THE BACTERIAL CONTENT OF ICE-CREAM IN RELATION TO MANUFACTURE, STORAGE AND STANDARDS OF PURITY

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Earlier studies on the bacterial content of ice-cream

Ice-cream has occasionally been proved to be the carrier of disease, and therefore a study of the ingredients used, the processes of manufacture and the bacterial content of the final product is an important branch of modern hygiene.

Between the years 1875 and 1909 there were in Great Britain eight outbreaks of disease which could definitely be traced to ice-cream consumption. Typhoid fever infections were reported by Turner (1892), Munro (1894), Hope (1897) and Barras (1904); Gaertner infections by Robertson (1905) and Peacock (1909); diarrhoea by Henry (1900); ice-cream "poisoning" by Collingridge (1902); and scarlet fever by Buchanan (1875).

Investigations into the bacterial content of ice-cream were carried out by MacFadyen & Colwell (1895), Anderson (1896), Nield-Cook (1896), Wilkinson (1899), Klein (1902), Rickards (1906), Pennington & Walter (1907) and Buchan (1910). Buchan made an exhaustive enquiry into the conditions of ice-cream manufacture in the Birmingham district. He described a scientific method of preparation which reduced the opportunities for bacterial contamination to a minimum and suggested certain standards of purity by means of which the food could be judged. No sample of ice-cream should contain more than 1,000,000 organisms per c.c. on nutrient agar after 2 days' incubation at 37° C. or on nutrient gelatin after 3 days' incubation at 22° C. No sample should produce acid and gas in bile-salt broth with a less quantity than 0·1 c.c. or contain *Clostridium sporogenes* in less than 10 c.c. or streptococci in less than 0·001 c.c.

Anderson (1935) and Lampitt (1935) believed that consideration of standards should come after compulsory registration of manufacturing premises, pasteurization of ice-cream mix, a high standard of cleanliness in plant and operatives, and medical inspection of workers. They maintained that it is somewhat futile to discuss bacteriological standards for ice-cream while there are none for raw milk and cream which are consumed by many more people and which are the raw materials for the ice-cream industry. Anderson suggested, however, that if there must be a standard it would be advisable to use the one for pasteurized milk until raw milk and cream are similarly controlled.

https://doi.org/10.1017/S0022172400011396 Published online by Cambridge University Press

Benson (1933) also stated that ice-cream mix should be pasteurized and that the finished product should not contain more than 100,000 organisms per c.c. and no *Bact. coli* in 0·1 c.c. Thierens (1930) preferred the Grade A milk standard, which was then in force, for ice-cream in which the total count should not exceed 200,000 bacteria per c.c. and *Bact. coli* must be absent in 0·01 c.c.

Since the publication of Buchan's paper and the stricter supervision of factories which naturally followed the detection of ice-cream as a vehicle of infection there have been few outbreaks in this country which could definitely be traced to this food. Copeman (1910) reported 142 cases of enteric fever in the Eccles district in which 108 of the patients were known to have eaten ice-cream. This outbreak was traced to two itinerant Italian vendors who made ice-cream on the ground floor of a Manchester tenement house. A lesser outbreak occurred at the same time in the Ancoats and Harpurhey districts of Manchester where these men also had a round. More recently Soothill & Leggat (1927) reported 63 cases of paratyphoid B fever in Norwich in which 57 patients had had ice-cream from a stall or were immediate contacts with others who had done so.

In spite of the general improvement in conditions of ice-cream manufacture there are still many samples which fall below Buchan's standard, while some are grossly polluted and may well be the cause of summer diarrhoea and mild intestinal disturbances in children, although unfortunately in the majority of cases it is very difficult to get the necessary proof.

The present investigation is a study of the bacterial content of ice-cream in relation to ingredients used in manufacture, method of preparation, storage and standards of purity.

