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Factors influencing the risk of *Clostridium perfringens* germination and growth in hot ready-to-eat meals during courier delivery

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Abstract

The purpose of this study was to evaluate the risk of the spore germination and subsequent multiplication of *Clostridium perfringens* during the distribution of hot ready-to-eat (RTE) meals by the courier services. This evaluation was based on the ability of *C. perfringens* vegetative cells growth in the model RTE meals (minced chicken meat, minced pork, and minced beef) and on determination of the rate of internal temperature decrease in identical meals during a simulated distribution. After 4 h of incubation at 40 °C and 50 °C, *C. perfringens* NCTC 8798 counts reached 3.54 and 4.45 log CFU/g, respectively. No significant differences ($P > 0.05$) in *C. perfringens* NCTC 8798 counts were observed among the tested meals after 4 and 6 h of incubation at 40 °C, 50 °C, or 60 °C. It was concluded that an average time interval of the internal dish temperature decline to the hazardous zone between 40 °C and 50 °C was 60–90 min, which corresponded to a negligible growth of *C. perfringens* during this time. Even a 4-h delay in meal delivery did not increase *C. perfringens* counts above 5 log CFU/g which thus remained below the threshold for causing gastrointestinal illness (with limit of detection 1.7 log CFU/g).

Food safety, danger zone, specific heat capacity, thermal insulation, courier service

One of the current trends in gastronomy regards an increase of food delivery services that have become widespread in large cities across Europe (Kim and Chung 2024). As far as hot ready-to-eat (RTE) meals are concerned, one of the key factors is the meal's temperature at the time of delivery, which is instrumental in terms of food safety. Though the delivery time is usually not strictly specified, the food should reach the consumer as soon as possible to ensure a minimum temperature of 60 °C (Decree No. 121/2023 Coll. 2023).

However, securing this temperature is extremely problematic when meals are distributed over longer distances and periods of time. In this context, it is important to consider the risk of vegetative cell growth of pathogenic bacteria as well as the factors affecting the internal dish temperature decrease from production until delivery time (Komprda et al. 2025).

As far as the foodborne bacterial hazards are concerned, *Clostridium perfringens* currently poses a non-negligible threat for human health. In 2023, 2,934 outbreaks of foodborne diseases with a known causative agent were reported in the European Union (EU; 51.6 % of all foodborne outbreaks), of which 140 outbreaks (4.8%) were caused by the *C. perfringens* toxins (EFSA 2024).

Clostridium perfringens is ubiquitous in environments where vegetation decomposes, including soil (Li et al. 2019; Gohari et al. 2021), and is often isolated from faeces

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or wastewater (Ohnishi et al. 2025). The survival of *C. perfringens* in these environmental niches is associated with its ability to form resistant spores (Li et al. 2016). *Clostridium perfringens* is a component of normal gastrointestinal microbiota of humans and animals (Packer et al. 2020), but at the same time is also a significant pathogen in both humans and livestock (Gohari et al. 2021). The presence of *C. perfringens* in various environments, including the digestive tract, results in contamination of various types of food, such as chicken, beef or mutton, raw milk (Bendary et al. 2022), seafood (Ohnishi et al. 2025) and spices (Ohnishi et al. 2025). The classification of *C. perfringens* as an anaerobe remains ambiguous, as the bacterium exhibits varying degrees of oxygen tolerance and the capacity to either survive or grow under aerobic conditions depending on specific environmental factors (Savard et al 2026). It has been demonstrated that *C. perfringens* can grow in the presence of oxygen at temperatures ranging from 15 °C to 42 °C on meat (Juneja et al. 1994).

Based on the revised classification of *C. perfringens* isolates (seven types, A-G; Gohari et al. 2021), foodborne illnesses in humans are caused by type F strains. Approximately 70% of human isolates of this group carry the *cpe* gene on their chromosome, which encodes the ability to produce CPE enterotoxin. Most type F strains form highly resistant spores that are able to survive boiling for an hour or more, contrary to the vegetative cells that cannot survive even brief exposure to 55 °C (Li et al. 2016). When spores germinate, the short generation time of *C. perfringens* vegetative cells (under optimal conditions < 10 min; Li et al. 2019) allows a sufficient bacterial load (6–7 log vegetative cells/g) to be rapidly achieved to cause gastrointestinal disease (Gohari et al. 2021).

Reported outbreaks of foodborne illness caused by *C. perfringens* usually affect larger numbers of patients, smaller outbreaks often remain undiagnosed. The disease usually manifests itself with mild symptoms such as abdominal pain, nausea, and diarrhoea and lasts for approximately 24 h (Gkogka et al. 2020). Identified outbreaks often occur in facilities preparing large quantities of food in advance and then store it for a long time before serving (Jaloustre et al. 2013; Ohnishi et al. 2025). Inadequate temperature control during food preparation and inappropriate temperature conditions during storage of the hot RTE meals are among the contributing factors to outbreaks (Márquez-González et al. 2012; Packer et al. 2020; Coşkun et al. 2021; Glass et al. 2024). If hot meals are intended for longer storage after cooking, they must be cooled quickly to prevent the germination of *C. perfringens* spores that have survived heat treatment (Poumeyrol et al. 2014). Vegetative cells of *C. perfringens* can grow in a temperature range of 10 to 52 °C (Li et al. 2019). To prevent growth, it is therefore necessary to keep food outside this temperature range. The Czech legislation defines the minimum temperature for serving and distributing hot meals to be 60 °C (Decree No. 121/2023 Coll. 2023).

