The effect of ice-cream-scoop water on the hygiene of ice cream

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SUMMARY

A survey of unopened ice cream, ice cream in use, and ice-cream-scoop water (n = 91) was conducted to determine the effect of scoop water hygiene on the microbiological quality of ice cream. An aerobic plate count around 10^6 c.f.u. ml⁻¹ was the modal value for scoop waters. Unopened ice creams generally had counts around 10^3 – 10^4 c.f.u. ml⁻¹ and this increased by one order of magnitude when in use. Many scoop waters had low coliform counts, but almost half contained > 100 c.f.u. ml⁻¹. E. coli was isolated in 18% of ice creams in use, and in 10% of unopened ice creams. S. aureus was not detected in any sample. Statistical analysis showed strong associations between indicator organisms and increased counts in ice cream in use. EC guidelines for indicator organisms in ice cream were exceeded by up to 56% of samples.

INTRODUCTION

Ice cream may be of dairy (milk fat, sugar, eggs and cornflour) or non-dairy (vegetable fat, separated milk powder, gelatin, emulsifiers and water) composition [1]. Historically, it has been responsible for a number of outbreaks of food-borne illness [2]. UK legislation enacted in 1947 has dealt effectively with these problems by requiring pasteurization or sterilization and rapid cooling of ice cream mixes [3]. This has also improved the quality of the product through reducing spoilage by souring and rancidity which occurred because of prolonged storage before freezing and the incorporation of rancid fat [1]. Nevertheless, Aerobic Plate Counts and coliform counts in excess of those given in the EC criteria for frozen milk-based products [4, 5] are often found during routine food examination (personal observation).

An increasing variety of ice cream flavours and products are available and manufacturers are attempting to extend its consumption beyond the normal summer season. This study was conducted only on ice cream served by scooping to determine the influence of scoop hygiene on the microbiological quality of the ice cream. Previously (EC Co-ordinated Food Control Programme, 1993) primary pathogens were seldom found in scooped ice cream but the general hygiene was not good, particularly in unbranded hard ice creams [2]. This is due to poor manufacturing hygiene, contamination during the protracted period of serving, and from the ice cream scoop. The work reported here was an extension of that conducted as part of a national study (LACOTS/PHLS Co-ordinated Food Liaison Group Sampling Programme) on the microbiological quality of ice-cream-scoop water which will be reported elsewhere.

MATERIALS AND METHODS

Sampling

Three samples were taken by Environmental Health Officers (EHOs) at each shop or restaurant serving ice

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cream via a scoop. At each visit, samples were taken aseptically from an unopened container of ice cream, a container of ice cream of the same batch which was in use and scoop water in which the ice cream scoop was held between servings. Ice creams were a mixture of dairy and non-dairy, but this designation failed to be recorded for all samples. Ninety-one sets of three samples were taken. One hundred sets of three samples had been planned, but all three samples (scoop water, unopened and opened ice cream) were sometimes not available at all premises visited. The ice cream manufacturer sometimes failed to be recorded, but samples came from a variety of large and small producers. Some premises were revisited during the survey. Clean scoops were sterilized with ethanol before sampling ice cream. Scoop waters were sampled into sterile plastic water sampling bottles (Sterilin) containing sodium thiosulphate for the neutralization of chlorine.

The temperature of scoop water failed to be recorded for all samples and was not included in the analysis. Samples were transported to the laboratory in cool boxes with ice packs. The temperature was measured on receipt and confirmed to be < 4 °C. Generally ice cream samples were still frozen when received. Examination took place within 2 h of receipt.

Aerobic plate counts

Aerobic plate counts (APC) were performed using a spiral plater. Plate Count Agar plates were inoculated with 50 μ l of 1:10 diluted ice cream. For ice-creamscoop water samples, undiluted, 10^{-2} and 10^{-3} dilutions prepared in Maximum Recovery Diluent (MRD) were used. The plates were incubated at 30 ± 1 °C for 48 ± 4 h. Colonies were counted using the IUL Countermat as described previously [6].

