sup> CFU/g within four hours under aerobic conditions and from 2 x 10<sup>5</sup> to 3 x 10<sup>7</sup> 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 thermotolerant *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<sup>th</sup> percentiles of the maximum growth temperature  $T_{max}$  and the maximum growth rate  $\mu_{opt}$  for NVH-883-00 determined in the study by Carlin et al. (2013) were used (see Table 1).

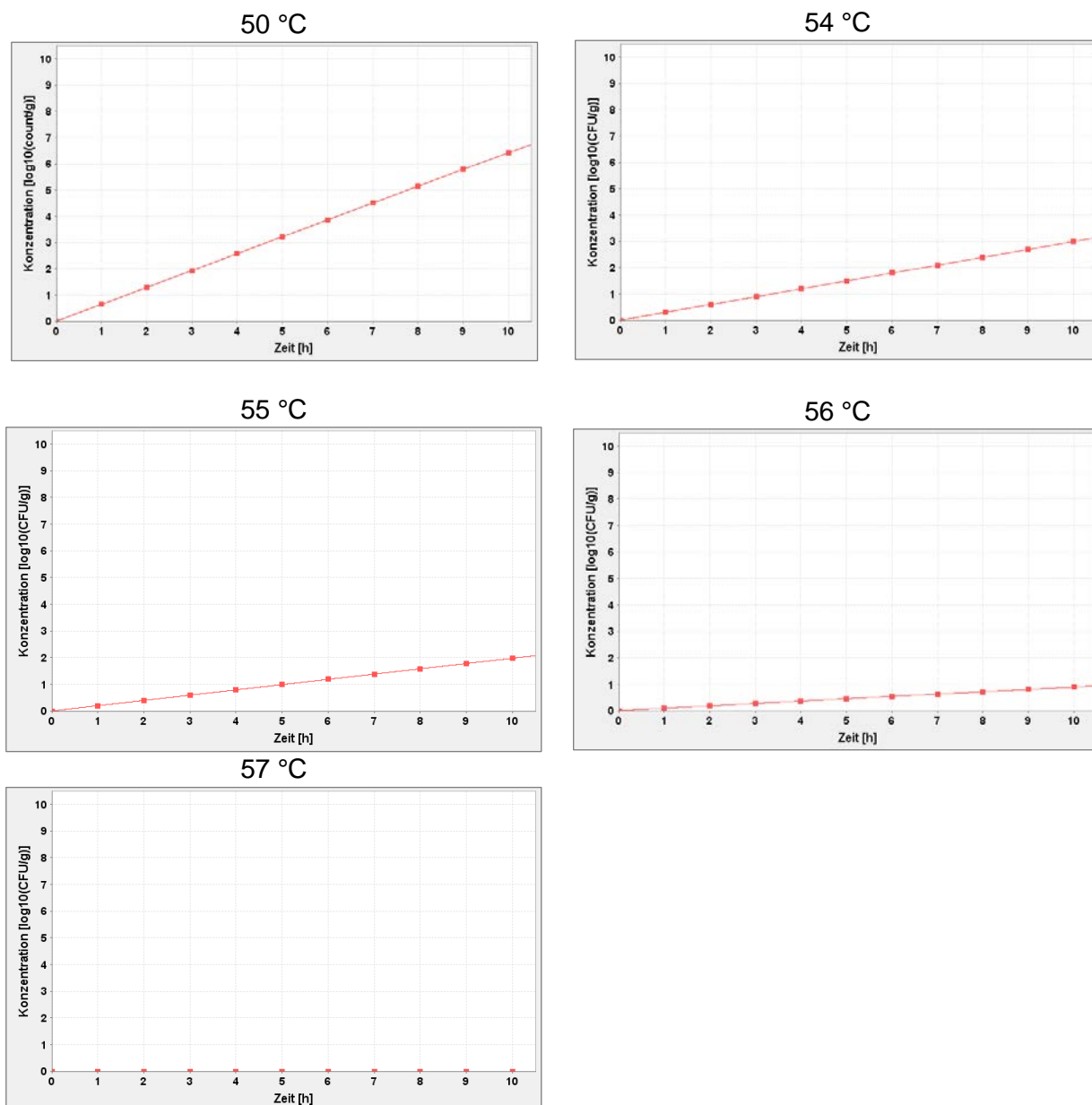


Figure 1: Prediction of the growth of *B. cytotoxicus* strain NVH-883-00 while holding hot over a period of 10 hours at 50 °C, 54 °C, 55 °C, 56 °C and 57 °C under conservative model assumptions

For each of the two relevant model parameters  $T_{max}$  and  $\mu_{opt}$ , we used the upper bound of the parameter's 95% confidence interval from the Carlin et al. 2013 publication as parameter values in the predictive model. 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.

