An investigation of the bacteriological quality of retail vanilla slices

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SUMMARY

One hundred and thirty-three vanilla slices, purchased from shops in the West Yorkshire Metropolitan County, were examined to determine the numbers and types of bacteria present at the time of purchase. The surface colony count at 37 °C was $> 10^3$/g in 67/133 (50 %) of the samples examined, *Bacillus cereus* being found at that concentration in 21·8 %, coliform bacilli including *E. coli* in 5·3 %, *Staphylococcus aureus* in 3·0 % and *Streptococcus faecalis* in 0·8 %. Thirty-four strains of *B. cereus* were serotyped and 11 (32 %) of these were typable with the sera available.

Preparation of custard mixes in the laboratory suggests that the milk or milk powder used in the mix may be the major source of *B. cereus* in the final product. Many of the present methods of manufacture, distribution and storage allow organisms present in the custard at manufacture the opportunity to multiply and possibly reach numbers which present a risk of food poisoning.

INTRODUCTION

The first well documented cases of food poisoning due to *Bacillus cereus* were described by Hauge (1950). After this, reports from a number of countries appeared in the literature confirming the role of *B. cereus* in food poisoning outbreaks (Hauge, 1955; Mossel, Koopman & Jongerius, 1967; Nikodemusz, 1958; Midura, Gerber, Wood & Leonard, 1970). Between 1960 and 1968, *B. cereus* was quoted as the third most common organism in food poisoning in Hungary (Ormay & Novotny, 1969). The subject of *B. cereus* food poisoning has been recently reviewed by Goepfert, Spira & Kim (1972). Many different foods were implicated as vehicles, including mashed potatoes, minced meat, liver sausage, rice dishes, puddings and soups (Mossel *et al.* 1967), meat dishes, vegetables, milk and cocoa (Ormay & Novotny, 1969). Recent outbreaks in the U.K. have followed the consumption of cooked rice (P.H.L.S., 1972, 1973, 1976; Mortimer & McCann, 1974; Gilbert & Taylor, 1975, 1976) and the organism in this country is frequently found in milk, cereals and similar products.

In the Leeds area in August 1975 a food poisoning incident was reported to the

local authority in which a vanilla slice was suspected by the patient as the vehicle of infection. The incubation period of 4 h was followed by nausea and, after a further 4 h, violent stomach pains and vomiting. There was some subsequent looseness of stools but no actual diarrhoea. None of the suspected food was available but a vanilla slice, purchased from the same shop the following day, was submitted to this laboratory for bacteriological examination. It was found to have an aerobic colony count of 3.5×10^6/g and a *B. cereus* count of 1.0×10^6/g. Two months later a second food complaint was received by the same local authority in which a vanilla slice, produced by the same bakery, was the suspected vehicle of infection. On this occasion the vanilla slice was not completely consumed because the complainant thought it tasted 'off' and examination of the remains showed an aerobic colony count of 7.5×10^8/g, the predominant organism being *Streptococcus faecalis*. As a result of the first complaint, the authority in which the bakery was situated had commenced taking routine samples from the bakery and the sample submitted from the batch implicated in the second complaint was found to have an aerobic plate count of 2.7×10^8/g and a *B. cereus* count of 1.3×10^7/g. This culture was found to be serotype 1.

It was therefore decided, with the co-operation of several local manufacturers, to assess the degree of bacterial contamination, particularly with *B. cereus*, in vanilla slices as purchased in shops and to study the methods and conditions of manufacture and possible sources of contamination with *B. cereus*.

Vanilla slices consist of puff pastry, usually filled with a vanilla-flavoured custard mix or sometimes with cream, and topped with fondant. Methods of manufacture differ considerably. The traditional method is to add boiling milk to a mixture of cold milk and custard powder. The milk used may be fresh or may be reconstituted dried milk. The largest bakeries make their own custard powder from cornflour, vanilla flavouring, colouring matter and sugar, although the majority of bakeries buy a ready-made product.

Another quicker method, increasingly used by the smaller bakeries, is the 'cold mix'. Cold water is added to the powder and the custard is ready for use within 10 min. Some bakeries add artificial cream to the custard, particularly to the cold mix preparation, to give it more flavour.