Manufacture of ice-cream

Ice-cream is prepared in different ways and does not always contain the same constituents. The ingredients other than milk are cornflour or similar thickening material and sugar, but sometimes eggs, gelatin or cream may be added. The use of gelatin, which has colloidal properties, has a smoothing effect on the ice-cream and it also acts as a stabilizer. Most manufacturers make use of one of the many proprietary ice-cream powders on the market. These usually consist of cornflour flavoured and coloured and sometimes even sweetened so that it is only necessary to add the milk. The manufacture of ice-cream may include boiling all the ingredients or boiling only the milk and sugar before freezing. It may also be produced entirely without heating.

Method I, in which all the ingredients are boiled

This is the usual method of manufacture in the Manchester district. The milk and sugar are placed in a large metal pan and brought to the boil when the rest of the ingredients are added. The whole mixture is stirred and boiled for a variable period usually not less than 3 and not more than 10 min. This is not necessarily sufficient to sterilize although it does destroy most of the non-sporing bacteria.

Method II, in which only the milk and sugar are boiled

In this method the boiling sweetened milk is poured with stirring over the cornflour or ice-cream powder which has previously been made into a thin paste with a little cold milk. If the powder is not sterile some of the bacteria are certain to survive and contaminate the ice-cream.

Cooling

The cooling of the heated ice-cream mixture is by standing at room temperature and is therefore very prolonged. The food is often heated one day and frozen the next, so that the cooling period is rarely less than 15 hr. and is often much more. Buchan found that the cooling time averages about $20\frac{3}{4}$ hr. Most manufacturers have covers of fine muslin or grease-proof paper over the cooling buckets to limit the chances of contamination as much as possible.

Method III, in which ice-cream is prepared without heating

Some manufacturers use a "cold mix" powder made of dried skimmed milk mixed with a kind of gum and possibly some finely powdered gelatin. Sugar and milk are added to the powder which is left for about an hour in a warm room to thicken and then frozen without any heating at all.

The freezing process

The oldest type of freezer consists of a bucket placed in a tub of freezing mixture, usually ice and salt. The bucket is revolved with one hand while the ice-cream is turned with a spade-like implement held in the other. The ice-cream is open to the atmosphere during this process and is very easily contaminated from the worker's hand sliding up and down the handle of the spade. This method was in use only a few years ago in the Manchester district and may persist in some places still.

Another type of freezer which is almost as undesirable is the open rotary drum. This drum is packed inside with ice and salt and is revolved either by hand or by electric power, while the ice-cream flows over the surface in a film so thin that almost every part of its substance is exposed to the air. Freezing is very rapid. The finished product is cut with a knife and falls into a receptacle placed under the drum.

The best type of freezer and the most usual one in this district is a vessel closed with a tightly fitting lid, standing in a tub of freezing mixture and rotated by hand or by electric power. Such freezers are fitted with a stirrer to hasten solidification and ensure evenness.

Ice-cream as a culture medium

Ice-cream, being a milk product, is during some stages of preparation a particularly good medium for bacterial growth and should therefore be guarded carefully from dust and dirt, should be manufactured as quickly as possible and should not be prepared from heavily infected materials.

If the ice-cream mixture is sufficiently heated the risk from heavily infected ingredients is small. After boiling the product should contain only a negligible number of bacteria, but during the further processes of manufacture other organisms find their way into it from the air, from the utensils and from the people who handle it. The air-borne bacteria are usually sporing bacilli of the *subtilis-mesentericus* group which may also have been present in the materials used in manufacture. These bacilli have no particular sanitary significance unless they are present in excessively large numbers. The bacteria derived from utensils are more difficult to define. Some of them may also be sporing aerobes and there may be cocci or coliform bacilli from sediment left in the ridges of the cooling buckets and freezer by careless washing. The bacteria which come directly from human sources may be pathogenic. The usual contaminants are streptococci of the enterococcus group and coliform bacilli which indicate faecal pollution. Whenever these organisms occur in appreciable numbers it is possible that bacteria of the colon-typhoid group may also gain access to the food, although harmful organisms are probably rare in ice-cream.