In summary, 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 not 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 thermotolerant *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)

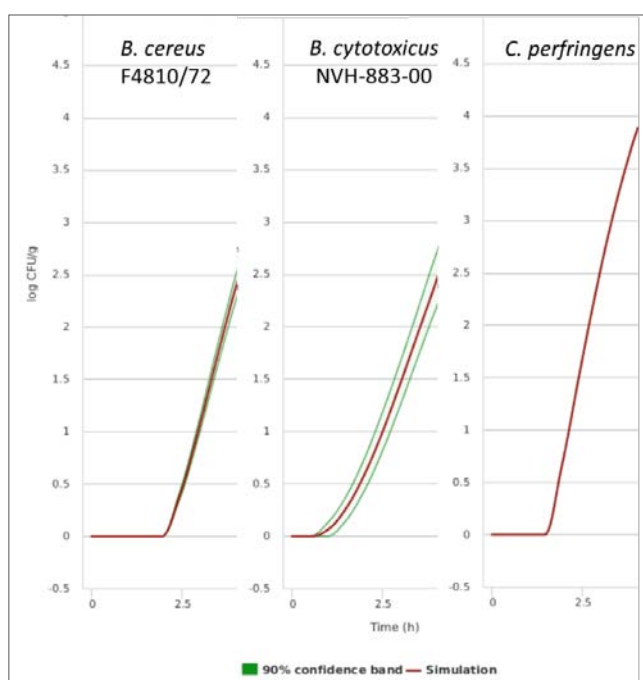


Figure 2a: Predicted growth of mesophilic, emetic *B. cereus* (strain F4810/72), thermotolerant *B. cytotoxicus* (strain NVH-883-00) and *C. perfringens* cells subject to cooling from 57 °C by 6 °C/h for 4 h with an assumed initial bacterial count of 1 CFU/g (0 log CFU/g). CFU: colony forming units

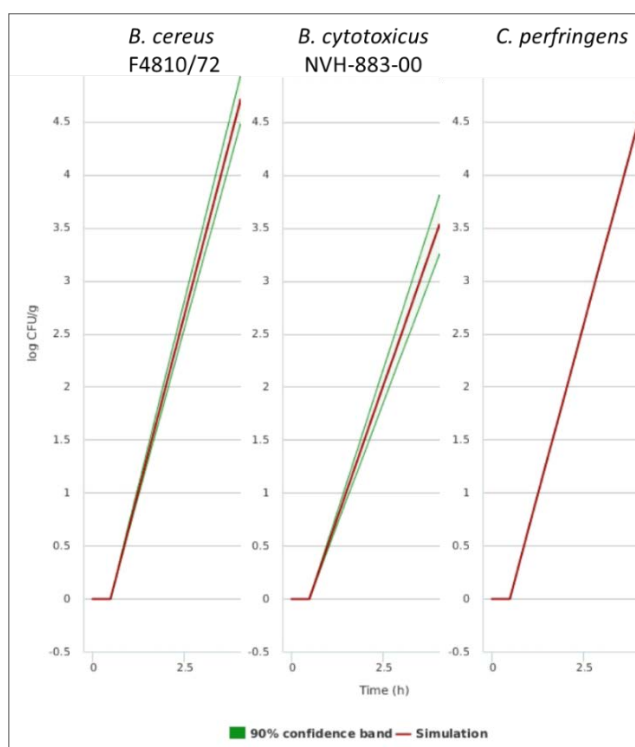


Figure 2b: Predicted growth of mesophilic, emetic *B. cereus* (strain F4810/72), thermotolerant *B. cytotoxicus* (strain NVH-883-00) and *C. perfringens* cells subject to cooling from 57 °C to 37 °C in 30 min and then storing for 3.5 h at 37 °C with an assumed initial bacterial count of 1 CFU/g (0 log CFU/g). CFU: colony forming units