In order to keep the internal temperature of a hot RTE meal out of the danger zone allowing the *C. perfringens* vegetative cells growth, not only the speed of the meal delivery, but also the thermal performance of the pertinent packaging materials should be considered. However, not only insulation properties and thermal efficiency of the packaging (Saha et al. 2022), but also their environmental impact (Athira et al. 2021) and chemical safety (Zemanová 2020; Rantuch 2022) should be taken into account. Leaching of endocrine disruptors (such as bisphenol A or phthalic acid esters) under exposure to heat (Zemanová 2020) on one hand and the less sufficient insulating properties of the more sustainable materials leading to a faster cooling (Rantuch 2022) on the other exemplify the unavoidable trade-off between efficiency and food safety.

The aim of the presented study was i) to determine the rate of temperature decline in model hot RTE meals under conditions simulating distribution by courier services; ii) to evaluate the ability of *C. perfringens* vegetative cell growth in the identical model hot RTE meals after artificial contamination with spores; iii) and to combine the results of these

two parts of the study to evaluate the risk of *C. perfringens* germination and multiplication during the distribution of hot RTE meals by courier services.

The tested hypotheses were as follows. *Clostridium perfringens* spores will be able to germinate and vegetative cells to multiply at temperatures of 40 °C and 50 °C, respectively, but not anymore at a temperature of 60 °C. The *C. perfringens* counts will not reach the minimal infective dose of 6–7 log CFU/g after the 4-h culturing (a presumed maximum, but still reasonable, time of hot RTE meal delivery) even when the internal dish temperature cools down to values between 50 °C and 40 °C. The *C. perfringens* counts during culturing period up to 6 h at temperatures of 60 °C, 50 °C, and 40 °C, respectively, will be affected by the type of the model meal. Both the type of the model meal and the packaging material used for a simulated meal delivery will significantly affect the time interval of the internal dish temperature cool down to the values between 50 °C and 40 °C.

Materials and Methods

Dish preparation

Three types of meat were used for preparation: beef, pork, and chicken. Beef (boneless neck) and pork (boneless shoulder) were delivered directly from the meat producer (Steinhauser s.r.o., Tišnov, Czech Republic) in a chilled state 5 days after the slaughter. Chicken meat (meat from the upper thigh) was purchased at a retail store. Each type of meat was ground in an electric grinder (Kenwood PRO 2000 Excel; Maso-profit Ltd., Prague, Czech Republic) through a plate with a hole size of 5 mm. Sodium chloride (NaCl) was added to the minced meat to a concentration of 1% and minced-meat dishes weighing 150 g were formed.

Cooking of all meat samples was performed in a RATIONAL SCC61 combi oven (version SCC-07-00-09.1; Rational AG, Landsber am Lech, Germany) using the grilling program guaranteeing an internal temperature of 71 °C. Altogether 128 portions of each type of the dish were prepared.

One half of the samples (64 of each type of a dish) was used within the part of the experiment concerning a contamination of a dish with *C. perfringens* spores, packaging and storage of contaminated samples and enumeration of *C. perfringens* counts in contaminated samples; this part of the experiment included also determination of physicochemical properties (pH, water activity, NaCl content).

The time intervals of the internal dish temperature decrease to a hazardous threshold value in terms of *C. perfringens* growth during a simulated hot ready-to-eat meal delivery was measured in the second part of the experiment using the remaining part of the dish samples.

Dish sample contamination, packaging, and storage

A commercial mixture of BIOBALL HighDose 10K spores (Biomérieux, Lyon, France) was used to contaminate the prepared meals. The dishes were contaminated with a commercially obtained suspension of *C. perfringens* NCTC 8798 spores at a level of 40 spores per g of the meal (a concentration corresponding to one lyophilized pellet). The spores in the contaminated dishes were activated by heating to 80 °C for 15 min.

Contaminated dish samples and uninoculated control samples of each dish were placed in polypropylene (PP) trays (100% PP; Bittner Packaging Sp.j., Ożarów Mazowiecki, Poland) and packaged without atmosphere replacement on a T-190 packaging machine (MetalPack; Maso-profit, Ltd.) using 185 mm polyethylene terephthalate/PP top film (thickness 52 µm; Maso-profit Ltd.) and a sealing temperature of 180 °C. The samples were then tempered in a thermostat (Venti-Line; VWR International, Ltd., Czech Republic) at temperatures of 40 °C, 50 °C, and 60 °C, respectively, and subsequently stored at these temperatures. Individual samples were taken for a monitoring of the *C. perfringens* growth activity immediately after packaging and then after 1.0, 2.0, 2.5, 3.0, 4.0 and 6.0 h within each of the 40 °C, 50 °C, and 60 °C setting. The testing at these constant temperatures was chosen as a 'worst-case scenario' to identify growth limits. The experiment was performed in triplicate with each dish.

Microbiological analysis

Twenty-five g of each sample was weighed into the sterile homogenization bags using sterile instruments. The samples were diluted at a ratio of 1:9 with buffered peptone water (Oxoid, Basingstoke, UK) and homogenized in a Stomacher Star Blender LB 400 (VWR, Radnor, USA). From this primary dilution (homogenate), further tenfold dilutions of the samples were prepared.

Enumeration of *C. perfringens* was performed according to ISO 15213-2:2023. Tryptone-sulphite-cycloserine agar (TSC agar; Oxoid) was used to enumerate counts of *C. perfringens* bacteria at 37 °C/48 h anaerobically. Anaerostat and AnaeroGen 2.5 l atmosphere generator (Oxoid) was used to create an anaerobic environment.