It is particularly important to insist that the ingredients should not be grossly polluted when dealing with ice-cream made from cold mix powders because the bacteria are never subjected to the destroying action of heat. Such food contains all the bacteria originally present in the milk, in the powder and other ingredients, together with any extraneous organisms gathered during manufacture. The period of thickening, though short, affords a further opportunity for contamination and helps to raise the bacterial content by multiplication of the organisms. Ice-cream produced by this method will be grossly polluted unless the milk is graded, sterilized or at least pasteurized and unless the greatest possible care is used in manufacture. The method finds favour with many manufacturers because the food is very quickly made and can be produced in small quantities as required.

Refreezing

It is the custom in many places to bring in unsold ice-cream from the streets or from the seashore where it has been exposed at intervals to blowing dust and sand, and to refreeze it if necessary for sale the following day. This is also the practice in many shops and cafés, and if the food is produced in cleanly fashion so that the bacterial count is low there is probably no great harm in keeping it for a very limited time. Buchan believed that no ice-cream should be exposed for sale 48 hr. after boiling, which means that in cases where the cooling period is prolonged the ice-cream should not be put on the market for a second day. Sometimes unsold ice-cream which has almost certainly become contaminated by constant opening of the freezer for the purpose of serving is mixed with freshly made ice-cream. This cannot be considered satisfactory because bacteria are introduced into the fresh food, and also portions of the ice-cream may in this manner be carried over for several successive days.

INSPECTION OF ICE-CREAM FACTORIES

In Buchan's paper it is stated that ice-cream is often manufactured and stored under filthy conditions in overcrowded houses and is subjected to extremely careless handling, particularly by the Italian population. This is not true of the Manchester district at the present time. Every large-scale producer is required to have suitable manufacturing premises quite separate from the living and sleeping quarters. These premises must have at least two rooms, one for boiling the mixture and storing the cooling custard, the other for freezing. The floors must be concreted, the walls tiled or cemented. There must be an adequate water supply and some means of sterilizing the utensils. The best factories have a steamer, but some use the boiler. Every factory must also be fitted with a refrigerator for storage purposes.

It is true that during the summer months a few small shops make ice-cream, but more and more of them are buying their supplies in wrapped portions from wholesale manufacturers who also supply suitable refrigerating containers. The Manchester authorities object to ice-cream being made for sale on domestic premises unless there is an enclosed automatic refrigerator which is placed away from the living quarters. It is a much easier task to control a few large factories with a steady output than innumerable shops producing small quantities when the weather conditions create an occasional demand.

SAMPLING ICE-CREAM

The bacteriological examination of ice-cream affords some indication of the care with which the food has been produced and handled, but many authorities find it more satisfactory to exercise control by regular inspection and improvement of the factories rather than by biological tests, and so comparatively few samples are received for examination in proportion to the enormous number of factories which exist.

Seventy-five samples of ice-cream were submitted for examination in 1935, 64 in 1936 and 87 in 1937. A further 11 were collected privately, making a total of 237 samples. All were manufactured in Lancashire or Cheshire, were taken at random and were of approximately the same size but they were not by any means of the same age. The samples were taken in wide-mouthed sterile bottles fitted with screw caps and were collected with the vendor's own server.

Technique of examination of ice-cream

The technique used in the bacteriological examination of ice-cream is similar to the method recommended by the Ministry of Health for water analysis (1934). The ice-cream is warmed slightly (by standing in a water-bath for a few minutes), is thoroughly mixed by shaking and then diluted. Portions of the dilutions and of the undiluted sample are then inoculated into (*a*) MacConkey's bile-salt lactose broth, incubated at 37° C. for 2 days for the detection of coliform bacilli and faecal streptococci of the enterococcus group, (*b*) litmus milk incubated anaerobically at 37° C. for 2 days for the detection of *Cl. welchii*, and (*c*) Petri plates poured with Yeastrel agar (with 1 % milk) for counting purposes. Some of these are incubated for 2 days at 37° C., others for 3 days at 22° C.