The model-based forecasts shown were created using a modelling tool (<https://symprevius.eu>). In the case of the simulations described above for the mesophilic, emetic *B. cereus* strain F4810/72 and for the thermotolerant *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 97.5<sup>th</sup> percentile). All simulation calculations are conservative because they assume no lag phase and optimal pH and  $a_w$  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: If food is cooled down below 57 °C in an uncontrolled manner, significant microbial growth can occur already within four hours. This is 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 thermotolerant *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 thermotolerant *B. cytotoxicus* ( $\leq 56$  °C), *C. perfringens* ( $\leq 50$  °C) and mesophilic *B. cereus* ( $\leq 48$  °C) is possible. Depending on the spore and vegetative cell content at the start of the hot holding period (initial bacterial count) and the temperatures actually prevailing in the food over the four hours, bacterial counts 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 disease 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 above, 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 limits for growth of thermotolerant 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 growth of *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, thermotolerant *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 ( $\leq 56$  °C,  $\leq 50$  °C or  $\leq 48$  °C). How fast the growth is and whether critical microbial levels are reached

depends, among other things, on the initial bacterial count in the food and the temperatures that actually prevail in all parts of the food during the four hours.

The assumptions that underlie 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 content of *B. cereus* or *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 which limits the growth of *B. cereus* or *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 only 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 evaluation of the literature and modelling by the BfR suggest that, depending on the temperature conditions, growth of *B. cereus*, *B. cytotoxicus* and *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](https://www.bfr.bund.de/en/a-z_index/food_hygiene-129858.html)



BfR "Opinions app"

## 5 References

Afchain, A.L., Carlin, F., Nguyen-The, C., Albert, I., 2008. Improving quantitative exposure assessment by considering genetic diversity of *B. cereus* in cooked, pasteurised and chilled foods. *International Journal of Food Microbiology* 128, 165-173.

Agata, N., Ohta, M., Yokoyama, K., 2002. Production of *Bacillus cereus* emetic toxin (cereulide) in various foods. *International Journal of Food Microbiology* 73, 23-27.

Antolinos, V., Munoz, M., Ros-Chumillas, M., Aznar, A., Periago, P.M., Fernandez, P.S., 2011. Combined effect of lysozyme and nisin at different incubation temperature and mild heat treatment on the probability of time to growth of *Bacillus cereus*. *Food Microbiology* 28, 305-310.

Apetroaie-Constantin, C., Shaheen, R., Andrup, L., Smidt, L., Rita, H., Sallinoja-Salonen, M., 2008. Environment driven cereulide production by emetic strains of *Bacillus cereus*. *International Journal of Food Microbiology* 127, 60-67.

Aryani, D.C., den Besten, H.M., Hazeleger, W.C., Zwietering, M.H., 2015. Quantifying strain variability in modeling growth of *Listeria monocytogenes*. *International Journal of Food Microbiology* 208, 19-29.

Auger, S., Galleron, N., Bidnenko, E., Ehrlich, S.D., Lapidus, A., Sorokin, A., 2008. The genetically remote pathogenic strain NVH391-98 of the *Bacillus cereus* group is representative of a cluster of thermophilic strains. *Applied and Environmental Microbiology* 74, 1276-1280.

Bauer, T., Stark, T., Hofmann, T., Ehling-Schulz, M., 2010. Development of a stable isotope dilution analysis for the quantification of the *Bacillus cereus* toxin cereulide in foods. *Journal of Agricultural and Food Chemistry* 58, 1420-1428.

Becker, B., Schillinger, U., Bohringer, B., Untucht, T., Izykovski, N., Franz, C., 2011. Presence of bacilli in pasteurized packaged foods and determination of heat resistance of vegetative cells and spores of selected *Bacillus* isolates. *Archiv Fur Lebensmittelhygiene* 62, 205-211.

Berthold-Pluta, A., Pluta, A., Garbowska, M., 2015. The effect of selected factors on the survival of *Bacillus cereus* in the human gastrointestinal tract. *Microbial Pathogenesis* 82, 7-14.

Blankenship, L.C., Craven, S.E., Leffler, R.G., Custer, C., 1988. Growth of *Clostridium perfringens* in cooked chili during cooling. *Applied and Environmental Microbiology* 54, 1104-1108.

Bradshaw, J.G., Stelma, G.N., Jones, V.I., Peeler, J.T., Wimsatt, J.C., Corwin, J.J., Twedt, R.M., 1982. Thermal inactivation of *Clostridium perfringens* enterotoxin in buffer and in chicken gravy. *Journal of Food Science* 47, 914-916.