Obviously each method of manufacture exposes the ingredients to a different degree of heat for a different period. As will be seen later this can have a considerable bearing on the bacteriological condition of the finished product.

MATERIALS AND METHODS

Examination of vanilla slices purchased from retailers

Vanilla slices purchased by the local environmental health authorities from retail outlets in the West Yorkshire Metropolitan County were examined for the degree of bacterial contamination and particularly for the incidence of *Escherichia coli, Streptococcus faecalis, Staphylococcus aureus* and *Bacillus cereus*.

The samples were kept at ambient temperature but were transported at once to this laboratory and immediately examined. Ten gram portions were removed

aseptically and placed in sterile stomacher bags. Ninety millilitres of sterile $\frac{1}{4}$ strength Ringer's solution was added and the suspension homogenized for 30 s using a Colworth Stomacher. Further decimal dilutions were made in $\frac{1}{4}$ strength Ringer's solution. Counts were carried out using a modified Miles and Misra surface drop technique on blood agar plates which were incubated aerobically at 37 °C for 18–24 h and the surface colony count recorded. Colonies showing the appearance of *B. cereus* on blood agar were further subcultured on Nagler plates for evidence of an egg yolk reaction after incubation at 37 °C for 24 h and identity was confirmed by biochemical tests.

Laboratory preparation and examination of custard mixes

To determine the time required for high bacterial counts to develop in the custard of vanilla slices, a series of small batches of cold and hot mix custard were made in the laboratory using ingredients and recipes provided by local bakers. Cold mix custard was prepared by adding 90 g of cold water to 30 g of commercial cold mix powder. Hot milk custard was made by adding 135 ml of boiling, fresh milk to 15 g of commercial custard powder which had been mixed to a smooth paste with a small volume of milk taken from the 135 ml before boiling. A hot water mix was also prepared using the same commercial custard powder but substituting 135 ml of boiled tap water for the boiled, fresh milk. All the mixes were stored at ambient room temperature (21–23 °C) and sampled after 0, 3, 6, 9, 12, 18, 21 and 24 h. At each sampling 10 g of mix was removed aseptically and the surface colony count and *B. cereus* count determined as described above.

Serotyping of B. cereus strains

This was carried out using antisera supplied by the P.H.L.S. Food Hygiene Laboratory (Taylor & Gilbert, 1975). Four antisera pools and 18 individual antisera were used. H antigen suspensions were prepared by inoculating *B. cereus* cultures into Craigie tubes containing semi-solid nutrient agar and incubating at 37 °C for 18 h. Two successive subcultures were made to ensure active motility. The surface growth from the final Craigie tube was inoculated into 100 ml of nutrient broth in a 250 ml conical flask and incubated at 37 °C on a rotary shaker (100 rev./min). After 4–5 h at 37 °C, the opacity of the suspensions was checked and the cells were examined in a hanging drop preparation for active motility. Suitable suspensions were formalized by the addition of 1 ml of formalin and then stored at 4° C.

Agglutination tests were carried out by making doubling dilutions of 0·25 ml volumes of antisera in 0·85 % sterile saline in Dreyer tubes and adding equal volumes of antigen suspension to each tube. After incubation at 50 °C for 2 h, positive reactions were shown by the formation of a floccular precipitate in the tube.

Table 1. *Surface colony counts of aerobic bacteria in vanilla slices*

Surface colony count/g at 37 °C	No. of samples	% of total samples
$< 10^3$	66	50
$10^3 < 10^6$	33	25
$10^6 - 10^8$	31	23
$> 10^8$	3	2

Table 2. *Occurrence of specific organisms in vanilla slices at counts of $> 10^3$/g*

Organism	Proportion of samples with counts of $> 10^3$/g	Incidence (%)
B. cereus	29/133	21.8
Coliforms/*E. coli*	7/133	5.3
Staph. aureus	4/133	3·0
Strept. faecalis	1/133	0·75

RESULTS

Examination of retail vanilla slices

One hundred and thirty-three vanilla slices were examined and the surface colony counts obtained can be seen in Table 1.