The production of acid and gas in the MacConkey's medium is presumptive evidence of the presence of coliform bacilli. Faecal streptococci of the enterococcus group produce acid in this medium, and any tube showing acid or acid and gas is examined for enterococcus. These organisms are heat-resistant and often escape destruction in the manufacture of ice-cream, since they survive heating at 60° C. for ½ hr. and may resist higher temperatures if they are sufficiently protected by the medium. They are commonly found in dried milk powder and in ice-cream powder. The presence of enterococcus in large numbers in ice-cream or any heated substance in the absence of *coli* usually means that the food has been made of polluted materials. The coliform and enterococcus results are expressed as the probable number of organisms present per 100 c.c. of sample. These figures are obtained by the use of McCrady probability tables supplied by the Ministry of Health for water analysis.

Results considered on the basis of samples

(*a*) *Coliform bacilli*

Among the 237 samples examined there were 209 which contained coliform bacilli, and the McCrady figure varied between 1 and 180,000,000 organisms per 100 c.c. of sample. Table I summarizes the *coli* results. 119 samples (50·21 %) had no coliform bacilli present in less than 0·1 c.c. and would in this respect satisfy the standard for graded milk. If, however, the standard is lowered to include McCrady values up to 25,000 organisms per 100 c.c., 169 samples (71·33 %) showing no *coli* in less than 0·01 c.c. would be regarded as satisfactory.

The arithmetic mean of the coliform figures on the whole series of 237 samples was approximately 1,774,605 organisms per 100 c.c., the arithmetic mean on the 209 positive samples was 2,012,351 and the geometric mean (excluding zero results) was 6606 organisms per 100 c.c. of sample. It is probable that the geometric mean (which is simply the nth root of n observations) is the fairer estimate of the average coliform content in the present series, not

Table I. *Coliform results obtained from 237 samples of ice-cream*

No. of samples	McCrady figure per 100 c.c.	Coliform organisms: Found in (c.c.)	Not found in (c.c.)
28	0	—	100
14	1–25	10	1
39	26–250	1	0·1
38	251–2,500	0·1	0·01
50	2,501–25,000	0·01	0·001
35	25,001–250,000	0·001	0·0001
17	250,001–2,500,000	0·0001	0·00001
14	2,500,001–25,000,000	0·00001	0·000001
2	25,000,001–250,000,000	0·000001	0·0000001

only because this method of calculation brings the figures closer together and stresses the value of those which occur most frequently (Robertson, 1932), but also because in this series of experiments the samples were collected at different times and were of different ages when submitted for analysis. The McCrady figure of 6606 coliform organisms per 100 c.c. indicates the probability of finding these bacilli in not more than two out of five tubes of MacConkey broth inoculated with 1 c.c. of a 1 in 100 dilution of ice-cream. This would be interpreted as *coli* present in 0·1 c.c., not present in 0·01 c.c., or alternatively *coli* present in 0·1 c.c. occasionally present in 0·01 c.c.

(*b*) *Enterococcus*

A total of 216 samples yielded enterococcus. The McCrady figures obtained were slightly lower than those for *coli* (Table II). The arithmetic mean of the enterococcus figures on all 237 samples was 849,400; the arithmetic mean on the

Table II. *Enterococcus results obtained on 237 samples of ice-cream*

No. of samples	McCrady figure per 100 c.c.	Enterococcus: Found in (c.c.)	Not found in (c.c.)
21	0	—	100
15	1–25	10	1
40	26–250	1	0·1
66	251–2,500	0·1	0·01
44	2,501–25,000	0·01	0·001
19	25,001–250,000	0·001	0·0001
22	250,001–2,500,000	0·0001	0·00001
9	2,500,001–25,000,000	0·00001	0·000001
1	25,000,001–250,000,000	0·000001	0·0000001

216 positive samples was 931,981 organisms per 100 c.c. of sample. The geometric mean (on the positive samples) was 5005 organisms per 100 c.c., which gives an enterococcus-*coli* ratio of rather less than 1. The usual ratio of enterococcus to *coli* in water, milk and faeces is about 1 : 10 or even 1 : 100. In ice-cream the higher ratio is probably due to the use of milk and ice-cream powders which often have high enterococcus counts.

