SAFETY OF THE HOT READY-TO-EAT MEAL DELIVERY CONSIDERING AN OCCURRENCE OF *BACILLUS CEREUS*

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ABSTRACT

An effect of the type of a meal (cooked rice; mashed potatoes; mushroom sauce), inner primary packaging (sugarcane bagasse [SB] tray; polypropylene [PP] tray), secondary container (polyester/polyethylene foam/aluminum foil [PPA] bag; PP box) on the time interval of the internal hot ready-to-eat (RTE) meal temperature decrease to the value critical for *Bacillus cereus* growth (40 °C) was tested during a simulated delivery. *B. cereus* growth was quantified in aliquot samples of the same meals. Type of a meal had no effect on the tested time interval ($p > 0.05$). Using PP tray instead of bagasse tray, and PP box instead of PPA bag delayed ($p < 0.05$) the internal meal temperature decrease by 50 and 15 minutes, respectively. Average *B. cereus* counts in the naturally contaminated meals after the four-hour culturing at 40 °C was < 3 log CFU·g^{-1}. It was concluded that the delivery of a hot RTE meal up to four hours under the tested conditions is unlikely to facilitate *B. cereus* growth above unacceptable levels.

Keywords: food safety, pathogenic bacteria, cooked rice, sugarcane bagasse, polypropylene

INTRODUCTION

Regarding hot ready-to-eat (RTE) meal delivery, keeping temperature of the food until delivery out of the zone of rapid microbial growth is not only a matter of the delivery speed, but also of the thermal performance of the packaging material (Ricci et al., 2020). Selecting appropriate packaging involves a trade-off between thermal efficiency, environmental impact, and chemical safety (Zemanová, 2020). The present study focuses on analyzing the impact of packaging materials on the thermal stability of the hot RTE meals during a prolonged (up to 4 hours) delivery and evaluates how packaging choices can influence a microbial safety during transport. Counts of *Bacillus cereus* in the meal were chosen as a safety marker.

B. cereus sensu lato is a Gramm-positive, facultative anaerobic, motile, spore-forming, rod-shaped opportunistic human pathogen causing gastrointestinal illnesses due to the ability of producing emetic and/or diarrheal toxins. Emetic and diarrheal strains are able to grow at the pH range of 4.5–9.5, temperature range of 4–48 °C, minimal water activity of 0.93 and NaCl concentration up to 7 % (Woh and Ng, 2024). *B. cereus* infective dose is reported to be > 10^5 CFU·g^{-1} of food (Schoeni and Wong, 2005). Due to the ability to form spores, it is practically impossible to prevent *B. cereus* contamination of foods. Cereal products, rice, seeds, dairy products, poultry, vegetables, herbs, spices and seafood are usually listed as the risky products, including RTE foods (Samapundo et al., 2011).

The following null hypotheses were tested in the present study: type of a meal, packaging material and external temperature during the delivery has no effect on the time interval of the meal internal temperature decrease to the risky value of 40 °C; counts of *B. cereus* in naturally contaminated tested hot RTE meals do not exceed risky levels of 10^4 colony forming units ($CFU \cdot g^{-1}$) after four hours at the temperature of 40 °C.

MATERIALS AND METHODS

Three meals considered risky from the viewpoint of a microbiological safety were tested: cooked rice (long-grain rice, onion); mashed potatoes (fresh potatoes, milk); mushroom sauce (dried mushrooms, beef broth, milk, cream, flour). Cooking temperature at least 90 °C was applied during meals production.

The original portion of each of the prepared meal was then divided into two aliquots. Effect of selected conditions on the maintenance of the internal hot RTE meal temperature during a simulated delivery was evaluated using one part of each meal. Growth of *B. cereus* in the second aliquot of the same meal was quantified in the laboratory.

A 150 g portion of each meal was put into each of the two tested primary wrappings: a polypropylene (PP) tray and a sugarcane bagasse (SB) tray, respectively. The trays were immediately heat-sealed with an overlying heat-sealing foil (polyethylene/polypropylene) at the heat-sealing temperature of 180 °C.

Immediately after the heat-sealing, the trays with the meals were inserted into each of the two types of the secondary containers: a three-layered bag consisting of a polyester external layer and two inner layers, polyethylene foam and aluminum foil (PPA bag; overall thickness of 17 mm); a polypropylene thermobox (PP box; thickness of 30 mm).

The heat-sealed bowls were inserted into the secondary containers in one layer (altogether 2 trays), three layers (6 trays) and six layers (12 trays), respectively. A sensor of a datalogger was stuck into each layer of trays and the internal temperature on each layer was recorded during a time interval from inserting the meals into the secondary container (time 0 min) to 420 min. A closed secondary container was exposed to the temperature conditions of a surrounding environment and the external temperature was recorded using a customary external thermometer. The simulation of the meal delivery proceeded during all seasons of the year in the range of external temperatures from –2 °C to +33 °C.