Bursova, S., Necidova, L., Harustiakova, D., 2018. Growth and toxin production of *Bacillus cereus* strains in reconstituted initial infant milk formula. *Food Control* 93, 334-343.

Carlin, F., Albagnac, C., Rida, A., Guinebretière, M.-H., Couvert, O., Nguyen-the, C., 2013. Variation of cardinal growth parameters and growth limits according to phylogenetic affiliation in the *Bacillus cereus* Group. Consequences for risk assessment. *Food Microbiology* 33, 69-76.

Carlin, F., Fricker, M., Pielat, A., Heisterkamp, S., Shaheen, R., Salonen, M.S., Svensson, B., Nguyen-The, C., Ehling-Schulz, M., 2006. Emetic toxin-producing strains of *Bacillus cereus* show distinct characteristics within the *Bacillus cereus* group. *International Journal of Food Microbiology* 109, 132-138.

Carlin, F., Girardin, H., Peck, M.W., Stringer, S.C., Barker, G.C., Martinez, A., Fernandez, A., Fernandez, P., Waites, W.M., Movahedi, S., Leusden, F.v., Nauta, M., Moezelaar, R., Torre, M.D., Litman, S., 2000. Research on factors allowing a risk assessment of spore-forming pathogenic bacteria in cooked chilled foods containing vegetables: a FAIR collaborative project. *International Journal of Food Microbiology* 60, 117-135.

Ceuppens, S., Boon, N., Uyttendaele, M., 2013. Diversity of *Bacillus cereus* group strains is reflected in their broad range of pathogenicity and diverse ecological lifestyles. *FEMS Microbiology Ecology* 84, 433-450.

Ceuppens, S., Rajkovic, A., Heyndrickx, M., Tsilia, V., van De Wiele, T., Boon, N., Uyttendaele, M., 2011. Regulation of toxin production by *Bacillus cereus* and its food safety implications. *Critical Reviews in Microbiology* 37, 188-213.

Ceuppens, S., Uyttendaele, M., Drieskens, K., Rajkovic, A., Boon, N., Wiele, T.V., 2012. Survival of *Bacillus cereus* vegetative cells and spores during in vitro simulation of gastric passage. *J Food Prot* 75, 690-694.

Clavel, T., Carlin, F., Lairon, D., Nguyen-The, C., Schmitt, P., 2004. Survival of *Bacillus cereus* spores and vegetative cells in acid media simulating human stomach. *Journal of Applied Microbiology* 97, 214-219.

Delbrassinne, L., Andjelkovic, M., Rajkovic, A., Botteldoorn, N., Mahillon, J., Van Loco, J., 2011. Follow-up of the *Bacillus cereus* emetic toxin production in penne pasta under household conditions using liquid chromatography coupled with mass spectrometry. *Food Microbiology* 28, 1105-1109.

Delbrassinne, L., Andjelkovic, M., Rajkovic, A., Dubois, P., Nguessan, E., Mahillon, J., Van Loco, J., 2012. Determination of *Bacillus cereus* Emetic Toxin in Food Products by Means of LC-MSA(2). *Food Analytical Methods* 5, 969-979.

den Besten, H.M., Mataragas, M., Moezelaar, R., Abee, T., Zwietering, M.H., 2006. Quantification of the effects of salt stress and physiological state on thermotolerance of *Bacillus cereus* ATCC 10987 and ATCC 14579. *Applied and Environmental Microbiology* 72, 5884-5894.

den Besten, H.M.W., van der Mark, E.-J., Hensen, L., Abee, T., Zwietering, M.H., 2010. Quantification of the Effect of Culturing Temperature on Salt-Induced Heat Resistance of *Bacillus* Species. *Applied and Environmental Microbiology* 76, 4286-4292.

Desai, S.V., Varadaraj, M.C., 2010. Behavioural pattern of vegetative cells and spores of *Bacillus cereus* as affected by time-temperature combinations used in processing of Indian traditional foods. *Journal of Food Science and Technology-Mysore* 47, 549-556.

Dierick, K., Van Coillie, E., Swiecicka, I., Meyfroidt, G., Devlieger, H., Meulemans, A., Hoedemaekers, G., Fourie, L., Heyndrickx, M., Mahillon, J., 2005. Fatal family outbreak of *Bacillus cereus*-associated food poisoning. *Journal of Clinical Microbiology* 43, 4277-4279.