https://doi.org/10.1017/S0022172400011396 Published online by Cambridge University Press

(*c*) *Cl. welchii*

There are no probability figures available for *Cl. welchii*. Only 43 samples out of 237 were tested for this organism and 16 of them gave positive results; 8 samples had *Cl. welchii* in 10 c.c., 7 samples in 1 c.c. and 1 sample in 0·1 c.c. All contained *coli* and enterococcus. The geometric mean coliform figure for these samples was 2203 organisms per 100 c.c. which is lower than the mean for all samples, but the corresponding figure for enterococcus was higher (7209). This indicates that samples which contain *Cl. welchii* also contained an undue proportion of enterococcus but not necessarily an excess of *coli*. This difference is probably accounted for by the heat resistance of *welchii* and enterococcus as compared with *coli* and the likelihood that polluted materials were used in manufacture.

(*d*) *Total counts*

Table III shows that among the 237 samples there were 115 (48·52 %) which had not more than 200,000 organisms present per c.c. at 22° C., and there were 138 (58·23 %) which had not more than 200,000 organisms per c.c. at 37° C. If the limiting count is raised to 500,000 per c.c. there were 147 samples (62·03 %) at 22° C. and 612 samples (68·35 %) at 37° C. which satisfied the required standard.

Table III. *Total counts obtained from* 237 *samples of ice-cream*

Total counts	No. of samples giving the total counts indicated in the left column at 22° C.	37° C.
1–500	7	12
501–1,000	7	5
1,001–10,000	26	34
10,001–100,000	59	61
100,001–200,000	16	26
200,001–500,000	32	24
500,001–1,000,000	18	14
1,000,001–10,000,000	49	49
10,000,001–100,000,000	22	12
100,000,001–1,000,000,000	1	0

The arithmetic mean of the count at 22° C. was 4,072,677 per c.c. and the arithmetic mean of the count at 37° C. was 2,655,754 organisms per c.c. of sample. The geometric means were 173,700 at 22° C. and 109,200 at 37° C. The ratio of the two counts is very different in ice-cream from the ratio obtained from similar counts in water where the 22° C. count is usually about ten times, rarely less than five times, the 37° C. count. The relatively high ratio of the 37° C. to the 22° C. count suggests that many of the contaminating organisms in ice-cream are of human or at least of faecal origin.

(e) Results considered on the basis of factories from which the samples were collected

The samples of ice-cream were collected from 125 factories. Table IV shows how many samples were taken from each. It is possible to consider the purity of ice-cream from the point of view of the factory, and the results have been analysed on this basis. When more than one sample was taken from a factory the mean of the results of all the samples from that factory has been used, and indicates the general standard of cleanliness. Table V summarizes the coliform and the enterococcus results.

Table IV. *Distribution of* 237 *samples among* 125 *factories*

No. of factories	No. of samples collected from each factory
71	1
24	2
14	3
9	4
4	5
1	6
2	7

Table V. *Coliform and enterococcus results obtained from* 125 *factories*

	No. of factories giving the McCrady figure indicated in the left column for	
McCrady figures per 100 c.c.	Coliform organisms	Enterococcus
0	7	10
1–25	10	6
26–250	17	16
251–2,500	20	31
2,501–25,000	23	23
25,001–250,000	21	13
250,001–2,500,000	17	17
2,500,001–25,000,000	8	7
25,000,001–250,000,000	2	2

The coliform arithmetic mean for all factories was 1,220,170. Omitting the seven factories which gave a zero result the arithmetic mean was 1,292,583 and the geometric mean was 9317 organisms per 100 c.c. Similarly the enterococcus arithmetic mean for all factories was 1,164,755 and on the 115 positive factories was 1,266,038. The geometric mean was 9322.

The arithmetic means of the total counts were 3,301,579 at 22° C. and 2,330,575 organisms per c.c. at 37° C. The geometric means were 337,000 at 22° C. and 211,900 at 37° C. These figures do not correspond to those shown for all samples because the figures for many of the factories were themselves arithmetic means (Table VI).

