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Microbiological examination of ready-to-eat foods and ready-to-bake frozen pastries from university canteens

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ABSTRACT

During a 10-year inspection survey (2001–2010), a microbiological study of ready-to-eat (RTE) foods and ready-to-bake frozen pastries from 15 canteens of the university campus was undertaken to determine their microbiological quality. The cumulative study revealed that the aerobic colony counts for the RTE product groups were as follows: from 10^6 to 10^8 CFU/g for 50% of sandwiches; under the detection limit (<10 CFU/g) for 88.6% of oven baked pastries; $<10^5$ CFU/g for 86.5% of desserts oven baked; from 10^3 to 10^9 CFU/g for desserts with dairy cream. The highest mean Enterobacteriaceae counts were recorded for desserts with dairy cream. The highest percentages of foodborne pathogens were: 20% *Listeria monocytogenes* and 12.5% *Staphylococcus aureus* in desserts with dairy cream; 17.5% *Salmonella* spp. and 8.5% presumptive *Escherichia coli* O157 in sandwiches; 14.6% *Bacillus cereus* in oven baked pastries. Aerobic colony counts were in the range 10^7–10^8 CFU/g for 48.8% of frozen pastries; whereas Enterobacteriaceae counts between 10^3 and 10^4 CFU/g were detected in 35.3%. Foodborne pathogens prevalences for frozen pastries were as follows: *B. cereus*, 31.8%; *Salmonella* spp., 28.6%; presumptive *E. coli* O157, 25%; *S. aureus*, 8.7%; *L. monocytogenes*, 8.7%. Improved sanitary conditions in the processing plants and precautionary measures are necessary for consumer protection.

1. Introduction

Changing life styles-including more convenience foods for on-the-go consumption had as a consequence a broad range of emerging microbiological issues in food safety. In food service establishments, in addition to refrigerated ready-to-eat (RTE) foods (like sandwiches and desserts) are provided to the consumers hot-held products mainly oven baked pastries which are prepared from frozen pastries (ready-to-bake) heat processed at the point of sale. Ready-to-bake frozen pastries occupy an increasingly great portion of the food market covering sectors such as institutional and catering businesses, supermarkets, and restaurants (Kennedy, 2000). They are subjected to prolonged storage under freezing conditions before baking. These products are characterized by quick preparation time and look and taste as if they are freshly made. Although the market of frozen bakery products has been increased in the last years, the safety issues of the products have not been studied extensively.

RTE foods, especially salads and sandwiches have been implicated in foodborne illness outbreaks, as these foods are often prepared by hand and this direct contact may lead to an increased incidence of contamination with potential foodborne pathogens (Colombari et al., 2007). *Staphylococcus aureus* is an opportunistic pathogen, most likely to grow on foods with reduced water activity and increased percentage of water-phase salt, like the ingredients of sandwiches, i.e. cooked meat products, dry-fermented sausage and cheese. Presence of *S. aureus* is associated to contamination of raw material or cross-contamination occurring as a consequence of mishandling during processing and storage (Pérez-Rodríguez et al., 2007). *Bacillus cereus* is a spore-forming pathogenic bacterium widespread in nature which may be hazardous in oven baked pastries. Due to its resistant endospores may survive in heat-treated, starchy products, where growth of the organism may occur as a consequence of improper cooling of the products after heat treatment (Rosenquist et al., 2005).

Listeria monocytogenes can cause serious illness in susceptible individuals (pregnant women, cancer and AIDS patients, organ transplant recipients as well as adults >65 years old) (Chan and Wiedmann, 2009). Storage at slight abuse temperatures (e.g. 7–10 °C) of refrigerated RTE foods that permit *L. monocytogenes* growth (i.e. products with pH > 4.4 or a_w > 0.92, products with pH > 5.0 and a_w > 0.94 and products with a shelf life of more than five days according to European Commission Regulation, 2073/2005 & 1441/2007) greatly increases the risk that

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L. monocytogenes, if present, will reach numbers that could cause human disease (Chan and Wiedmann, 2009). In the European Union for RTE foods in which *L. monocytogenes* growth will not occur under conditions of storage of the product, the microbiological limit for acceptable lots is 100 CFU/g. Instead, for RTE foods in which growth of *L. monocytogenes* can occur, the microbiological limit is "absence in 25 g". In the United States a zero tolerance policy has been adopted with respect to *Listeria* in RTE foods. But, the U.S. Food and Drug Administration has published a proposal for *L. monocytogenes* in RTE foods not able to support growth of this pathogen, to be permitted up to the level of 100 CFU/g (FDA, 2012). In the European Union an acceptance of a tolerance up to 100 *L. monocytogenes* CFU/g is widely permitted in RTE products served from catering establishments, as most products have a shelf life of under 5 days. According to the EFSA BIOHAZ Panel, listeriosis (predominantly foodborne and sporadic) has increased between 2000 and 2007 in Europe mostly concerning persons aged above 60 years (EFSA, 2007).

The incidence of *Salmonella* infections in humans constitutes the most frequently recorded cause of diarrheal illnesses in almost all industrialized countries. A majority of cases of human salmonellosis are due to the consumption of contaminated poultry, pork, beef, dairy products, eggs and seafood (D'Aoust and Maurer, 2007). The infectious dose of nontyphoidal *Salmonella* spp. for humans is generally described as 10^2–10^3 organisms (Musher and Musher, 2004). However, doses of 10 organisms or fewer than 10 organisms have caused illness (Cruickshank and Humphrey, 1987).