Dommel, M.K., Lucking, G., Scherer, S., Ehling-Schulz, M., 2011. Transcriptional kinetic analyses of cereulide synthetase genes with respect to growth, sporulation and emetic toxin production in *Bacillus cereus*. *Food Microbiology* 28, 284-290.

Doyle, E., 2002. Survival and Growth of *Clostridium perfringens* during the Cooling Step of Thermal Processing of Meat Products A Review of the Scientific Literature.

EFSA BIOHAZ Panel, 2005a. Opinion of the Scientific Panel on Biological Hazards on *Bacillus cereus* and other *Bacillus* spp in foodstuffs. *EFSA Journal* 175, 1-48.



EFSA BIOHAZ Panel, 2005b. Opinion of the Scientific Panel on Biological Hazards on the request from the Commission related to *Clostridium* spp in foodstuffs. EFSA Journal 199, 1-65.

EFSA BIOHAZ Panel, 2016. Scientific opinion on the risks for public health related to the presence of *Bacillus cereus* and other *Bacillus* spp. including *Bacillus thuringiensis* in foodstuffs. EFSA Journal 14, 93.

Ellerbroek, L., 2008. Hot holding period of dishes in food holding equipment. *Archiv Fur Lebensmittelhygiene* 59, 137-141.

Fagerlund, A., Brillard, J., Fürst, R., Guinebretiere, M.H., Granum, P.E., 2007. Toxin production in a rare and genetically remote cluster of strains of the *Bacillus cereus* group. *Bmc Microbiology* 7.

Fagerlund, A., Ween, A., Lund, T., Hardy, S.P., Granum, P.E., 2004. Genetic and functional analysis of the *cytK* family of genes in *Bacillus cereus*. *Microbiology-Sgm* 150, 2689-2697.

Feijoo, S.C., Cotton, L.N., Watson, C.E., Martin, J.H., 1997. Effect of storage temperatures and ingredients on growth of *Bacillus cereus* in coffee creamers. *Journal of Dairy Science* 80, 1546-1553.

Finlay, W.J.J., Logan, N.A., Sutherland, A.D., 2000. *Bacillus cereus* produces most emetic toxin at lower temperatures. *Letters in Applied Microbiology* 31, 385-389.

Gilbert, R.J., Stringer, M.F., Peace, T.C., 1974. The survival and growth of *Bacillus cereus* in boiled and fried rice in relation to outbreaks of food poisoning. *Journal of Hygiene* 73, 433-444.

Gounina-Allouane, R., Broussolle, V., Carlin, F., 2008. Influence of the sporulation temperature on the impact of the nutrients inosine and l-alanine on *Bacillus cereus* spore germination. *Food Microbiology* 25, 202-206.

Granum, P.E., Skjelkvale, R., 1977. Chemical modification and characterization of enterotoxin from *Clostridium perfringens* type-a. *Acta Pathologica Et Microbiologica Scandinavica Section B-Microbiology* 85, 89-94.

Guerin, A., Dargaignaratz, C., Clavel, T., Broussolle, V., Nguyen-the, C., 2017. Heat-resistance of psychrotolerant *Bacillus cereus* vegetative cells. *Food Microbiology* 64, 195-201.

Guinebretiere, M.H., Auger, S., Galleron, N., Contzen, M., De Sarrau, B., De Buyser, M.L., Lamberet, G., Fagerlund, A., Granum, P.E., Lereclus, D., De Vos, P., Nguyen-The, C., Sorokin, A., 2013. *Bacillus cytotoxicus* sp nov is a novel thermotolerant species of the *Bacillus cereus* Group occasionally associated with food poisoning. *International Journal of Systematic and Evolutionary Microbiology* 63, 31-40.

Guinebretiere, M.H., Thompson, F.L., Sorokin, A., Normand, P., Dawyndt, P., Ehling-Schulz, M., Svensson, B., Sanchis, V., Nguyen-The, C., Heyndrickx, M., De Vos, P., 2008. Ecological diversification in the *Bacillus cereus* Group. *Environmental Microbiology* 10, 851-865.