It is evident that among the 125 factories tested the general standard of cleanliness was not sufficient to produce ice-cream which would conform to the graded milk standard. This is discussed further in relation to standards on p. 545.

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Table VI. *Total counts obtained from* 125 *factories*

Total counts	No. of factories giving the total counts indicated in the left column at 22° C.	37° C.
1–500	1	3
501–1,000	2	1
1,001–10,000	8	12
10,001–100,000	28	29
100,001–200,000	9	13
200,001–500,000	17	15
500,001–1,000,000	16	14
1,000,001–10,000,000	32	30
10,000,001–100,000,000	12	8
100,000,001–1,000,000,000	0	0

Manufacture of ice-cream in the laboratory

A small "reliance" freezer of the closed type and having a capacity of about 2 qt. was used in making ice-cream in the laboratory. The food was prepared in three different ways: (*a*) by boiling the whole mixture, (*b*) by boiling the milk and sugar only, (*c*) by the cold mix method.

(*a*) *By boiling the whole mixture*

Two experiments were carried out in which ice-cream was made by boiling the whole mixture before freezing (Table VII). The same ice-cream powder was used for both samples, and it was not by any means free from contamination having a total count of 1180 organisms per g. at 22° C. and of 230 per g. at 37° C., with *Bact. coli* present in 100 g. and *Cl. welchii* in 0·1 g. No special precautions were taken to protect the food during preparation, only ordinary domestic cleanliness was observed. In the first experiment pasteurized milk was used, in the second fresh mixed milk.

In each case mere boiling of the mixture destroyed most of the bacteria. From the pasteurized milk a *coli* figure of 16,000 and an enterococcus figure of 18,000 organisms per 100 c.c. was obtained, but the heated mixture cooled at

Table VII. *Ice-cream manufactured in the laboratory by boiling all the ingredients before freezing*

Ingredient	Coliform: Found in	Coliform: McCrady	Enterococcus: Found in	Enterococcus: McCrady	*Cl. welchii* found in	Total counts at 22° C.	Total counts at 37° C.
Pasteurized milk	0·01 c.c.	16,000	0·01 c.c.	18,000	—	181,000	179,500
Ice-cream powder	100 g.	1	—	—	0·1 g.	1,180	230
Custard, boiled and cooled	—	—	10 c.c.	25	10 c.c.	90	40
Ice-cream (sampled after serving)	10 c.c.	25	0·1 c.c.	1,800	10 c.c.	210	150
Fresh mixed milk	0·0001 c.c.	1,800,000	0·1 c.c.	1,800	10 c.c.	2,900,000	254,750
Ice-cream powder	100 g.	1	—	—	0·1 g.	1,180	230
Custard, boiled and cooled	—	—	—	—	10 c.c.	240	8
Ice-cream (sampled before serving)	—	—	100 c.c.	3	10 c.c.	770	110

https://doi.org/10.1017/S0022172400011396 Published online by Cambridge University Press

room temperature yielded no *coli* and only 25 enterococcus. The total counts also were reduced from 181,000 to 90 at 22° C. and from 179,500 to 40 at 37° C. With fresh milk there was a high *coli* figure of 1,800,000 and an enterococcus figure of 1800 per 100 c.c. In the boiled cooled mixture both *coli* and enterococcus were absent in 100 c.c. The counts were reduced from nearly 3,000,000 to 240 organisms per c.c. at 22° C. and from 254,750 to 8 organisms per c.c. at 37° C. The greater reduction in numbers in the second experiment may have been due to cleaner vessels or to a slightly longer boiling period. It did not appear to make any difference whether polluted or unpolluted milk was used because boiling seemed to destroy most of the organisms.

