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Toxin production and growth of pathogens subjected to temperature fluctuations simulating consumer handling of cold cuts

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ABSTRACT

It is crucial for the quality and safety of ready-to-eat (RTE) foods to maintain the cold chain from production to consumption. The effect of temperature abuse related to daily meals and elevated refrigerator temperatures on the growth and toxin production of *Bacillus cereus*, *Bacillus weihenstephanensis* and *Staphylococcus aureus* and the growth of *Listeria monocytogenes* and *Yersinia enterocolitica* was studied. A case study with temperature loggings in the domestic environment during Easter and Christmas holidays was performed to select relevant time and temperature courses. A model for bacterial surface growth on food using nutrient agar plates exposed to variations in temperatures was used to simulate food stored at different temperatures and exposed to room temperature for short periods of time. The results were compared with predicted growth using the modeling tool ComBase Predictor.

The consumers exposed their cold cuts to room temperatures as high as 26.5 °C with an average duration of meals was 47 min daily for breakfast/brunch during the vacations. Short (≤2 h) daily intervals at 25 °C nearly halved the time the different pathogens needed to reach levels corresponding to the levels associated with human infection or intoxication, compared with the controls continuously stored at refrigerator temperature. Although the temperature fluctuations affected growth of both *B. weihenstephanensis* and *S. aureus*, toxin production was only detected at much higher cell concentrations than what has been associated with human intoxications. Therefore, growth of *L. monocytogenes* and *Y. enterocolitica* was found to be the limiting factor for safety. In combination with data on temperature abuse in the domestic environment, modeling programs such as ComBase Predictor can be efficient tools to predict growth of some pathogens but will not predict toxin production.

1. Introduction

For many foods, low temperature storage from production to consumption is necessary to maintain quality and safety. Although temperature abuse may occur in every stage in the food chain, the least controllable part is at the consumer stage. This is especially important for ready-to-eat (RTE) foods with a long shelf life based on an unbroken cold chain. If RTE food is stored periodically or constantly at a too high temperature or eaten after the "last day of consumption," the producers cannot guarantee for the safety of the product. In a study conducted in Norway in 2009 where over 2000 randomly selected consumers were surveyed, 44% reported they continued using cold cuts even after the expiry date. The majority of these consumers, 62%, considered their own sensory apparatus as an important tool to evaluate the quality of cold cuts after the last day of consumption (Jacobsen and Lavik, 2011; Røssvoll et al., 2013, 2012). This is consistent with a survey conducted in the United States, where nearly two-thirds stated that they relied on their senses whether to eat refrigerated food or not (Kosa et al., 2007). This is a hazardous practice, as growth of pathogens will normally not destroy the organoleptic quality of the food (Farber, 1991).

It is important to get a better understanding of the consequence of consumer food-handling practices for the growth and toxin production of pathogens under domestic conditions. Laws and regulations are implemented in the food chain to reduce risk and improve food safety all the way from the primary producer to retail, but as soon as the product is purchased by the consumer, there is little knowledge of how the product is treated. Garrido et al. (2010) conducted a study of *Listeria monocytogenes* growth on sliced RTE ham stored at temperatures simulating temperature variations in refrigerators. However, they did not investigate how the consumers treated the sliced RTE ham outside of the refrigerators (Garrido et al., 2010). Many studies describe the growth of pathogenic bacteria in food stored at varying temperatures. But to our knowledge nobody has collected information about rapid temperature

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variations caused by consumers' handling of food, and tested how these temperature variations influence the growth and toxin production of pathogenic bacteria.

When the product is taken out of the cooling systems in the supermarkets, it is out of the producers' control. We were therefore interested in looking at the temperatures that RTE foods are exposed to at the consumer stage over a longer period of time. Breakfast and lunch in Norway are traditionally based on bread and different cooked meat and fish products served cold. Vacations and holidays were chosen as the domestic setting in this study, as such occasions often entail longer meals as people eat more slowly and leave the food at room temperature for extended periods of time, and could therefore represent a possible "worst case" scenario. Also, food may be stored longer before consumption during Christmas and Easter holidays than in the weekends, since food stores are closed or not accessible and traditional home-made food is often prepared in advance. Temperatures from purchase to refrigerator storage by the consumers were not considered in this study. The aim of this study was to i) measure the occurrence of repeated temperature abuse connected to serving RTE food in the domestic environment, using vacations as a worst case scenario, and ii) investigate both pathogenic growth and toxin production under such temperature abuse. We focused on bacteria that are known to cause problems either by growing at low temperatures or by producing toxins after moderate temperature abuse: *L. monocytogenes* (Farber and Peterkin, 2000), *Yersinia enterocolitica* (Nesbakken, 2000) and the toxin producers *Bacillus cereus* (Granum and Baird-Parker, 2000), *Bacillus weihenstephanensis* (emetic toxin) (Stenfors et al., 2002) and *Staphylococcus aureus* (Baird-Parker, 2000). *B. weihenstephanensis* is a psychrotolerant species which belongs to the *B. cereus* group (Lechner et al., 1998). There has not been any documented foodborne outbreaks caused by *B. weihenstephanensis*, however it has been found that the organism has the ability to produce several pathogenicity factors and it was therefore included in this study (Stenfors et al., 2002).

