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Incidence and Growth of *Bacillus cereus* in Retail Pumpkin Pies[1]

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ABSTRACT

Pumpkin pies were sampled and screened for presence of *Bacillus cereus*. Pies were obtained from a cross-section of distribution outlets including: two major chain supermarkets, an independently owned supermarket with in-store bakery and a major chain supermarket that distributes products baked at a central distribution center. Water activity (a_w) and pH were determined on each sample. *B. cereus* was isolated on KG agar incubated at 30 C for 24 h. Intraperitoneal injections in mice were used to determine pathogenicity of the isolates. Pumpkin pies inoculated with *B. cereus* were incubated at 4, 25 or 35 C. *B. cereus* grew well at 25 C. D-values in minutes for *B. cereus* in pumpkin pie were 40 at 100 C, 10.5 at 108 C, and 7 at 124 C. Under normal baking conditions, the internal temperature of the pie reaches 108 C for approximately 1 min. Therefore it appears that the spores would survive baking. Potassium sorbate (0.25%) or refrigeration temperature inhibited growth of *B. cereus*.

Bacillus cereus is a well-known agent for bacterial foodborne disease (*10*). Two different types of illnesses have been attributed to consumption of foods containing large numbers of this organism. The first and best known type is characterized by abdominal pain and diarrhea which occurs within 8-16 h after ingestion of the contaminated food. A variety of foods have been incriminated, including cooked meats, poultry, meat, vegetable soups, raw and cooked vegetables and vanilla sauces. The second type of illness is characterized by nausea and vomiting which occurs within 30 min to 5 h after consumption of the contaminated food. Boiled and fried rice have been the most frequently implicated foods (*3*). The incubation period and symptoms of the vomiting type of outbreak closely resemble those for staphylococcal food poisoning. In contrast, the diarrheal type of outbreaks of *B. cereus* food poisoning resembles that of *Clostridium perfringens* food poisoning with respect to incubation period and symptoms. One can only speculate how many outbreaks of food poisoning caused by *B. cereus* have been misclassified because of this similarity and the fact that most foods are routinely examined only for the presence of salmonellae, staphylococci, clostridia and more recently vibrios.

An investigation of the incidence and growth of *B. cereus* in retail pumpkin pies was conducted to determine the possible hazard associated with the present food handling and distribution practices in the retail bakery industry.

MATERIALS AND METHODS

Four retail outlets in Corvallis, OR, representing a cross section of food distribution practices, were selected for sampling bakery products. The retail outlets included two major supermarket chains with in-store bakeries, an independently owned supermarket with an in-store bakery and a major supermarket that distributes bakery products centrally baked. The products were purchased directly from the retail outlets. The a_w and pH were measured on all samples, using a Beckman model HMP-1 Laboratory Moisture Measuring System and a Perkin-Elmer Metrion IV pH meter, respectively.

The microbial quality of the products was determined by enumerating for aerobic plate count (APC), coliforms, *B. cereus* and other pathogens (*18*). The products were stored at 25 C, simulating conditions of the food distribution system. Portions were taken at 24-h intervals for 72 h. A 50-g sample was blended in a Waring Blender for 1 min with 450 ml of 0.1% sterile peptone water. A duplicate 50-g sample was blended with 450 ml of lactose broth. Subsequent 10-fold dilutions were made as necessary in 90-ml sterile dilution blanks containing 0.1% peptone. One ml of each dilution was poured onto Plate Count Agar (PCA) incubated at 35 C for 48 h (*11*). Coliforms were determined by inoculating Lauryl Tryptose Broth with 1 ml of the first three dilutions (1:10, 1:100, 1:1000), incubated 35 C for 24 to 48 h and examined for gas production. A loopful from each gassing tube was transferred to Brilliant Green Bile Broth, incubated at 35 C for 24-48 h and examined for gas production (*18*).

B. cereus was isolated from the 1:10 dilution. A 0.1-ml portion was spread on plates of KG Agar and incubated at 30 C for 24 h (*13*). Following incubation, the plates were examined for typical colonies which are surrounded by a zone of turbidity. Presumptive *B. cereus* colonies were transferred to Nutrient Agar (BBL) slants and incubated at 30 C for 24 h. The gram and spore stains were done on each isolate. Confirmation was based upon carbohydrate utilization pattern, reduction of nitrate, acetyl methylcarbinol production and gelatin liquefaction (*5,8*).