Enterohemorrhagic *Escherichia coli* O157:H7 is an important pathogen that is predominantly associated with hemorrhagic colitis, hemolytic uremic syndrome, mild to bloody diarrhea and thrombotic thrombocytopenic purpura. An outbreak of *E. coli* O157 linked to cooked, sliced meats supplied to schools across Wales involved 157 cases. This was the rationale for carrying out a survey of the general microbiological quality of RTE food served in schools (Meldrum et al., 2009). As contamination of RTE foods by pathogenic bacteria can be a serious problem the application of stringent standards and specifications is required.

The provision of food to students is an area that has generated a significant amount of public interest in recent years. The aim of this survey is to identify foods that might be associated with foodborne disease and generate data that can be used in order to develop and implement control measures. The Aristotle University of Thessaloniki is the largest university in Greece with approximately 99,000 undergraduates and postgraduates students, and staff members. During a 10-year inspection program (2001–2010) 15 canteens of the university campus were surveyed to determine the microbiological load of RTE foods and ready-to-bake frozen pastries which were intended to be baked at the point of sale. The aim of this study was to obtain the bacteriological profile of RTE foods sold at the canteens and of the ready-to-bake frozen pastries in order to study the microbiological quality of the purchased products for on-the-go consumption by the premises of the university campus. Aerobic colony counts were determined and Enterobacteriaceae, enterococci, *Staphylococcus* spp. were enumerated to assess the levels of hygiene and contamination. The incidence of the foodborne pathogens *Salmonella* spp., *L. monocytogenes, E. coli* O157, *S. aureus,* and *B. cereus* was also examined.

2. Materials and methods

2.1. Sample collection

A total of 135 sandwiches, 140 oven baked bakery products, 52 desserts oven baked, 30 desserts with dairy cream, 122 ready-to-bake frozen pastries were collected from the 15 canteens of the university campus and examined in our laboratory in a 10-year period (2001–2010). All food samples were purchased and transported to the laboratory in sterile plastic pouches. The core temperature over all food samples were recorded at the point of sale. All samples were stored at 10 °C in a portable cooling container during transportation and microbiological analysis was carried out within 4 h of purchase. The plastic pouches containing the frozen bakery products were sealed and placed in a booth with a constant temperature of 25 °C for 3 h in order to thaw before sampling.

2.2. Sample processing and analysis

For RTE foods and frozen pastries (after thawing), 20 g samples including all constituents were taken aseptically, transferred to sterile plastic pouches and homogenized for 60 s with 180 ml of sterile diluent containing peptone (1 g/l), NaCl (0.85 g/l) and Tween 80 (1 ml/l) using a Stomacher Lab-Blender 400 (Seward Medical, London, UK). Appropriate dilutions of the sample homogenates were prepared in sterile peptone water (0.1%) and inoculated in duplicate in growth media using standard pour- and spread-plate techniques to estimate microbial counts, i.e. aerobic colony count, Enterobacteriaceae, enterococci, *Staphylococcus* spp., *S. aureus,* and *B. cereus* as described in Table 1. *L. monocytogenes*, *Salmonella* spp. and presumptive *E. coli* O157 were detected as described in Table 1 after homogenizing 25 g of sample in 225 ml appropriate enrichment broths.

2.3. Statistical analysis

All analysis were performed in triplicate. Data were analyzed using Microsoft Excel and the SPSS 17.0 (SPSS Inc., Chicago, IL, USA) program for Windows. Box-plots were constructed in SPSS graphing software. The boxes represent the 25th percentile (lower quartile, lower margin of box), median (solid center line), and 75th percentile (upper quartile, upper margin of box). The whiskers may extend up to 1.5 interquartile range of the lower and the upper quartile and points outside of that (outliers) are represented by white circles followed by the number of the sample analyzed. The width of the box indicates the degree of variability in the data. If the median does not appear near the center of the box, it indicates that the data are not normally distributed.

3. Results

3.1. Bacterial counts in RTE foods

The ingredients included in the sandwiches analyzed (135 samples) are presented in Table 2. Sandwiches examined were displayed at temperatures ranging from 4 to 65 °C (data not shown). A percentage of 29.6% were stored between 4 and 8 °C, 60% were stored at >8 °C and below 17 °C, whereas 10.4% were displayed at temperatures between 58 and 65 °C (either hot-held sandwiches or major ingredient of sandwiches). The results of the microbiological analysis of the sandwiches are presented in box-plots, because the distribution and median can be displayed by this graph type. As indicated in Fig. 1 the aerobic colony count in the middle half of the samples range from 10^6 to 10^8 CFU/g, whereas Enterobacteriaceae range from 3×10^3–10^5 CFU/g. In the samples analyzed, the counts of both bacterial groups (i.e. aerobic colony count and Enterobacteriaceae) are fairly symmetrically distributed, as the median is located in the middle of the central box. In a percentage of 25% of the sandwiches the aerobic colony count was between 10^8 and 10^{10} CFU/g. On the other hand, there are extreme low values for aerobic colony counts for two samples containing