After freezing the bacterial counts increased. Buchan noticed a rise in count in ice-cream during freezing and reports that in one case the gelatin count rose from 327,000 to 611,000 organisms per c.c. and the agar count from 93,000 to 139,000 organisms per c.c. after 12 hr. exposure to temperatures varying between 28 and 28·8° F. In the present experiments where freezing lasted not more than $\frac{3}{4}$ hr. the increase may have been due to the washed but not sterilized freezer and to air contamination while pouring the liquid from one vessel to another, for the total counts in both were well below 1000 organisms per c.c. In one experiment the finished product was sampled immediately on opening the freezer and was found to be free from *coli* and to have only three enterococcus per 100 c.c. In the other experiment the food was sampled after it had been served with a clean but not sterile spoon, and in this case there was a low *coli* count.

All food made from this particular ice-cream powder contained *Cl. welchii* in 10 c.c. which would represent approximately 0·1 g. of the powder. Spores of this bacillus evidently resisted the short boiling period.

It is interesting to compare the results obtained from two heated and cooled mixtures collected from factories with those obtained from similar material prepared in the laboratory (Table VIII). In one examination the

Table VIII. *Results obtained from examination of heated and cooled ice-cream mixtures*

Source of sample	Coliform: Found in	Coliform: McCrady	Enterococcus: Found in (c.c.)	Enterococcus: McCrady	*Cl. welchii* found in (c.c.)	Total counts at 22° C.	Total counts at 37° C.
Factory 1 (all ingredients boiled)	—	—	—	—	—	—	—
Factory 2 (all ingredients boiled)	—	—	0·1	900	—	10,000	2,000
Laboratory 1 (all ingredients boiled)	—	—	10	25	10	90	40
Laboratory 2 (all ingredients boiled)	—	—	—	—	10	240	8
Laboratory 3 (milk and sugar boiled)	—	—	10	35	50	100	95
Laboratory 4 (milk and sugar boiled)	—	—	1	250	50	310	125
Laboratory 5 (milk and sugar boiled)	—	—	50	1	10	1,250	1,093

https://doi.org/10.1017/S0022172400011396 Published online by Cambridge University Press

cold mixture from a factory appeared to be completely sterile, in the other, although there was no *coli*, the enterococcus figure was 900 organisms per 100 c.c. and the counts were approximately 10,000 organisms at 22° C. and 2000 at 37° C. None of the cooled custards from the laboratory was completely sterile.

(*b*) *Ice-cream made by boiling the milk and sugar only*

This method is recommended by the makers of the laboratory freezer as a cheap and easy way of producing ice-cream. It consists simply of freezing a cold custard made from custard powder and milk. In preparing the custard a portion of the milk is used to mix the powder into a thin paste, and the remainder of the milk, when boiling, is poured over it. Thus part of the milk and the powder are never boiled at all, although they are raised in temperature sufficiently to burst the starch grains and give the necessary smoothness. This method is probably not much used on the large scale as the results are not very pleasing to taste.

Three experiments were carried out in this way, using pasteurized milk and fresh milk (Table IX). The cold custard was always free from *coli* in 100 c.c. and the enterococcus counts and total counts were low (well below 1500 organisms per c.c.). The bacterial content of the finished product was shown to

Table IX. *Ice-cream manufactured in the laboratory by boiling the milk and sugar only*

	Coliform		Enterococcus		*Cl. welchii*	Total counts at	
Ingredients	Found in	McCrady	Found in	McCrady	found in	22° C.	37° C.
Sterilized vessels and utensils:							
Pasteurized milk	0·01 c.c.	18,000	0·1 c.c.	1,800	50 c.c.	40,000	3,650
Custard powder	1 g.	250	1 g.	250	50 g.	12,867	33,000
Vanilla essence	—	—	—	—	—	—	—
Custard after cooling	—	—	10 c.c.	35	50 c.c.	100	95
Ice-cream	—	—	10 c.c.	50	50 c.c.	170	140
Scalded vessels and utensils:							
Pasteurized milk	0·1 c.c.	600	0·01 c.c.	18,000	50 c.c.	32,000	2,850
Custard powder	1 g.	250	1 g.	250	50 g.	12,867	33,000
Custard after cooling	—	—	1 c.c.	250	50 c.c.	310	125
Ice-cream	—	—	1 c.c.	250	50 c.c.	385	335
Ordinary domestic cleanliness:							
Fresh mixed milk	—	—	0·01 c.c.	9,000	—	181,500	12,450
Custard powder	1 g.	250	1 g.	250	50 g.	12,867	33,000
Custard after cooling	—	—	50 c.c.	1	10 c.c.	1,250	1,093
Ice-cream	50 c.c.	1	10 c.c.	25	10 c.c.	2,500	1,500