The mouse lethality test was conducted to determine the pathogenicity of the *B. cereus* isolates. Intraperitoneal injection of 0.75 ml of either a broth or filtrate was used. Four white mice (18-22 g) were used for each treatment. Mice were observed for 30 h to determine

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if death occurred. Broth was prepared by inoculating tubes of brain heart infusion (BHI) broth from the Nutrient Agar slants and incubating overnight at 30 C. A filtrate was prepared by taking 0.5 ml of the broth, adding it to 50 ml of fresh beef infusion broth (*15*) and incubating it on a rotating shaker at 37 C, 90 rpm, for 12 h. The broth was centrifuged at 2,300 rpm for 10 min and the supernatant fluid was filtered through a glass filter. Positive and negative controls were prepared in the same manner. The number of organisms in the broth was determined by plating 0.1 ml of the appropriate dilution onto BHI agar and incubating for 24 h at 30 C.

The ability of pumpkin pies to support growth of the pathogen was determined by inoculating the cooked filling with a pure culture of *B. cereus*. The inoculum was prepared by transferring the organism in BHI broth and incubating it overnight at 35 C. The broth was then centrifuged for 10 min at 2,300 rpm. The cells were resuspended in sterile phosphate buffer. Cells were washed repeatedly by centrifugation to remove any contaminating medium. The cell density was adjusted by optical density at 500 nm with a Spectronic 20 spectrophotometer. The exact number of microorganisms inoculated was further determined by incubating the suspension on BHI agar at 35 C for 24 h (*14*). After complete dispersion of the inoculum, the product was stored at 4, 25 and 35 C. Growth was measured from the portions taken at intervals over a 10-day period and tested with BHI agar.

The effectiveness of potassium sorbate to inhibit growth of *B. cereus* was examined by adding 0.25% potassium sorbate to the product mix, baking and storing it at 25 C. Portions taken at regular intervals for up to 3 days were tested for microbial growth, using KG agar.

D-values for the *B. cereus* isolates were determined by the thermal death time (TDT) tube method (*9*). Five ml of the uncooked filling, inoculated with *B. cereus*, were heated in a mineral oil bath at 100 C for 5, 10, 30 and 60 min; 108 C, for 1, 5, 15 and 25 min and at 124 C for 1, 5, 10, and 15 min. After heat treatment, the tubes were immediately placed in an ice-bath. After cooling, the organisms were enumerated by plating appropriate dilutions on KG agar, incubating for 24 to 48 h at 30 C. Biochemical confirmation was made in addition to the spore stain. One tube from each treatment was stored at 30 C for 48 h to determine if recovery from the thermal injury had occurred. The D-values were calculated from the graph.

RESULTS AND DISCUSSION

The pH of the pumpkin pies from different retail outlets ranged from 5.6 to 6.6 and the a_w was 0.97. The proposed FDA model retail food store sanitation ordinance (*4*) states that products having a_w above 0.85 and a pH of 4.5 or above shall be required to be refrigerated. The current distribution practice of the stores sampled showed only two of the five outlets displayed their products in refrigerated cases. In the retail outlet where the pies were baked in a central bakery, the products were transported in a refrigerated truck, but they were displayed at room temperature on the bakery shelf.

There was some variation among the different outlets as to the kind of ingredients used, but basically, all products were prepared with canned pumpkin, milk (whole, powder or condensed), frozen whole eggs or egg-o-mix, sugar, brown sugar or honey, fat, salt and spices.

The microbial quality of the product sampled from four different retail outlets stored at 25 C for 72 h is shown in Table 1. The pull date practices of the stores were as follows: Store A, decreased price after 2 days for one additional day and then removed and destroyed; Store B, removed after 2 days; Store C, removed after 3 days; Store D, removed after 5 days. When stored at 25 C, all pies showed a significant increase in APC. After 72 h, a significant change in odor and occasionally mold growth was noticed. All products showed the same rate of APC increase; however, the initial count for product D was lower and consequently did not reach the APC level after 72 h shown by other products. The store D products was made with the condensed milk rather than whole milk and egg-o-mix instead of whole frozen eggs. Perhaps this was the reason for the lower count. After storage for 72 h at 25 C, the pH decreased in all samples (Table 1). Milk and eggs could act as buffers to counter the acid produced by the bacteria; however, as the microbial count reached the higher levels, the pH would eventually decrease. Sample D did not show the same magnitude of pH change due to the lower initial number. The coliform growth was insignificant with the exception of sample C which showed a coliform count greater than 2400 after 48 h. It is unlikely that the coliforms could have survived the cooking temperature of 108 C, therefore the high coliform level was due to multiplication of the bacteria after post-processing contamination. Jay (*12*) reported that coliforms could grow at temperatures as high as 50 C.