Coliform and E. coli counts

The coliform/E. coli method is that required by the EC Dairy Products Directive. The method has been adopted for all dairy products required to meet the Dairy Product (Hygiene) Regulations [7]. For coliform and E. coli counts, undiluted, 10^{-1} and 10^{-2} dilutions of scoop water and 10^{-1} , 10^{-2} and 10^{-3} dilutions of ice cream in MRD were used. Lauryl tryptose broth medium (LTMUG) containing 4-methylumbelliferyl- β -D glucuronide (MUG) allowed the combined detection of coliforms and presumptive E. coli. In-

cubation was performed at 30 ± 1 °C for 48 ± 4 h. Enumeration was by the most probable number method (MPN) and allowed detection at low levels (< 100 c.f.u. g⁻¹ or 10 ml⁻¹).

One ml of undiluted scoop water and 10^{-1} and 10^{-2} dilutions were prepared in MRD and pipetted into each of three tubes containing 10 ml LTMUG and Durham tubes. After the inoculum and medium were mixed carefully to avoid introduction of air into the Durham tubes, the racks of tubes were incubated at 30 ± 1 °C for 48 ± 2 h. A 1:10 suspension of ice cream and 10^{-2} and 10^{-3} dilutions were processed similarly.

Tubes were examined for gas production at 24 and 48 h. Those containing gas were presumptive coliforms. Each tube containing gas was subcultured upon detection to brilliant green bile broth (BGBB) and incubated at 30 °C for 24 ± 2 h. The presence of gas confirmed the identification of coliforms. The number of positive tubes was converted into a coliform count using MPN tables [8].

At 48 h, 0.5 ml of NaOH was added to each tube of LTMUG and the tubes examined under u.v. light for the presence of blue-white fluorescence. Then 0.5 ml of Kovac's indole reagent was added to each tube, mixed and examined after 1 min. The presence of indole was indicated by red colour in the alcoholic phase. Presumptive *E. coli* was indicated by the presence of both fluorescence and indole. Counts were calculated by using MPN tables as before. *E. coli* NCTC 10418 and *Klebsiella aerogenes* NCTC 9528 were used as positive and negative controls respectively.

Staphylococcus aureus counts

Tests for *Staphylococcus aureus* were carried out by spread plating from two decimal dilutions of samples in MRD using Baird Parker Agar incubated at 37 ± 1 °C for 48 ± 4 h. Neat and 10^{-1} dilutions of scoop water, and 10^{-1} and 10^{-2} dilutions of ice cream were used. For confirmation, five typical colonies were examined using tube coagulase (human plasma) and DNase agar tests.

Statistical methods

Results were analysed using Microft Specimen Control System and Microsoft Excel. Counts were analysed with Systat software. Indefinite values outside the ranges of the tests' sensitivities and

Table 1. Statistical groupings for APCs

	Group					
Sample	Low	Medium	High			
Ice-cream-scoop water (ICSW)	< 10 ³	$\geqslant 10^3 \text{ and } < 10^6$	≥ 10 ⁶			
Ice cream (open) (OPEN)	$< 10^4$	$\geqslant 10^4 \text{ and } < 10^5$	$\geqslant 10^5$			
Ice cream (unopened) (CLOSED)	$< 10^{3}$	$\geqslant 10^3 \text{ and } < 10^4$	$\geqslant 10^4$			
OPEN-CLOSED (DIFF)	$< 10^{0.25}$	$\geqslant 10^{0.25} \text{ and } < 10^1$	≥ 10¹			

Table 2. Aerobic plate counts for opened and unopened ice cream and ice-cream-scoop water

	Aerobic plate count range (c.f.u. ml ⁻¹)									
Sample	< 20	20-< 200	< 200	$200 - < 10^3$	$10^3 - < 10^4$	$10^4 - < 10^5$	$10^5 - < 10^6$	$10^6 - < 10^7$	> 10 ⁷	Total
Opened	NT*	NT	5	20	36	22	8	0	0	91
Unopened	NT	NT	14	39	29	9	0	0	0	91
ICSW†	16	1	NT	21	15	10	21	7	0	91

^{*} NT, not tested.

dilutions were dealt with by the following method. To meet the assumptions of the statistical analysis the 91 samples for each variable were divided into three groups of approximately equal numbers; low, medium and high. These are defined in Table 1 for APCs. Cross-classification tables (not shown) were examined for all variable pairs. Pearson's chi-square was calculated for each table. This treatment enabled values above the dilution limit and below the sensitivity levels of the tests to be accounted for without introducing major bias to the results.