Three presumptive *C. perfringens* colonies from each TSC agar plate were processed using the ethanol-formic acid extraction protocol and subsequently analysed by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry analysis (UltrafleXtreme instrument, Bruker Daltonics, Bremen, Germany; FlexControl software version 3.4; BioTyper software version 3.1; Bruker Daltonics; BioTyper database version 10.0,

9607 entries). Only identification outputs with a BioTyper log (score) above 2.0, which means highly reliable identification at the species level, were taken into consideration.

Physicochemical analyses

The pH value was measured in an aqueous solution of the 1/10-diluted sample using a combined ion-selective electrode (TipTrode; Hamilton, Switzerland) and pH meter 211 (Hanna Instruments, USA) at a temperature of 25 ± 1 °C. Water activity (a_w) was determined in a homogenized sample using a LabMaster a_w meter (Novasina AG, Switzerland) at a temperature of 25.0 ± 0.1 °C.

Sodium content was determined by atomic absorption spectrometry: 0.5 g of the sample, 6 ml of concentrated nitric acid (65% v/v) and 1 ml of hydrogen peroxide (30% v/v) were mixed, and mineralization was performed using an Ethos SEL microwave laboratory station (Milestone, Italy) at 200 °C for 30 min. The sodium content was then measured by air-acetylene flame atomization using a contrAA 700 atomic absorption spectrometer (Analytik Jena, Germany). All samples were measured in triplicate and the values obtained were processed using Aspect CS version 2.1 software, resulting in a single final value for each sample (product batch). The Na-based NaCl content in the sample (in %) was calculated using a conversion factor of 2.5 in accordance with EU Regulation No. 1169 (2011).

Dry matter was determined by drying of 5 g sample at 105 °C until constant weight (Komprda et al. 2021). Fat content was measured as a hexane/2-propanol extract according to Komprda et al. (2017).

Simulated delivery of dishes in the form of hot ready-to-eat meals

Second half of the samples of RTE meals prepared from minced chicken meat (n = 64), minced pork (n = 64), and minced beef (n = 64) was used in this part of the experiment. A 150 g portion of each meal was put into either a PP tray or a sugarcane bagasse (SB) tray (Ecoware Solutions Private Limited, New Delhi, India; Plate VI, Fig. 1c left and right, respectively. The trays were immediately heat-sealed as described above.

Immediately after heat-sealing with the overlying foil, the trays with meals were inserted into a three-layered bag consisting of a polyester external layer and two inner layers of polyethylene foam and aluminium foil (PPA) of overall thickness of 17 mm (Guangzhou A.C.T. Products Co., Ltd., Guangzhou, China) and a PP thermal box (extruded PP; thickness of 30 mm; Polibox Srl, Arluno, Italy; Figs. 1a and 1b), respectively. The heat-sealed trays were inserted into secondary containers in one layer (altogether 2 trays), three layers (6 trays), and six layers (12 trays), respectively. A temperature sensor of a data logger (Extech SDL200; Teledyne FLIR, France; Fig. 1d) was stuck into one of the trays within each layer. The internal dish temperature was monitored at 10-min intervals, and the total time interval from the moment of insertion of a meal into the secondary container until the time when the internal temperature cooled down to 50 °C and 40 °C, respectively, was recorded.

A customary external thermometer was used for the recording of an external temperature during the simulated hot RTE meal delivery of the closed secondary containers.

Statistical evaluation

Normality of the data distribution was evaluated by Kolmogorov-Smirnov test. Effects of the tested independent variables on variability of *C. perfringens* counts and on variability of the time interval of the internal dish temperature decrease were assessed by ANOVA of main factors. Kolmogorov-Smirnov pair test was used for evaluation of differences between counts of *C. perfringens* strains, and Kruskal-Wallis test with *post hoc* Nemenyi test for evaluation of differences between *C. perfringens* counts in meals cultured at 40 °C, 50 °C, and 60 °C and between *C. perfringens* counts in minced chicken meat, minced pork, and minced beef, respectively.

One-way ANOVA with *post hoc* Tukey's test was used for evaluation of differences at the time intervals of the internal dish temperature cooldown to a target temperature between the dishes, between primary trays, between secondary containers and between numbers of layers of primary trays in secondary container. Dependence of the *C. perfringens* counts on the storage time was evaluated by polynomial regression. Statistica 14 software (TIBCO Software Inc., Santa Clara, CA, USA) was used for the evaluations.

Results

Clostridium perfringens growth

In the first part of the experiment, growth of a selected strain of *C. perfringens* in three dishes under the influence of some internal (water activity, pH, fat content) and external (storage temperature, storage time) factors was evaluated. Samples of the same dishes, which were not artificially contaminated with spore suspension, were used as control. Values of the physicochemical characteristics of RTE meals prepared from chicken minced meat, minced pork, and minced beef are shown in Table 1.

As is apparent from Fig. 2, natural contamination by *C. perfringens* was not detected in any of the samples of any tested dish at any storage temperature (from all tested temperatures 0, 1, 2, 2.5, 3, 4, 6 h, only three pivotal time intervals are shown in Fig. 2; the

values were calculated regardless of the meal). Only data regarding *C. perfringens* strain NCTC 8798 are therefore presented in the following text.

Table 1. Physicochemical properties of minced chicken meat (C), minced pork (P) and minced beef (B).