Guinebretiere, M.H., Velge, P., Couvert, O., Carlin, F., Debuyser, M.L., Nguyen-The, C., 2010. Ability of *Bacillus cereus* group strains to cause food poisoning varies according to

phylogenetic affiliation (groups I to VII) rather than species affiliation. *Journal of Clinical Microbiology* 48, 3388-3391.

Hägglom, M.M., Apetroaie, C., Andersson, M.A., Salkinoja-Salonen, M.S., 2002. Quantitative analysis of cereulide, the emetic toxin of *Bacillus cereus*, produced under various conditions. *Applied and Environmental Microbiology* 68, 2479-2483.

Heini, N., Stephan, R., Ehling-Schulz, M., Johler, S., 2018. Characterization of *Bacillus cereus* group isolates from powdered food products. *International Journal of Food Microbiology* 283, 59-64.

International Commission on Microbiological Specifications for Foods (ICMSF), 1996. *Microorganisms in foods 5 : Characteristics of microbial pathogens*. Blackie Academic & Professional, London.

Jääskeläinen, E.L., Hägglom, M.M., Andersson, M.A., Vanne, L., Salkinoja-Salonen, M.S., 2003. Potential of *Bacillus cereus* for producing an emetic toxin, cereulide, in bakery products: quantitative analysis by chemical and biological methods. *Journal of Food Protection* 66, 1047-1054.

Jaloustre, S., Guillier, L., Morelli, E., Noel, V., Delignette-Muller, M.L., 2012. Modeling of *Clostridium perfringens* vegetative cell inactivation in beef-in-sauce products: a meta-analysis using mixed linear models. *Int J Food Microbiol* 154, 44-51.

Jessberger, N., Rademacher, C., Krey, V.M., Dietrich, R., Mohr, A.K., Bohm, M.E., Scherer, S., Ehling-Schulz, M., Martlbauer, E., 2017. Simulating Intestinal Growth Conditions Enhances Toxin Production of Enteropathogenic *Bacillus cereus*. *Frontiers in Microbiology* 8.

Kalinowski, R.M., Tompkin, R.B., Bodnaruk, P.W., Pruett, W.P., 2003. Impact of cooking, cooling, and subsequent refrigeration on the growth or survival of *Clostridium perfringens* in cooked meat and poultry products. *Journal of Food Protection* 66, 1227-1232.

Kim, H.S., Choi, S.J., Yoon, K.S., 2018. Efficacy Evaluation of Control Measures on the Reduction of *Staphylococcus aureus* in Salad and *Bacillus cereus* in Fried Rice Served at Restaurants. *Foodborne Pathogens and Disease* 15, 198-209.

Knaysi, G., 1964. Effect of temperature on the rate of germination in *Bacillus cereus*. *Journal of Bacteriology* 87, 619-622.

Kranzler, M., Stollewerk, K., Rouzeau-Szynalski, K., Blayo, L., Sulyok, M., Ehling-Schulz, M., 2016. Temperature Exerts Control of *Bacillus cereus* Emetic Toxin Production on Post-transcriptional Levels. *Frontiers in Microbiology* 7.

Labbe, R.G., Juneja, V.K., 2017. *Clostridium perfringens*, *Foodborne Diseases*. Elsevier, pp. 235-242.

Laurent, Y., Arino, S., Rosso, L., 1999. A quantitative approach for studying the effect of heat treatment conditions on resistance and recovery of *Bacillus cereus* spores. *International Journal of Food Microbiology* 48, 149-157.

Lovdal, I.S., Hovda, M.B., Granum, P.E., Rosnes, J.T., 2011. Promoting *Bacillus cereus* Spore Germination for Subsequent Inactivation by Mild Heat Treatment. *Journal of Food Protection* 74, 2079-2089.

Lücking, G., Dommel, M.K., Scherer, S., Fouet, A., Ehling-Schulz, M., 2009. Cereulide synthesis in emetic *Bacillus cereus* is controlled by the transition state regulator *AbrB*, but not by the virulence regulator *PlcR*. *Microbiology-Sgm* 155, 922-931.

Messelhäußer, U., Ehling-Schulz, M., 2014. *Bacillus cereus* - Vorkommen, Nachweis und Präventionsstrategien. B. Behr's Verlag, Hamburg.

Messelhäußer, U., Ehling-Schulz, M., 2018. *Bacillus cereus* - a Multifaceted Opportunistic Pathogen. *Current Clinical Microbiology Reports* 5, 120-125.