In two of the samples with colony counts of $> 10^8$/g, coliform bacilli were present in numbers of $> 10^7$/g and in one of those *E. coli* was present at a concentration of $4·5 \times 10^7$/g. In the third sample which was the vanilla slice implicated in the food complaint the predominant organism was *Streptococcus faecalis*. These findings would appear to indicate a simple lack of hygiene in preparation or handling.

Table 2 shows that, while other organisms did occur, *B. cereus* was the organism most frequently found in significant numbers. In addition, *B. cereus* was present in smaller numbers in a further 26 samples representing an overall incidence of 55/133 (41·4 %).

Serotyping of B. cereus *strains*

The results of typing 34 strains obtained from 34 separate vanilla slices can be seen in Table 3. While the majority of strains were untypable, 32 % were typable with the sera available.

Examination of laboratory prepared custard

Two batches of hot milk custard and two batches of hot water custard were prepared in the laboratory and stored at ambient room temperature (20–23 °C) for 24 h. In Table 4 the bacterial counts obtained on sampling after the indicated storage periods are compared. Immediately after preparation, bacterial counts were found to be extremely low in both types of custard. After 9 h the bacterial population of the hot milk custard was found to be increasing, and at 24 h was

Table 3. *Serotypes found among* 34 *strains of* B. cereus
from 34 *samples of vanilla slices*

Serotype	Number of strains	%
Non-typable	23	68
1	5	15
12	3	9
4	1	3
8	1	3
11	1	3
Total	34	

Table 4. *Effect of storage on the bacterial population of laboratory
prepared hot milk and water custard mixes*

Time after preparation (h)	Hot milk custard mix (surface colony count/g at 37 °C)		Hot water custard mix (surface colony count/g at 37 °C)	
	Batch 1	Batch 2	Batch 1	Batch 2
0	$< 10^2$	$2 \cdot 0 \times 10^2$	$1 \cdot 0 \times 10^2$	$5 \cdot 0 \times 10^2$
3	$< 10^2$	$1 \cdot 0 \times 10^2$	$< 10^2$	$3 \cdot 0 \times 10^2$
6	$< 10^2$	$< 10^2$	$< 10^2$	$2 \cdot 0 \times 10^2$
9	—	$4 \cdot 0 \times 10^2$	—	$< 10^2$
12	—	$5 \cdot 0 \times 10^3$	—	$< 10^2$
18	$7 \cdot 5 \times 10^3$	$2 \cdot 0 \times 10^5$	$< 10^2$	$1 \cdot 0 \times 10^2$
21	$1 \cdot 5 \times 10^5$	$3 \cdot 75 \times 10^5$	$< 10^2$	$< 10^2$
24	$2 \cdot 25 \times 10^5$	$> 5 \cdot 0 \times 10^5$	$< 10^2$	$1 \cdot 6 \times 10^2$

—, Not determined.

considerable. In contrast, the hot water custard was found to have a low bacterial count throughout the 24 h storage period.

DISCUSSION

In the survey of bacterial contamination found in retailed vanilla slices, a variety of organisms was found. *Staphylococcus aureus*, coliform bacilli including *E. coli*, and *Streptococcus faecalis* were demonstrated in a small proportion of the samples and may be taken to be a reflexion of mishandling and poor storage during the manufacturing process. *B. cereus*, however, was found in a considerable proportion of the samples (41.4 %) and was present in significant numbers ($> 10^3$/g) in 21·8 %.

The frequency and degree of *B. cereus* contamination in food samples has been the subject of a number of surveys in recent years. Nygren (1962) carried out a comprehensive study of 3888 samples and found that 51·6 % of 1546 food ingredient samples, 43·8 % of 1911 cream and pudding samples and 52·2 % of 431 meat and vegetable products contained *B. cereus* although, in most instances, the count was less than 10^2/g. In 1971, Kim & Goepfert examined 170 samples of dried food products purchased in shops and isolated *B. cereus* from 25·3 % of the

products. They found that 37·5 % of milk powder samples and 16·7 % of flour and starch samples were contaminated. However, the degree of contamination in any positive sample did not exceed 4×10^3 B. cereus/g and, in most instances, was < 10^3/g. In a survey carried out in Russia, Akimova (1969) reported that 13·6 % of canned foods, 7·7 % of sausage products and, most important from the point of view of the present survey, 6·7 % of confectionery items contained B. cereus.