Indicator	Type of dish (mean ± SEM)		
	C	P	B
Fat content (%)	$1.48^{b} \pm 0.06$	$2.31^{c} \pm 0.08$	$0.89^{a} \pm 0.04$
Dry matter (%)	$38.10^{a} \pm 0.73$	$44.10^{b} \pm 0.62$	$37.40^{a} \pm 0.58$
pH	$6.30^{b} \pm 0.02$	$6.06^{a} \pm 0.02$	$6.09^{a} \pm 0.02$
a_w	$0.96^{a} \pm 0.00$	$0.97^{b} \pm 0.00$	$0.97^{b} \pm 0.00$
NaCl (%)	$1.46^{a} \pm 0.06$	$1.46^{a} \pm 0.01$	$1.50^{b} \pm 0.02$

SEM – Standard error of the mean; a_w – water activity

[a,b,c] – Means with different superscripts within rows differ at $P < 0.05$, one-way ANOVA with *post hoc* Tukey's test, n = 64

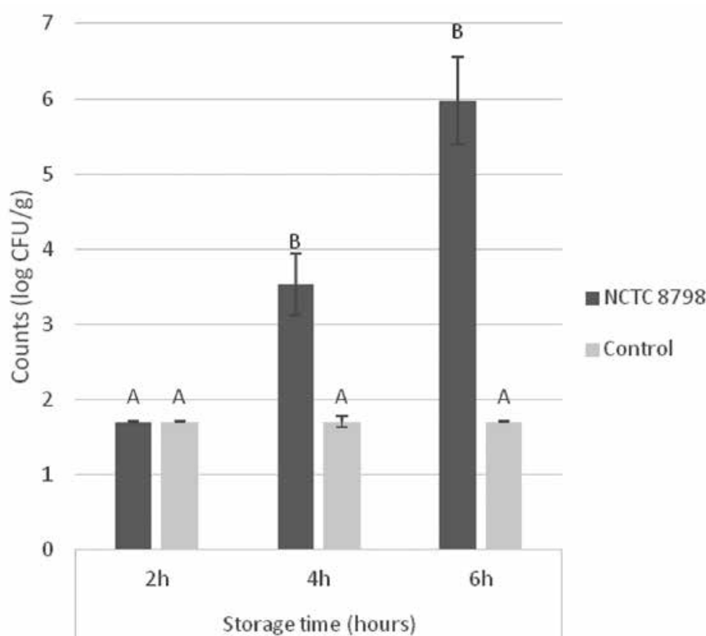

Fig. 2. Comparison of the counts of *Clostridium perfringens* strains cultured for 2, 4, and 6 h in all tested meals (minced chicken; minced pork, minced beef) at 40 °C

The results were calculated regardless of the type of meal tested; A, B – means with different letters within a given storage time differ at $P < 0.05$, Kolmogorov-Smirnov pair test, n = 11; all control samples and the NCTC 8798 2h sample were below the limit of detection (< 1.7 log CFU/g)

Table 2. Effects of the tested variability factors on total/explained variability of *Clostridium perfringens* NCTC 8798 counts (n = 192).

Factor	Variability (%) Total	Explained	*P*
Type of dish	1	1	0.56
Storage temperature	11	37	< 0.01
Storage time	46	52	< 0.01
NaCl	4	7	< 0.01
a_w	1	2	0.35
pH	1	1	0.58
Residual	36	/	

a_w – Water activity

Effects of the tested independent variables on differences in *C. perfringens* NCTC8798 counts are shown in Table 2. Storage temperature and storage time accounted for the majority of variability in the *C. perfringens* counts ($P < 0.01$). Apart from these two indicators, the only significant tested factor affecting *C. perfringens* growth was the NaCl content in the dishes ($P < 0.01$). However, differences in the NaCl concentration in the dishes were caused by weight losses during cooking. As far as the other two internal factors of the meals are concerned, neither water activity nor pH affected *C. perfringens* growth in the meals ($P > 0.05$). The effect of the meals themselves was also negligible ($P > 0.05$). It is also apparent from Table 2 that the tested independent variables explained more than 60% of total variability in the *C. perfringens* counts.

As far as storage temperature is concerned, *C. perfringens* NCTC 8798 counts after 2 h of storage were below the detection limit (< 1.7 log CFU/g) at all three tested temperatures (Fig. 3). It is also worth mentioning that counts of *C. perfringens* NCTC 8798 in the meals (regardless of a meal) stored at 40 °C and 50 °C did not differ ($P > 0.05$) either after 4 or 6 h of storage.

Dependences of the *C. perfringens* NCTC 8798 counts on storage time, calculated for storage temperature of 50 °C and 40 °C, respectively, were highly significant ($P < 0.001$; Fig. 4), which corresponds with the data of Table 2 as far as storage time is concerned (52% of explained variability of *C. perfringens* NCTC 8798 counts). Though a polynomial

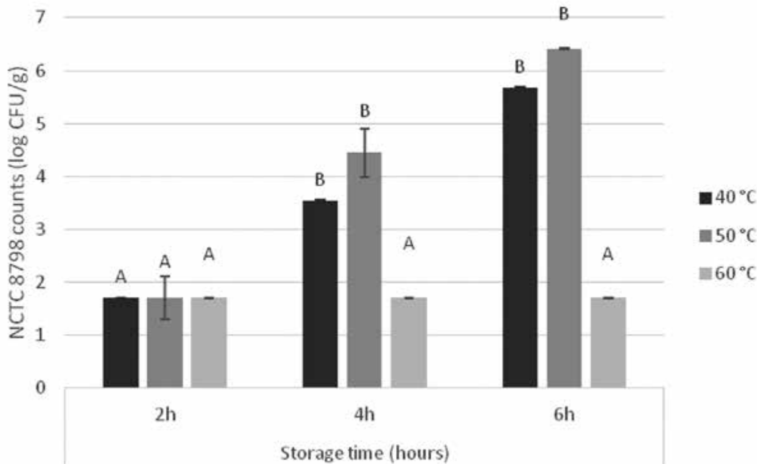

Fig. 3. Comparison of the counts of *Clostridium perfringens* NCTC 8798 cultured in all tested meals (minced chicken; minced pork, minced beef) at 40 °C, 50 °C, and 60 °C.