Microbiological safety of the hot RTE meals was assessed using aliquots of the same meals, in which a natural contamination of the meals with *B. cereus sensu lato* was evaluated in laboratory conditions. Six 150 g portions of each meal prepared as mentioned above were inserted into the PP trays. The trays were immediately heat-sealed as mentioned above and then two trays of each meal were incubated for 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 h at 40 °C, 50 °C and 60 °C, respectively. Subsequently, 25 g of each sample was weighed into sterile homogenization bags and samples were diluted 1:9 with buffered peptone water and homogenized in a stomacher. The homogenate was inoculated on Mannitol Yolk Polymyxin agar and cultivated for 24 h at 30 °C. Suspected colonies of *B. cereus* were reinoculated onto blood agar and isolates showing a complete hemolysis were identified by Matrix-Assisted-Laser-Desorption-Ionization-Time-of-Flight mass spectroscopy (MALDI-TOF MS).

Statistical evaluation. The data regarding effects of type of a dish, material of a primary wrapping, type of a secondary container, number of layers of the primary bowls stored in the secondary container and external temperature on the time interval of the internal dish temperature decrease during a simulated delivery were assessed by factorial ANOVA. Differences in the time intervals of the internal dish temperature decrease between the dishes, between the primary wrappings, between the secondary containers and between the numbers of layers, respectively, were evaluated by one-way ANOVA with *post-hoc* Tukey's test. Regression analysis was used for evaluating a dependence of the time interval of the internal dish temperature

decrease on the external temperature during the simulated delivery, and for evaluating a dependence of *B. cereus* counts on the time interval of the internal dish temperature decrease. The differences between counts of *B. cereus* growing at three tested temperatures and in the three different dishes were evaluated by Kruskal-Wallis test with *post-hoc* Nemenyi test. Statistica 14 software (TIBCO Software Inc., Santa Clara, CA, USA) was used for all statistical evaluations.

RESULTS

No differences in the tested time interval between dishes ($p > 0.05$) were found out. The time of the temperature decrease to 40 °C ranged between 100 and 110 minutes (calculated irrespective of primary wrapping, secondary container and number of layers).

On the other hand, significant differences ($p < 0.05$) between primary tray materials and between types of a secondary container, respectively, were established. Inserting a meal into a polypropylene tray instead of a sugarcane bagasse tray prolonged a temperature decrease to 40 °C by nearly 50 minutes and putting the primary trays into polypropylene box prolonged the temperature decrease by approximately 15 minutes as compared to the polyester/polyethylene foam/aluminum foil bag.

The time of the internal dish temperature decrease to 40 °C (calculated irrespective of the type of a dish, type of a primary tray or material of a secondary container) was approximately 45 minutes when only one layer of trays was put into a secondary container, but 100 minutes when three layers were applied, and 140 minutes in the case of six layers ($p < 0.05$).

The time of the internal dish temperature decline to 40 °C (Y; minutes) decreased linearly with decreasing external temperature during the simulated hot dish delivery (X; °C): $Y = 61.0 + 2.58X$ ($R^2 = 0.12$; $p < 0.001$).

The dependence of the *B. cereus* growth (Y; log CFU·g^{-1}) on the time of the internal hot RTE meal temperature decrease to 40 °C (X; hours) was described by the following equation: $Y = 1.43 – 0.17X + 0.14X^2$ ($R^2 = 0.49$; $p < 0.001$). It follows from the equation that *B. cereus* counts reached an average value of 2.99 log CFU·g^{-1} of meal after the four-hour culturing at 40 °C. However, in some cases, *B. cereus* counts reached nearly 4 log CFU·g^{-1}.

Though the culturing temperature was able to affect *B. cereus* counts, the counts at 40 °C were significantly higher ($p < 0.05$) in comparison with both 60 °C and 50 °C only in mashed potatoes. Only tendency ($p = 0.09$) was found out in cooked rice, and *B. cereus* counts were practically the same in mushroom sauce cultured in the all three temperatures ($p > 0.05$).

As far as the comparison of the meals is concerned, no differences in *B. cereus* counts between mashed potatoes, cooked rice and mushroom sauce cultured at 60 °C were established ($p > 0.05$). The same was true regarding the temperature of 50 °C ($p > 0.05$). On the other hand, as far as the temperature of 40 °C is concerned, *B. cereus* counts in mashed potatoes was higher ($p < 0.05$) as compared to mushroom sauce and tended to be higher ($p = 0.06$) in comparison with cooked rice.

DISCUSSION

The negligible effect of a meal on the time interval of the internal meal temperature decrease is explained by the high-water content in all three dishes, water having one of the highest specific heat capacities (approximately 4186 J·kg^{-1}·K^{-1}).

Two materials were used for primary food packaging in the study: conventional PP and an advanced material known also as sugarcane bagasse. The thermal conductivity of bagasse (0.05 W·m^{-1}·K^{-1}; Mahapatra et al., 2017) is lower in comparison with PP (0.2 W·m^{-1}·K^{-1}), which implies better thermal insulation properties

of bagasse. This is contrary to the results of our study, where the internal temperature of the meal wrapped in the sugarcane bagasse tray and the PP tray decreased to 40 °C within an average time interval of 60 minutes and 110 minutes, respectively. This result, however, does not question environmental benefits of bagasse, including compostability. When considering a choice of a proper packaging material for the hot RTE meal delivery, their toxicological attributes should also be taken into account: PP may release chemical substances such as bisphenol A at elevated temperatures (Rantuch, 2022).