TABLE 1. *Microbial quality[a] of retail pumpkin pie stored at 25 C.*

Store	Time (h)	APC/g	Coliforms (MPN/g)	*B. cereus*	pH
A	0	2.5×10^2	< 3	N.D.[b]	6.6
	24	9.0×10^7	< 3	+	-
	48	3.0×10^9	< 3	N.D.	-
	72	4.2×10^9	< 3	N.D.	4.2
B	0	7.5×10^1	< 3	N.D.	5.6
	24	1.3×10^5	< 3	N.D.	5.6
	48	9.9×10^6	< 3	N.D.	5.5
	72	7.0×10^7	< 3	N.D.	4.3
C	0	7.5×10^1	< 3	N.D.	6.1
	24	3.4×10^5	6.7	N.D.	6.1
	48	6.2×10^7	> 2400	N.D.	6.0
	72	1.9×10^8	> 2400	N.D.	4.8
D	0	< 10	< 3	N.D.	6.1
	24	< 10	< 3	N.D.	6.0
	48	6.6×10^3	< 3	N.D.	5.9
	72	4.5×10^5	< 3	+	5.9

[a]Average of duplicate samples.
[b]N.D. = none detected.

B. cereus was isolated from two of the four samples. *B. cereus* is a large gram-positive rod-shaped aerobic sporeforming organism capable of growing under anaerobic conditions. The organism could grow in the temperature range of 10 to 48 C, with optimum growth occurring between 28 and 35 C. Vas and Proszt (*21*) reported destruction of *B. cereus* spores was not logarithmic. Approximately 1 spore in 10^8 possessed an

unusually high resistance to heat. Similar results were obtained by Franklin (*6*) who demonstrated that 1 spore in 10^5, 10^6 survived at 135 C for 4 h. Bradshaw et al. (*3*) isolated *B. cereus* from commercially canned foods. It is likely that the *B. cereus* isolated in this study survived the cooking process. *B. cereus* has been found in milk (*17*). Nygren (*16*) reported a high incidence of *B. cereus* in dried egg yolks and vanilla.

The D-values of *B. cereus* isolated from pumpkin pie were 40 min at 100, 10.5 min at 108 and 7 min at 124 C. The temperature of 108 C approximates the internal temperature reached during a normal baking process. Under laboratory conditions the product reached the maximum temperature in 39 min at 450 F or in 49 min at 325 F, was held at that temperature for approximately 1 min and then the pies were removed from the oven to cool. It took approximately 120 min for pies to cool to room temperature. There apparently was no appreciable thermal injury to *B. cereus*, as the growth of *B. cereus* always took place during subsequent incubation.

The *B. cereus* isolated from pies from two retail outlets was examined by the mouse lethality test. Results of this test are shown in Table 2. With the broth culture, death occurred within 2 to 3 h, and the symptoms were typical as described by Bonventre and Eckert (*1*). The filtrate also caused typical death symptoms within 3 to 11 h. The reason for failure of the positive control to produce death is not known. Repeated effort could not induce death with the filtrate with the reference culture. This culture had been maintained as a reference culture for years at the Microbiology Department, Oregon State University and its pathogenicity had not been previously tested.

TABLE 2. *Toxicity of* B. cereus *isolate from pumpkin pie.*

Store	Treatment	Number of deaths
A	Broth	4/4
D	Broth	4/4
Positive control	Broth	4/4
Negative control	Broth	0/4
A	Filtrate	3/4
D	Filtrate	4/4
Positive control	Filtrate	0/4
Negative control	Filtrate	0/4

Feeding tests with Rhesus monkeys have shown that the cultural conditions to obtain reproducible responses in animals were difficult to establish. Goepfert (*9*) reported whole-cell cultures of *B. cereus* strain B-4 ac, isolated from a diarrheal type outbreak, elicited a diarrheal response in Rhesus monkeys. Similar tests, using a broth culture from vomiting type outbreaks, did not induce vomiting. The response was either diarrhea or no effect. *B. cereus* produces a wide range of extracellular metabolites, largely during the exponential growth phase. These products and a toxin lethal to mice were reviewed in detail by Bonventre and Johnson (*2*). No attempt was made to determine the type of toxin produced from our isolates.