The results were calculated regardless of the type of meal tested; A,B – means with different letters within a given storage time differ at $P < 0.05$, Kruskal-Wallis test with *post hoc* Nemenyi test, n = 11. Values of 1.7 log CFU/g (40 °C, 60 °C) represented in the graph correspond to the detection limit (< 1.7 log CFU/g).

regression was used for calculation of the dependences, the *C. perfringens* NCTC 8798 growth between the time intervals of 3 and 6 h seems to be exponential (Fig. 4) and the counts (calculated regardless of the tested dish) after 6 h exceeded the value of 5 log CFU/g. On the other hand, *C. perfringens* NCTC 8798 growth until 2.5 h of culturing was negligible. For that reason, comparison of *C. perfringens* NCTC 8798 growth in particular tested meals (minced chicken meat, minced pork, minced beef) is shown only for the 4-h and 6-h storage periods (Fig. 5).

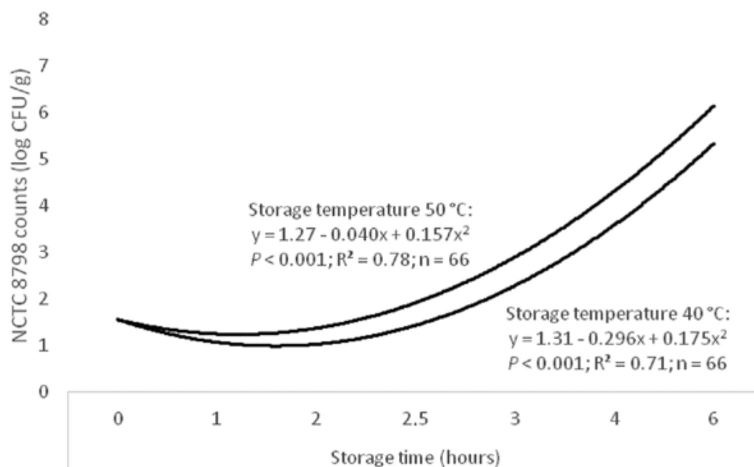

Fig. 4. Dependence of the counts of *Clostridium perfringens* NCTC 8798 on the storage time in dishes cultured at 40 °C and 50 °C

The regressions were calculated regardless of the type of meal tested (minced chicken; minced pork, minced beef).

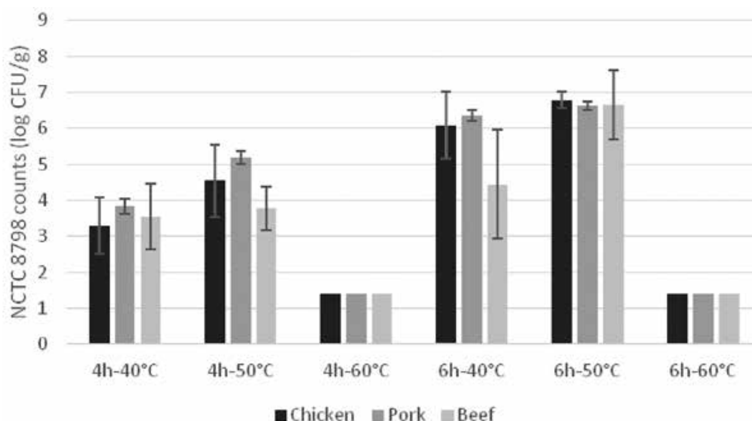

Fig. 5. Comparison of the counts of *Clostridium perfringens* NCTC 8798 in samples of minced chicken, minced pork, and minced beef cultured at 40 °C, 50 °C and 60 °C

Means within a given time-temperature combination do not differ ($P > 0.05$); Kruskal-Wallis test with *post hoc* Nemenyi test, n = 4

Counts of *C. perfringens* NCTC 8798 in all three types of the tested minced meat stored for 4 h and 6 h, respectively at the temperature of 60 °C were below the value of 1.7 log CFU/g (limit of detection). *Clostridium perfringens* NCTC 8798 counts in minced chicken meat, minced pork and minced beef did not differ ($P > 0.05$) either after 4-h storage at 40 °C (range of the values 3.30–3.84 log CFU/g) or at 50 °C (3.78–5.19 log CFU/g), or after 6-h storage at 40 °C (4.44–6.36 log CFU/g) or at 50 °C (6.63–6.79 log CFU/g), respectively (Fig. 5). The fact that no differences ($P > 0.05$) between the dishes regarding any of the time-temperature combinations were established is in a full agreement with the data in Table 2.

Factors affecting the interval of the internal dish temperature decrease

In the second part of the experiment, selected factors presumably affecting the time interval of the internal dish temperature decrease during the RTE meal delivery were evaluated, using aliquot parts of the meals used in the first part of the experiment.