Messelhäußer, U., Frenzel, E., Blochinger, C., Zucker, R., Kampf, P., Ehling-Schulz, M., 2014. Emetic *Bacillus cereus* are more volatile than thought: Recent foodborne outbreaks and prevalence studies in Bavaria (2007-2013). *Biomed Research International*.

Naik, H.S., Duncan, C.L., 1977. Enterotoxin formation in foods by *Clostridium perfringens* type A. *Journal of Food Safety* 1, 7-18.

Naik, H.S., Duncan, C.L., 1978. Thermal inactivation of *Clostridium perfringens* enterotoxin. *Journal of Food Protection* 41, 100-103.

Naranjo, M., Denayer, S., Botteldoorn, N., Delbrassinne, L., Veys, J., Waegenaere, J., Sirtaine, N., Driesen, R.B., Sipido, K.R., Mahillon, J., Dierick, K., 2011. Sudden Death of a Young Adult Associated with *Bacillus cereus* Food Poisoning. *Journal of Clinical Microbiology* 49, 4379-4381.

Phat, C., Kim, S., Park, J., Lee, C., 2017. Detection of Emetic Toxin Genes in *Bacillus cereus* Isolated from Food and their Production of Cereulide in Liquid Culture. *Journal of Food Safety* 37.

Planchon, S., Dargaignaratz, C., Levy, C., Ginies, C., Broussolle, V., Carlin, F., 2011. Spores of *Bacillus cereus* strain KBAB4 produced at 10 degrees C and 30 degrees C display variations in their properties. *Food Microbiology* 28, 291-297.

Quintavalla, S., Parolari, G., 1993. Effects of temperature, *aw* and pH on the growth of *Bacillus* cells and spores: a response surface methodology study. *International Journal of Food Microbiology* 19, 207-216.

Raevuori, M., Genigeorgis, C., 1975. Effect of pH and Sodium Chloride on Growth of *Bacillus cereus* in Laboratory Media and Certain Foods. *Applied Microbiology* 29, 68-73.

Rajkovic, A., Uyttendaele, M., Ombregt, S.A., Jaaskelainen, E., Salkinoja-Salonen, M., Debevere, J., 2006. Influence of type of food on the kinetics and overall production of *Bacillus cereus* emetic toxin. *Journal of Food Protection* 69, 847-852.

Rajkovic, A., Uyttendaele, M., Vermeulen, A., Andjelkovic, M., Fitz-James, I., in't Veld, P., Denon, Q., Verhe, R., Debevere, J., 2008. Heat resistance of *Bacillus cereus* emetic toxin, cereulide. *Letters in Applied Microbiology* 46, 536-541.

Ratkowsky, D.A., Lowry, R.K., McMeekin, T.A., Stokes, A.N., Chandler, R.E., 1983. Model for bacterial culture growth rate throughout the entire biokinetic temperature range. *Journal of Bacteriology* 154, 1222-1226.

Rosso, L., Lobry, J.R., Bajard, S., Flandrois, J.P., 1995. Convenient Model To Describe the Combined Effects of Temperature and pH on Microbial Growth. *Applied and Environmental Microbiology* 61, 610-616.

Rosso, L., Lobry, J.R., Flandrois, J.P., 1993. An unexpected correlation between cardinal temperatures of microbial-growth highlighted by a new model. *Journal of Theoretical Biology* 162, 447-463.

Rouzeau-Szynalski, K., Stollewerk, K., Messelhauser, U., Ehling-Schulz, M., 2020. Why be serious about emetic *Bacillus cereus*: Cereulide production and industrial challenges. *Food Microbiology* 85.

Roy, R.J., Busta, F.F., Thompson, D.R., 1981. Thermal inactivation of *Clostridium perfringens* after growth at several constant and linearly rising temperatures. *Journal of Food Science* 46, 1586-1591.

Samapundo, S., Heyndrickx, M., Xhaferi, R., de Baenst, I., Devlieghere, F., 2014. The combined effect of pasteurization intensity, water activity, pH and incubation temperature on the survival and outgrowth of spores of *Bacillus cereus* and *Bacillus pumilus* in artificial media and food products. *International Journal of Food Microbiology* 181, 10-18.

Setlow, P., 2013. Summer meeting 2013-when the sleepers wake: the germination of spores of *Bacillus* species. *Journal of Applied Microbiology* 115, 1251-1268.