For coliforms, scoop water results were divided into three groups, < 10, 10–100 and > 100. Unopened ice cream was divided into three groups, < 10, 10–110 and > 110 and opened ice cream into the three groups < 50, 50–1000 and > 1000. For *E. coli*, results were divided into two categories, detected and undetected, for opened and unopened ice cream and for scoop water. These intervals were based on the distribution of results and the formation of useful analytical groups rather than any health-based guidelines. Both coliforms and non-pathogenic *E. coli* are indicator organisms and have no direct relation to infection. Pearson χ^2 and p-values were calculated.

RESULTS

S.~aureus was not isolated from any of the samples. The modal order of magnitude for APCs was 10^6 c.f.u. ml $^{-1}$ for ice-cream-scoop water (Table 2). None was found

to exceed 3.7×10^7 c.f.u. ml⁻¹. For unopened ice creams most APCs were in the range 10^3 – 10^4 c.f.u. ml⁻¹. Ice creams which had been opened and were in use, exposed to contaminants from scoops and the environment showed that most APCs were in a range one order of magnitude higher than unopened ice creams, 10^4 – 10^5 c.f.u. ml⁻¹.

Many ice-cream-scoop waters contained low coliform counts, but almost half contained > 100 coliforms ml⁻¹ (Table 3). Ice cream which had been opened showed an increased number of higher coliform counts than unopened ice cream.

 $E.\ coli$ was present in only a small number of samples and the counts were generally low (Table 4). It was isolated more frequently from scoop water than from ice cream. Ice cream in use was more frequently contaminated with $E.\ coli$ than ice cream which was unopened. In no case did the $E.\ coli$ count exceed 500 c.f.u. ml⁻¹.

The APC data for ice-cream-scoop water (ICSW) showed a significant association with opened ice cream (open) (P < 0.01) and a closer association with the difference between the APCs of unopened and opened ice cream (diff) (P < 0.001) (Table 5). No significant association was found between the count of ICSW and unopened ice cream (closed) which was usually manufactured on different premises.

For both coliforms and *E. coli*, there is a significant relationship between ice cream scoop water and opened ice cream. The relationship between scoop

[†] ICSW, ice-cream-scoop water.

Table 3. Coliform counts for opened and unopened ice cream and icecream-scoop water

	Coliform counts (c.f.u. ml ⁻¹)								
Sample	ND*	< 10	< 50	< 100	≥ 110	< 500	> 500	Total	
Opened	8	0	25	7	NT†	19	32	91	
Unopened	26	8	28	6	NT	12	11	91	
ICSW‡	23	13	15	0	40	NT	NT	91	

^{*} ND, not detected.

Table 4. E. coli counts for opened and unopened ice cream and icecream-scoop water

Sample	E. coli counts (c.f.u. ml ⁻¹)								
	ND*	< 10	< 50	< 100	≥ 110	< 500	> 500	Total	
Opened	75	2	10	2	NT†	2	0	91	
Unopened	81	3	6	0	NT	1	0	91	
ICSW‡	66	17	1	0	7	NT	NT	91	

^{*} ND, not detected.

Table 5. *Chi-square and P-values for counts from samples*

	APC*†		Coliforn	ns†	E. coli‡		
Sample pairs	χ^2	P	χ^2	P	χ^2	P	
Open/ICSW§	18.506	< 0.01	23.754	< 0.001	22.008	< 0.001	
Closed/ICSW Diff/ICSW	7·342 25·172	0·119 < 0·001	7·498 —	0·112 —	2·861 —	0·091 —	

^{*} APC, aerobic plate counts.

water and unopened ice cream was not statistically significant.

DISCUSSION

In this study, we found statistically significant associations between APC, coliforms and *E. coli* in scoop water and ice cream. It is entirely reasonable to assume that the association is causal although other sources of contamination such as flying insects, skin and airborne micro-organisms may also contribute considerably to the raised counts of retail ice cream in use. Raised counts in scoop water are strongly

predictive of raised counts in ice cream in retail use compared with the same ice cream before use.

S. aureus was not found in any of the samples. This reflects the infrequent isolation of this organism from foods in the UK [9]. S. aureus is a much more important food pathogen in the USA than in the UK where it has caused less than 6% of reported foodborne disease outbreaks. In only one of these was ice cream the vehicle [9].