Table 3 shows that the external temperature during simulated dish delivery accounted for the greatest part (nearly 60%) of explained variability of the internal dish temperature cooldown to a threshold temperature (data presented in Table 3 were calculated regardless of the threshold temperatures of 40 °C and 50 °C; the target temperature of 60 °C was not considered here, based on the results of the first part of the experiment: see Figs 2 and 5). Type of the dish, primary tray, secondary container and the number of layers of primary trays in the secondary container accounted for similar parts of explained variability of the internal dish temperature decrease. Though the effects of the tested factors (except for the type of the primary tray) were significant ($P < 0.01$), all these factors combined explained only 38% of the total variability of the internal dish temperature decrease (Table 3).

Table 3. Effects of the tested variability factors on total/explained variability of the time interval of the internal dish temperature decrease; the data were calculated regardless of the particular threshold temperature of either 40 °C or 50 °C; n = 192.

	Variability (%)		
Factor	Total	Explained	P
Type of dish	3	9	< 0.01
Primary tray	1	8	0.11
Secondary container	2	12	0.01
Number of layers	6	12	< 0.01
External temperature	26	59	< 0.01
Residual	62	/	

Figure 6 shows that the internal temperature of minced beef (regardless of the material of the primary tray or the secondary container and the number of primary tray layers in the secondary container) cooled down more rapidly ($P < 0.05$) to the target value of 50 °C (38.3 min) compared to both minced chicken meat (50.8 min) and minced pork (51.3 min). The same was true regarding the threshold temperature of 40 °C: 64.4 min for minced beef was a significantly lower value ($P < 0.05$) compared to 90.3 min for minced chicken meat and 87.7 min for minced pork.

As far as the primary tray material is concerned, the internal temperature of a dish (regardless of the type of meal, secondary container material, and number of layers) placed in the SB tray cooled down more rapidly ($P < 0.05$) to the target temperature of both 50 °C and 40 °C compared to the PP tray (42.3 min vs 51.3 min and 71.5 min vs 81.6 min, respectively).

Significant differences in the time interval of the internal dish temperature decrease were also found between materials of the secondary containers. Regardless of the type of meal, primary tray material or number of layers, a dish put inside the PP box cooled down more slowly ($P < 0.05$) compared to the PPA bag in the case of both threshold temperature of 50 °C (53.1 min vs 40.4 min) and 40 °C (90.9 min vs 66.7 min; Fig. 6).

Unsurprisingly, filling up of the space within a secondary container (i.e., number of layers; regardless of the type of dish, primary tray or secondary container) significantly

($P < 0.05$) affected the time of the internal dish temperature decrease: the more layers, the slower the cooling down of a dish for both target temperatures of 50 °C (36.9 min, 46.3 min, and 53.9 min for one, three, and six layers, respectively) and 40 °C (56.5 min, 76.5 min, and 96.0 min for one, three, and six layers, respectively).

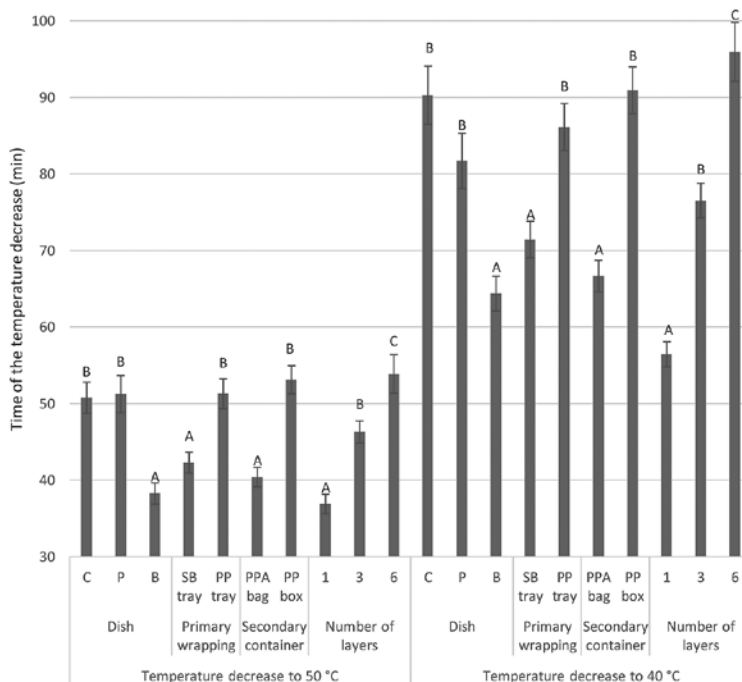

Fig. 6. The effect of a dish (minced chicken, C, n = 64; minced pork, P, n = 64; minced beef, B, n = 64); primary wrapping (sugarcane bagasse [SB] tray, n = 96; polypropylene [PP] tray, n = 96); secondary container (polyester/polyethylene foam/aluminium foil [PPA] bag, n = 96; PP box, n = 96); and the number of primary tray layers in the secondary container (one layer, n = 48; three layers, n = 72; six layers, n = 72) on the time interval of the internal dish temperature decrease to 40 °C and 50 °C, respectively

Data regarding a given independent variable were calculated regardless of other independent variables; A–C: means with different superscripts within a given independent variable differ at $P < 0.05$ (one-way ANOVA with *post hoc* Tukey's test)

Discussion

Clostridium perfringens growth in the dishes

Figure 2 shows that a natural contamination of RTE meals prepared from minced beef, minced pork, and minced chicken meat with *C. perfringens* in the present study was negligible. These data are in agreement with the findings of the United States Department of Agriculture Food Safety Inspection Service (USDA FSIS) that the spores of *C. perfringens* in commercial RTE meat products are typically present at less than detectable concentrations of 0.5 log CFU/g (Glass et al. 2024). *Clostridium perfringens*

concentrations in the samples of cooked meat and poultry tested by USDA FSIS on production lots of RTE meat products were mostly below 100 CFU/g, only 5% of the samples were within the range of 100–710 CFU/g (Kalinowski et al. 2003); the authors stated that *C. perfringens* in commercially processed RTE meat and poultry products presents very low public health risk.