Shigehisa, T., Nakagami, T., Taji, S., 1985. Influence of heating and cooling rates on spore germination and growth of *Clostridium perfringens* in media and in roast beef. *Japanese Journal of Veterinary Science* 47, 259-267.

Shiota, M., Saitou, K., Mizumoto, H., Matsusaka, M., Agata, N., Nakayama, M., Kage, M., Tatsumi, S., Okamoto, A., Yamaguchi, S., Ohta, M., Hata, D., 2010. Rapid Detoxification of Cereulide in *Bacillus cereus* Food Poisoning. *Pediatrics* 125, E951-E955.

Sinigaglia, M., Corbo, M.R., Altieri, C., Massa, S., 2002. Response surface model for effects of temperature, water activity and pH on germination of *Bacillus cereus* spores. *Journal of Food Safety* 22, 121-133.

Smith, A.M., Evans, D.A., Buck, E.M., 1981. Growth and survival of *Clostridium perfringens* in rare beef prepared in a water bath. *Journal of Food Protection* 44, 9-14.

Taormina, P.J., Dorsa, W.J., 2004. Growth potential of *Clostridium perfringens* during cooling of cooked meats. *Journal of Food Protection* 67, 1537-1547.

Tegiffel, M.C., Beumer, R.R., Hoekstra, J., Rombouts, F.M., 1995. Germination of bacterial spores during sample preparation. *Food Microbiology* 12, 327-332.

Valero, M., Leontidis, S., Fernández, P., Martínez, A., Salmerón, M.C., 2000. Growth of *Bacillus cereus* in natural and acidified carrot substrates over the temperature range 5–30°C. *Food Microbiology* 17, 605-612.

van Asselt, E.D., Zwietering, M.H., 2006. A systematic approach to determine global thermal inactivation parameters for various food pathogens. *International Journal of Food Microbiology* 107, 73-82.

van der Voort, M., Abee, T., 2013. Sporulation environment of emetic toxin-producing *Bacillus cereus* strains determines spore size, heat resistance and germination capacity. *Journal of Applied Microbiology* 114, 1201-1210.

van Melis, C.C., Almeida, C.B., Kort, R., Groot, M.N., Abee, T., 2012. Germination inhibition of *Bacillus cereus* spores: impact of the lipophilic character of inhibiting compounds. *International Journal of Food Microbiology* 160, 124-130.

Wang, J., Ding, T., Oh, D.H., 2014. Effect of temperatures on the growth, toxin production, and heat resistance of *Bacillus cereus* in cooked rice. *Foodborne Pathogens and Disease* 11, 133-137.

Warda, A.K., den Besten, H.M., Sha, N., Abee, T., Nierop Groot, M.N., 2015. Influence of food matrix on outgrowth heterogeneity of heat damaged *Bacillus cereus* spores. *International Journal of Food Microbiology* 201, 27-34.

Wei, J., Setlow, P., Hoover, D.G., 2009. Effects of moderately high pressure plus heat on the germination and inactivation of *Bacillus cereus* spores lacking proteins involved in germination. *Letters in Applied Microbiology* 49, 646-651.

Wells-Bennik, M.H.J., Eijlander, R.T., den Besten, H.M.W., Berendsen, E.M., Warda, A.K., Krawczyk, A.O., Groot, M.N.N., Xiao, Y.H., Zwietering, M.H., Kuipers, O.P., Abee, T., 2016. Bacterial Spores in Food: Survival, Emergence, and Outgrowth, in: Doyle, M.P., Klaenhammer, T.R. (Eds.), *Annual Review of Food Science and Technology*, Vol 7, pp. 457-482.

Wijnands, L.M., Dufrenne, J.B., van Leusden, F.M., Abee, T., 2007. Germination of *Bacillus cereus* spores is induced by germinants from differentiated caco-2 cells, a human cell line mimicking the epithelial cells of the small intestine. *Applied and Environmental Microbiology* 73, 5052-5054.

Ziane, M., Desriac, N., Le Chevalier, P., Couvert, O., Moussa-Boudjemaa, B., Leguerinel, I., 2014. Identification, heat resistance and growth potential of mesophilic spore-forming bacteria isolated from Algerian retail packaged couscous. *Food Control* 45, 16-21.

## 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.

*This text version is a translation of the original German text which is the only legally binding version.*