As far as secondary containers are concerned, the two types of packaging usually employed by food delivery companies were used in the present study, and significant difference between their abilities to maintain internal temperature of the transported meal above 40 °C was demonstrated. Polypropylene thermobox prolonged the temperature decrease by 15 minutes compared to secondary container consisting of polyester/polyethylene foam/aluminium foil. However, it should be noted that the PP box has a wall thickness of 35 mm, while the PPA bag, though three-layered, was only 17 mm thick. With comparable material thicknesses, the layered packaging would be more suitable, as the thermal conductivity of its sandwich wall is 4-6 times lower (0.035–0.05 $W \cdot m^{-1} \cdot K^{-1}$) than that of pure PP (0.2 $W \cdot m^{-1} \cdot K^{-1}$; Jing et al., 2022).

The significant differences in the time interval of the internal meal temperature decrease to 40 °C between numbers of layers of the primary bowls stacked in secondary container should be expected: total heat (Q) is directly proportional to the total mass of the substance m (in this case mass of the meal), specific heat capacity (c) and the temperature difference (ΔT) according to the equation $Q = m \cdot c \cdot \Delta T$ (Teggar et al., 2025). The more food is there in the secondary container, the higher is the specific heat capacity of the entire internal system, because the specific heat capacity of the meal is always higher than that of air (1000 $J \cdot kg^{-1} \cdot K^{-1}$).

Three naturally contaminated model RTE meals were used in the present study, which is different in comparison with other similar experiments, where the tested foods were usually inoculated with the heat-shocked *B. cereus* spores (Juneja et al., 2019).

A threshold, below which the inner temperature of the hot RTE meals should not decrease, was fixed to 40 °C in the present study. A value of 30–40 °C is often mentioned as an optimum for *B. cereus* growth, maximum growth temperature being 55 °C (Juneja et al., 2019). On the other hand, a sufficient temperature, where *B. cereus* spores are not able to germinate and vegetative forms cannot multiply, is ≥ 60 °C (Farber and Hughes, 1995). This value is based on the often-reported threshold value for *B. cereus* counts of 5 log $CFU \cdot g^{-1}$ of food (Yang et al., 2023). However, the most important threat for human health as far as *B. cereus* is concerned are not its counts as such, but a production of the emetic toxin cereulide. Based on the current scientific knowledge, cereulide production stops at 40 °C (Rouzeau-Szynalski et al., 2020). So, it could be concluded that the temperature of 40 °C can be considered a satisfactory lower limit for handling the hot RTE meals as far as *B. cereus* is concerned.

Regarding *B. cereus* counts in the RTE foods, 10^3–10^5 $CFU \cdot g^{-1}$ or ml are considered acceptable, the value exceeding 10^5 already unsafe (Soares et al., 2020). Juneja et al. (2019), reported *B. cereus* counts in cooked rice to be 4 log $CFU \cdot g^{-1}$ after 4 hours of storing at 40 °C. This value is by 2 log $CFU \cdot g^{-1}$ higher in comparison with our data as far as cooked rice is concerned, probably due to the fact that the quoted authors inoculated the tested dish with the heat-shocked spores, but natural contamination was evaluated in the present study.

Apart from cooked rice, the literature data regarding other two meals tested in the present study (mashed potatoes, mushroom sauce) are rather scanty as far as *B. cereus* growth is concerned. From the viewpoint of comparable time/temperature values, no useful data are available regarding hot RTE mushroom sauce delivery. As far as thermally treated potato puree is concerned, it is possible to extrapolate from the results of Rajkovic et al. (2006) an increase of *B. cereus* counts from 3.5 log $CFU \cdot g^{-1}$ to 4.0 $CFU \cdot g^{-1}$ in the spore-

inoculated samples stored at 20 °C for 4 hours. This is similar to the *B. cereus* counts in mashed potatoes cultured at 40 °C in the present study.

CONCLUSIONS

Both inner primary packaging and outer secondary container significantly affected the time of keeping the temperature above the threshold value. Contrary to the expectation, the presumed better thermal properties of the sugarcane bagasse as compared to polypropylene (PP) was not confirmed in the present study. On the other hand, the hypothesis that counts of *B. cereus* in naturally contaminated hot RTE meals do not exceed unacceptable levels (10^4 CFU·g^{-1}) after the 4-hour culturing (and relatedly during the 4-hour simulated delivery) at the temperature of 40 °C was confirmed: the 4-hour time interval corresponded to *B. cereus* counts slightly below 3 log CFU·g^{-1}. It could be concluded that a hot RTE meal delivered up to four hours under the conditions tested in the present study is not likely to facilitate *B. cereus* growth above the unacceptable levels and so endanger a consumer.

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