Tessi et al. (2002) found no *C. perfringens* in any of the RTE meals with a delivery time of up to 80 min from the centralized food service establishment to the consumers. Meldrum et al. (2009) reported that no samples of RTE meals distributed in schools in Wales, United Kingdom, contained detectable concentrations of *C. perfringens*. Similarly, within the Australian red meat industry, although a proportion of the raw products contaminated with *C. perfringens* was 10%, the post processing contamination rate was nil (Sumner et al. 2005).

Although the health risk presented by *C. perfringens* seems to be low based on the above-mentioned data, it is worthy to mention an underrepresentation of the *cpe*-positive *C. perfringens* isolates in environments (less than 5% of the total population; Tran et al. 2026). We did not pursue *cpe*-positive *C. perfringens* in the present study, equally as the quoted authors (Tran et al. 2026), who nevertheless point out a higher likelihood of these strains to survive the control measures. Bearers of this enterotoxin gene are more difficult to eliminate from the food production process due to their adaptability to harsh environments and a high genomic flexibility.

In the present study, *C. perfringens* NCTC 8798 did not grow at 60 °C, but reached the values above 3.5 log CFU/g and nearly 4.5 log CFU/g after 4 h at 40 °C and 50 °C, respectively (Fig. 3). These results agree with the often-mentioned optimum growth temperature of *C. perfringens* of 37–45 °C (García and Heredia 2011). Huang and Li (2020) reported the minimal, optimal, and maximal growth temperature of *C. perfringens* in cooked chicken meat during cooling to be 14.8 °C, 42.9 °C, and 50.5 °C, respectively. *Clostridium perfringens* spores heat-activated at 75 °C reproduced rapidly in the range of 52 °C to 10 °C based on the mathematical modelling of temperature during germination and outgrowth in chilled chicken (Wang et al. 2023).

As far as the *C. perfringens* growth rate is concerned, pertinent studies are mostly concerned with the so-called safe cooling rates in order to reach the temperature values suitable for the safe storage of foods, i.e., below the minimal growth temperature of *C. perfringens* (15 °C; García and Heredia 2011). Therefore, the particular relationships concerning *C. perfringens* counts usually use much longer time intervals (tens of hours; Wang et al. 2023) compared to the present study, where the maximal time interval of 6 h for a simulated RTE meal delivery was considered. From this viewpoint, comparison of the results of the present experiment with similar studies is limited.

Nevertheless, data on the dependence of *C. perfringens* NCTC 8798 counts on the growth time at 40 °C and 50 °C, respectively (Fig. 4) correspond to the range of the generation times of 8–20 min at 40 °C and 50 °C as reported by Brynestad and Granum (2002). However, García and Heredia (2011) argue that the *C. perfringens* generation times between 41 °C and 46 °C can be less than 8 min. On the other hand, Andersen et al. (2004) reported germination plus outgrowth plus lag times of *C. perfringens* at 45 °C to be 1.2–1.5 h.

Cooling a dish to 40 °C poses a risk not only for the germination and growth of *C. perfringens* but also of another spore-forming bacterium, *Bacillus cereus*. However, this growth occurs no earlier than after 2.5 h. The growth rate of *B. cereus* is determined not only by temperature, pH values, and food composition but also by specific bacterial strains. Yet, in contrast to *C. perfringens*, *B. cereus* did not germinate at 50 °C even after 4 h (Kameník et al. 2025; Komprda et al. 2025).

Based on the mathematical modelling of temperature on germination and outgrowth of *C. perfringens* in chilled chicken, Wang et al. (2023) reported values of 4.5 log CFU/g and 7.5 log CFU/g after 4 h and 6 h, respectively, which is also comparable with our data (Fig. 4). The time required for an increase of *C. perfringens* counts by 1 log CFU/g was determined by Wang et al. (2023) as 3.7 h using the Gompertz model. *Clostridium perfringens* counts in ground beef reached the values of 4 log CFU/g after 5 h within a cooling rate challenge study by Smith et al. (2004), which is also not very different from our results (Fig. 4). Juneja et al. (2010), using a predictive dynamic model for *Clostridium perfringens* spore germination and outgrowth in cooked pork products, observed an increase of the mean *C. perfringens* counts of 0.24 log CFU/g and 2.16 log CFU/g, when the cooling time from 54.4 °C to 27 °C was 1.5 h and 3.0 h, respectively. Temperature abuse during cooling of noncured ground pork from 54.4 °C to 7.2 °C within 20 h resulted in a 2.8 log CFU/g increase in *C. perfringens* in a study by Márquez-González et al. (2012).

An effect of pH and NaCl on *C. perfringens* was investigated by Glass et al. (2024) during cooling of cooked (73 °C) turkey. An increase of 1–3 log CFU/g was observed during a 3-h cooling from 48.9 °C to 26.7 °C, where the pH and NaCl values were < 6.2% and > 1.2%, respectively; these values are comparable with our data, except for a higher pH of minced chicken meat (Table 1). The quoted authors (Glass et al. 2024) point out an impact of the low pH and high salt content on the inhibition of *C. perfringens*.