2. Materials and methods

2.1. Refrigerator temperatures and temperature variations at the consumer stage

In order to choose relevant conditions for the laboratory experiments, a model system for measuring the temperature profile for cooled RTE products served at breakfast/brunch was established. The case study is a descriptive study involving a convenience sample of 60 households recruited from the Oslo and Akershus area in Norway. Subjects were recruited by word-of-mouth and by the snowball effect. Criteria for inclusion of subjects were that they ate cold cuts and that they were preparing most of the meals themselves. One part of the study targeted pregnant women. The subjects were provided with a temperature logger (EL-USB data logger Lascar Electronics, UK) during Easter 2009, Christmas 2009 or Easter 2010. The subjects received the following written guidance on how to treat the temperature loggers (translated here from Norwegian):

> "The temperature logger is to be treated as cold cuts during the whole period. Put the temperature logger in your refrigerator where you also keep your cold cuts. When you take the cold cuts out of the refrigerator, take the temperature logger out as well. For instance, when laying the table for breakfast and/or lunch with cold cuts, the temperature logger is to be placed on the table as well. And when the meal is over and the cold cuts are put back into the refrigerator, the temperature logger is to be placed beside the cold cuts in the refrigerator."

The temperature was logged every minute over the whole period of 11 days.

2.2. Temperature effect on growth and toxin production

2.2.1. Experimental design of growth and toxin production experiments

The effect of the temperature abuse related to daily meals and elevated refrigerator temperatures found in the case study was studied on pathogenic growth and toxin production using nutrient agar plates as a food model. The results were compared with predicted growth using the modeling tool ComBase predictor.

2.2.2. Bacterial strains and culture conditions

The pathogens *B. cereus*, *B. weihenstephanensis* and two different strains of *L. monocytogenes*, *S. aureus* and *Y. enterocolitica* were investigated (Table 1). In the first experiment, strains isolated from foodborne outbreaks in Norway were chosen, that is *L. monocytogenes* 2583/92, *S. aureus* 50089 (toxin A), *Y. enterocolitica* 1106-0129-1 O:3 and an emetic toxin producing *B. weihenstephanensis* MC67. The experiment was performed three times on different weeks with newly prepared inocula, resulting in three biological replicates. In the subsequent experiment, the other strains were chosen for comparison, see Table 1. The purpose of the latter experiment was to investigate whether the isolates chosen deviated significantly from other strains, and it was performed only once.

Stock cultures were maintained in 20% glycerol at −80 °C. For inoculum preparation, frozen suspensions were streaked on tryptic soy agar (TSA; Oxoid, Basingstoke, UK), and incubated at 30 °C for 24 h, except for *B. cereus* F3605/73 and *B. weihenstephanensis* MC67. Preliminary experiments showed that *Bacillus* spp. formed large, indistinct colonies on TSA that were difficult to count, and that they grew better when preincubated at 20 °C. Standard plate count agar (PCA; Oxoid) and a preincubation temperature of 20 °C were therefore used for *Bacillus* spp. Three to five colonies from the agar plates were transferred to 5 mL tryptic soy broth (TSB; Oxoid) and cultured for 16–18 h at 30 °C, 200 rpm (*L. monocytogenes* was cultured without shaking because of a lack of a shaking incubator at the class 3 pathogen laboratory). The strains were transferred to 4 °C and incubated for 16–18 h for cold adaptation prior to the growth and toxin experiments.

2.2.3. Surface growth

The effect of rapidly fluctuating temperatures on the growth of the selected pathogenic strains was examined on the surface of TSA (PCA for *Bacillus* spp. strains). Every plate was inoculated with 100 μL of a 10^3 CFU/mL inoculum, which gave approximately 100 bacterial cells on each plate. The agar plates were incubated at 4 ± 1 °C and 8 ± 1 °C (Innova 4230, New Brunswick Scientific Co, Edison, USA) and exposed to a temperature of 25 ± 0.5 °C (Termaks Series B8000, Bergen, Norway) every day for 30 min, 1 h or 2 h, respectively. *S. aureus* was incubated at 8 ± 1 °C and 12 ± 1 °C (Innova 4230, New Brunswick Scientific Co, Edison, USA), as it does not proliferate at temperatures below 7 °C (Halpin-Dohnalek and Marth, 1989; Rørvik and Granum, 2007). Control plates were also inoculated with cells and incubated at a constant temperature (4, 8 or 12 °C). The experiments lasted for 9–10 days.

Samples were removed daily for viable cell count. The cells were washed off the agar surface using 2 mL cold (4 °C) buffered peptone water and a sterile L-shaped spreader (VWR International, West Chester, USA). The viable cell count analysis was performed using an automatic spiral plater (WASP (Whitley Automatic Spiral Plater); Don Whitley Scientific, West Yorkshire, UK). *Bacillus* sp. made cell aggregates and was difficult to homogenize in the buffered peptone water. Preliminary experiments showed that the aggregates could be dissolved by sonication (Joyce et al., 2003) or the use of Tween 80 (Sigma-Aldrich, St. Louis, USA) (Besse and Lafarge, 2001; Oh and Cox, 2010) in the washing water. 10 min of sonication of the buffered peptone water with the cells from agar plates with *Bacillus* sp. was compared with the use of 0.2% Tween 80 instead of buffered peptone water. No significant difference in viable cell count was found between the two methods, and Tween 80 was used in further experiments.