Coliforms were detected in almost three quarters of unopened ice creams. Around a quarter of scoop waters were free of coliforms but high coliform counts were found in many of the remaining samples. These

[†] NT, not tested.

[‡] ICSW, ice-cream-scoop water.

[†] NT, not tested.

[‡] ICSW, ice-cream-scoop water.

^{† 4} degrees of freedom.

^{‡ 1} degree of freedom.

[§] ICSW, ice-cream-scoop water.

were not enumerated above 110 c.f.u. ml⁻¹ and may have been considerably higher than this. Future studies should expand the range of dilutions for coliforms. Few ice creams in use remained free of coliforms and high counts were found in over half the samples. There was a significant association between high coliform counts in scoop water and opened ice cream, indicating the important role of scoop water in transferring these organisms to ice cream in service. Although the temperature of scoop water is higher than ice cream and may allow microbial multiplication, it is more likely that the bacteria in scoop water arise from contamination rather than growth. This is particularly true regarding the presence of coliforms and E. coli which should be absent from chlorinated water supplies. Bacterial numbers will depend on the ambient temperature and how often the water and disinfectant are changed. It is difficult to obtain reliable information on whether or not scoop water and disinfectant solution are changed at least once every hour as recommended by the Milk Marketing Board.

In an earlier EC survey E. coli was detected in only 3% of samples of hard and soft ice creams [2]. We detected E. coli in 18% of ice creams in use. Two ice cream samples had E. coli counts > 100 c.f.u. ml⁻¹. Considering the low infectious dose of verotoxigenic E. coli (VTEC) [10], this may be a cause for concern should these strains become more prevalent.

Dairy and non-dairy ice cream mixes [11] produced in the UK are required to be subjected to a heat treatment lethal to non-sporulating organisms. APC, coliform and E. coli counts were higher in a number of unopened samples than should be if such a treatment is performed properly. Routine examination of ice creams has shown that a considerable number exceed the EC criteria for APC and coliforms although these counts are generally not greatly in excess of the guideline numbers. These guidelines [4, 5] exclude non-dairy ice cream and are given in the form of a three-class plan [12] where n = 5, c = 2. For APC at 30 °C, $m = 1 \times 10^5$, $M = 5 \times 10^5$. For coliforms, m = 10, M = 100. (n, number of samples drawn; c, maximum permissible number of marginally acceptable samples; m, marginally acceptable count; M, unacceptable count for any number of samples.) Data for the dairy/non-dairy origin of samples in our survey were incomplete, but more recent guidelines do not distinguish between ice cream of dairy and nondairy origin [13]. Of the 91 sets of samples reported here, 9% of opened and 0% of unopened ice creams

exceeded *M* for APC. For coliforms, however, 56% of opened and 25% of unopened ice creams exceeded *M*, often by more than one order of magnitude. The microbiological quality of ice cream should not change significantly between manufacture and packaging and point of sale. The fact that more than a quarter of ice creams were unsatisfactory indicates that greater attention to hygiene is needed in areas under manufacturers' control. These results indicate inadequate pasteurization and/or recontamination before packaging as well as after opening. Greater attention to Hazard Analysis Critical Control Point (HACCP) and dairy authority guidelines [14, 15] would be advisable throughout the industry.

Although epidemiological evidence does not indicate ice cream as a significant vehicle of disease in the UK, the possibility of infection with enteropathogens from ice cream may arise occasionally, or be present continually at a very low level. This was illustrated by a massive outbreak of Salmonella enteritidis infection involving an estimated 224000 persons in the USA [16, 17]. Contamination had occurred from transport of pasteurized ice cream premix in an uncleaned tanker previously used to transport unpasteurized liquid egg and other products. S. enteritidis was isolated in low numbers (< 0.1c.f.u. g⁻¹) from 3% of ice cream samples examined and the attack rate among consumers of the brand was 6.6%. A low attack rate (150 cases reported over 2 months, in this case) could obscure the fact that an epidemic is in progress. The author suggested that small outbreaks are missed regularly when surveillance rates are low, and that immunocompromised sentinel populations may give a better indication of the number of foods contaminated with low doses of pathogens [17].

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