The growth of the *B. cereus* inoculated in pumpkin pie cooked filling is shown in Fig. 1. At 25 C, *B. cereus* grew well and a high level of the organism was reached within 24 h. No growth was observed at 4 or 35 C. Also 0.25% potassium sorbate was effective in inhibiting growth of *B. cereus*. Most methods recommend an incubation temperature of 30 C, thus we expected growth at 35 C, as the literature had indicated (*7*). Possibly our isolate had the lower maximum growth temperature.

Based upon the results of this study, use of potassium sorbate or refrigeration would be the effective means to prevent growth of *B. cereus*. *Bacillus*, being a heat resistant, spore former would survive the normal baking temperatures of pumpkin pie. It is not practical to recommend the complete heat inactivation of this organism.

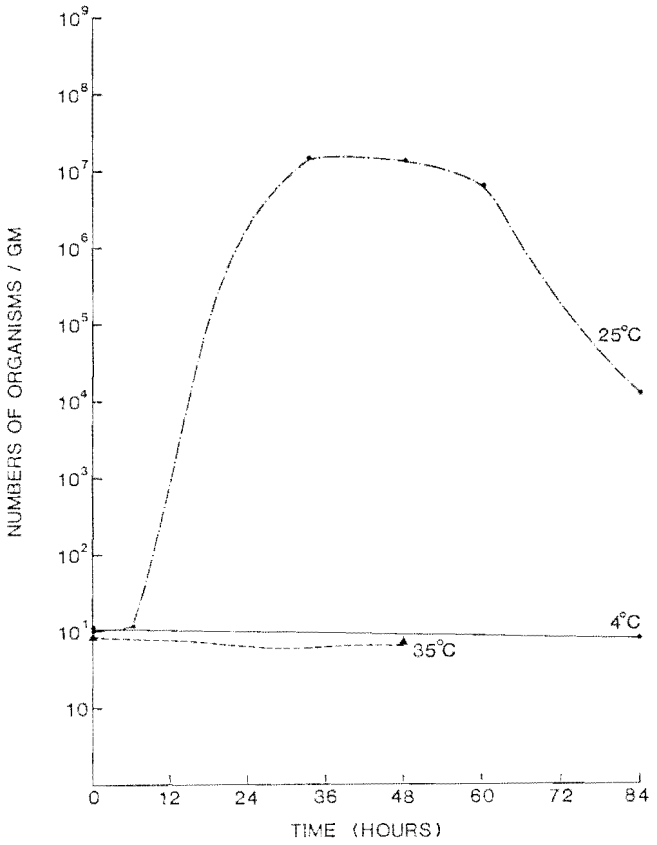

Figure 1. *Growth of* B. cereus *in pumpkin pie.*

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TABLE 6. *Sensory attributes of restructured steaks as affected by amount of pressure.*

Amount of pressure (psi)	Sensory properties[a]					
	Juiciness[b]	Flavor desirability[c]	Tenderness[d]	Texture desirability[c]	Texture description[e]	Overall palatability[c]
200	4.4f	5.5f	5.1f	4.9f	3.7f	5.0f
600	4.8f	5.6f	5.6f	5.1f	3.7f	5.2f
1000	4.6f	5.3f	5.4f	5.0f	3.6f	5.1f

[a]Means in the same column followed by a common letter are not different (P>0.05).
[b]Means based on an 8-point scale (8=extremely juicy; 1=extremely dry).
[c]Means based on an 8-point hedonic scale (8=like extremely; 1=dislike extremely).
[d]Means based on an 8-point scale (8=extremely tender; 1=extremely tough).
[e]Means based on a 7-point scale (7=extremely tough or rubbery; 4=excellent; 1=extremely mushy or crumbly).

pressure used to form restructured steaks had no significant effect on cooking characteristics or sensory attributes when comparing 200, 600 and 1,000 p.s.i., and that (*b*) flaking meat produced a more tender restructured steak as compared to steaks made from meat that was sliced as a particle production method. Further research is needed to identify the ideal particle size, and the optimum mixing time for the manufacture of restructured steaks.

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