Time interval of the internal dish temperature decrease

Based on the inner factors of the dishes tested in the present study (Table 1), the significantly shorter time interval of the internal temperature decrease in minced beef compared to minced pork or minced chicken meat (Fig. 6) is difficult to explain. Lower ($P < 0.05$) dry matter content of minced beef compared to minced pork implies higher water content and so higher heat capacity, which should slow down, not accelerate, the cooling of a dish. Water has a very high specific heat capacity (c_p; approximately 4 180 J/kg/K) that stays almost constant between 70 °C and 40 °C. One possible explanation could regard significantly lower ($P < 0.05$) fat content in minced beef compared to both minced pork and minced chicken meat (Table 1), despite the fact that fat has substantially lower specific heat capacity (approximately 2 060 J/kg/K) than water and gradually decreases as the dish cools from 70 °C to 40 °C (according to the equation $c_p = 1.9842 \times 10^3 + 1.4733t - 4.8008 \times 10^{-3}t^2$; Tun and Baranov 2019).

The fact that internal temperature of a dish (regardless of the type of dish) wrapped in the SB tray cooled down more rapidly to the target temperature of both 50 °C and 40 °C compared to the PP tray (Fig. 6) does not agree with the values of the thermal conductivities of these materials. Two materials were used for primary food packaging in our study: conventional PP and an advanced material made from sugarcane waste known also as SB. Information on SB can be found in several studies (Loh et al. 2013; Mahmud and Anannya 2021), including its thermal properties (Athira et al. 2021) and its use in packaging materials (Ghaderi et al. 2014). Bagasse, consisting of approximately 40–50% cellulose, 25–35% hemicellulose, 15–20% lignin and a low percentage of ash, has thermal conductivity around 0.05 W/m/K (dry) and 0.11 W/m/K (wet; Mahapatra et al. 2017). These values are likely to increase at the higher temperatures (40–90 °C) but they are still lower, implying better thermal insulation properties, compared to PP (0.2 W/m/K). Although this is contrary to the results of the present study, it is on the other hand in full agreement with the data of our previous study (Komprda et al. 2025), where the internal temperature of the meal wrapped in the SB tray cooled down to 40 °C within nearly a half-time interval compared to the PP tray.

When considering the packaging material for hot RTE meal delivery, the following factors should also be taken into account: the sustainability and eco-friendliness of SB which can

be upcycled (Aditya et al. 2024) and composted (Singh et al. 2021), and the very long decomposition time (hundreds of years) of PP packaging (Andler et al. 2022), including the possibility of toxic substance migration into the wrapped foods (Paiva et al. 2022).

As far as secondary containers are concerned, the PP thermal box slowed down significantly the internal dish temperature decrease compared to the secondary PPA container. As is the case for the primary wrappings, the results for secondary containers also agree with the data of our previous study employing similar containers (Komprda et al. 2025). Although the thermal conductivity of the PPA bag's sandwiched wall is substantially lower than that of pure PP (0.035–0.05 W/m/K and 0.2 W/m/K, respectively; Jing et al. 2022; Yin et al. 2024), the difference in the internal dish temperature decrease was probably caused by smaller wall thickness of the PPA bag compared to the PP box (17 mm vs 35 mm).

The significant differences in the time interval of the internal dish temperature cooldown to both 40 °C and 50 °C between numbers of layers of the primary bowls inserted in a secondary container (Fig. 6) were to be expected based on the equation of $Q = m \times c \times \Delta T$ (Teggar et al. 2025), describing a direct proportion of the total heat (Q) to the total mass of substance m (meal in this case), specific heat capacity (c), and the temperature difference (ΔT). Thus, the larger the amount of food in the secondary container, the higher the specific heat capacity of the entire internal system, due to the fact that the specific heat capacity of a meal is always higher than that of air (1 000 J/kg/K).

In conclusion, the type of dish did not have any significant effect on the spore germination and outgrowth of *C. perfringens* (one of the tested hypotheses was not confirmed). In contrast, the inability of *C. perfringens* NCTC 8798 spores to germinate and multiply at 60 °C was confirmed, as well as the hypothesis regarding the potential of this strain to grow at 40 °C and 50 °C, and, after 6-h culturing at 50 °C, to reach values above 6 log CFU/g. Nevertheless, considering a maximal but still reasonable delivery time interval of 4 h for hot RTE meals by courier services, *C. perfringens* counts are unlikely to exceed 5 log CFU/g and therefore to reach hazardous levels.

The present study confirmed the differences between SB and PP primary trays, and between a PPA bags and PP boxes, in their ability to maintain internal dish temperature during simulated hot RTE meal delivery. Six layers of PP trays put in a PP box provided an optimal combination, keeping the internal dish temperature above 50 °C. However, thermal insulation properties of packaging materials should be considered together with their sustainability, eco-friendliness, and toxicological safety.

Combining the results of both parts of the present experiment, it can be concluded that the time interval for the internal dish temperature to decrease into the danger zone between 40 °C and 50 °C was approximately 60–90 min, which corresponds to negligible growth of *C. perfringens* during this period. Based on the results, it can be stated that even a dish delivery delayed by the courier service for up to 4 h does not increase *C. perfringens* counts above 5 log CFU/g which thus stay below the hazardous values of 6–7 log CFU/g.

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Fig. 1. Types of secondary containers and primary trays used in simulated dish delivery:

a - polyester/polyethylene foam/aluminium foil (PPA) bag (17 mm thickness); b - polypropylene (PP) thermal box (extruded PP, 30 mm thickness); c - PP tray (left), sugarcane bagasse (SB) tray (right) (Ecoware Solutions Private Limited, New Delhi, India); (d): internal temperature measurement using a puncturing sensor of a data logger (Extech SDL200, Teledyne FLIR, France).