The high frequency and the high counts of B. cereus recorded in vanilla slices in this study must cause some concern, particularly as a number of food poisoning incidents have been reported implicating B. cereus in similar preparations as the causative organism. Hauge (1950, 1955) described four outbreaks where the contaminated food was a vanilla sauce prepared and stored at room temperature before being served. Samples of the sauce examined after the outbreak contained 25–110×10^6 B. cereus/ml. Christiansen, Koch & Madelung (1951) reported an outbreak in which abdominal pains and diarrhoea were produced in 15/18 adults and 106/180 children after consumption of a yellow pudding desert which, on examination, contained $1·3 \times 10^7$ B. cereus/g. Cream pastries have been implicated in outbreaks reported by Nikodemusz (1961) and Nikodemusz et al. (1962), the concentration of B. cereus ranging from $3·6 \times 10^4$ to $9·5 \times 10^8$/g.

The present survey was instigated as the result of a food poisoning incident in which a vanilla slice appeared to be the vehicle of infection. B. cereus colony counts in the subsequent samples taken ranged from 10^3 to $5·5 \times 10^7$/g and many samples contained B. cereus at concentrations which have been reported to have caused food poisoning in other incidents. Furthermore, when 34 B. cereus strains were serotyped (Table 3), 15 % were found to be type 1, which is the serotype most commonly found with the vomiting type of B. cereus food poisoning associated with the consumption of cooked rice, and a further 18 % were typable with the sera available.

The high bacterial counts found in vanilla slices sampled at retail outlets are a reflexion of the conditions of time and temperature to which the custard content of the product is subjected after manufacture. The rise in the bacterial population of laboratory prepared hot milk custard mixes illustrates this (Table 4). Bacterial contamination in the newly mixed ingredients was found to be low ($< 5 \times 10^2$/g). However, during subsequent cooling and storage, bacterial multiplication was rapid and counts of $> 10^5$/g were present after 24 h. In bakeries using hot mix custard, large batches of custard are cooled over long periods giving ideal conditions for the germination and rapid multiplication of bacteria in the mix. Several samples, taken from a bakery using hot mix manufacture immediately before delivery, were found to have counts of $> 10^6$/g.

A comparison of custards made from hot milk and hot water suggests that the milk or milk powder used in the manufacture of the custard may be the main source of B. cereus in the custard mix. Hot milk custard mix developed a very high B. cereus count in 24 h whereas custard made from the same ingredients, but with boiled water in place of boiled milk, had very low total and B. cereus counts after 24 h storage. Several reports in the literature support the suggestion that B. cereus originates from the milk, indeed its occurrence in milk has been reported

repeatedly since the early investigations by Lawrence & Ford (1916). Franklin (1967) stated that *B. cereus* could be expected to occur in the majority of farm milks and in all bulk milks, while Kim & Goepfert (1971) reported its frequent occurrence in dairy products including dried milk. Ionescu, Ienistea & Ionescu (1966) found *B. cereus* at counts ranging from 10^1 to 10^4/ml in 72·4 % of fresh raw milk, 86·7 % of bottled pasteurized milk and 100 % of milk samples taken directly from the pasteurizer.

It is not, of course, suggested that vanilla slices are a major factor in causing clinical illness or contribute much to the statistics of food poisoning. They are, however, representative of a wide variety of confectionery and there is certainly evidence that they can cause food poisoning incidents. Gilbert & Parry (1977) find the significance of *B. cereus* and of its specific serotypes in food difficult to assess. Low numbers, < 100/g, they consider are probably of little significance but with larger numbers, especially > 10^5/g, there is a definite risk that an outbreak of food poisoning may occur.

As it is not practicable to handle, transport and store vanilla slices in refrigerated conditions, it must be accepted that, if certain organisms are present in the custard, they will have the opportunity to multiply and possibly reach significant numbers.

It is obviously important therefore that manufacture should be carried out so as to minimize the initial bacterial contamination. This must take into account the number of organisms in the constituent substances as well as the general hygienic standard of the process. It is hoped that, with the continued co-operation of the manufacturing industry, progress may be made.

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