depend upon the state of the utensils. When all utensils coming into contact with the food were steam sterilized the ice-cream was free from *coli*, had enterococcus only in 10 c.c. and counts of less than 200 organisms per c.c. When the vessels were scalded in boiling water immediately before use the ice-cream was still free from *coli*, but enterococcus was found in 1 c.c. and the counts were between 300 and 400 organisms per c.c. When the vessels were washed with ordinary domestic cleanliness *Bact. coli* occurred in 50 c.c. and the counts were nearer 2500 organisms per c.c. If careless washing had been the rule presumably the bacterial content would have risen in proportion.

Thus if it were possible to get every manufacturer to steam sterilize his cooling buckets, freezer and other utensils or even to scald them, it is reasonable to suppose that a decided improvement in the bacterial content of ice-cream would result.

(c) *Cold-mix method*

A weighed quantity of ice-cream powder was mixed with sugar and cold milk. The whole mixture was left to stand for about $1\frac{1}{2}$ hr. to thicken and then was frozen in the ordinary way.

Four experiments were carried out in this way (Table X). In the first one the milk was pasteurized, in the second and third it was fresh, in the fourth it was sterilized. The powder was by no means sterile. *Bact. coli* was found in

Table X. *Ice-cream manufactured in the laboratory by the "cold mix" method*

Ingredients	Coliform: Found in	Coliform: McCrady	Enterococcus: Found in	Enterococcus: McCrady	*Cl. welchii* found in	Total counts at 22° C.	Total counts at 37° C.
Pasteurized milk	0·1 c.c.	1,600	0·01 c.c.	18,000	10 c.c.	197,300	24,300
Cold-mix powder	50 g.	1	0·0001 g.	1,800,000	—	455,000	705,000
Mixture after thickening	0·1 c.c.	1,800	0·01 c.c.	18,000	10 c.c.	202,000	45,000
Ice-cream	0·1 c.c.	1,800	0·01 c.c.	18,000	10 c.c.	222,250	65,000
Fresh mixed milk	0·01 c.c.	18,000	0·01 c.c.	18,000	10 c.c.	615,000	25,775
Cold-mix powder	50 g.	1	0·0001 g.	1,800,000	—	455,000	705,000
Mixture after thickening	0·01 c.c.	9,000	0·01 c.c.	18,000	10 c.c.	665,000	50,300
Ice-cream	0·01 c.c.	35,000	0·01 c.c.	18,000	10 c.c.	720,000	94,000
Fresh mixed milk	0·00001 c.c.	16,000,000	0·0001 c.c.	1,800,000	—	1,900,000	920,000
Cold-mix powder	50 g.	1	0·0001 g.	1,800,000	—	455,000	705,000
Mixture after thickening	0·00001 c.c.	7,000,000	0·0001 c.c.	1,800,000	—	2,753,000	2,817,000
Ice-cream	0·00001 c.c.	9,000,000	0·00001 c.c.	9,000,000	—	7,100,000	6,350,000
Sterilized milk	—	—	—	—	—	—	—
Cold-mix powder	50 g.	1	0·0001 g.	1,800,000	—	455,000	705,000
Mixture after thickening	—	—	0·01 c.c.	18,000	—	29,600	24,233
Ice-cream	—	—	0·01 c.c.	18,000	—	36,500	32,875

50 g. giving a McCrady figure of 1 per 100 g., enterococcus was found in 0·0001 g. with the high McCrady figure of 1,800,000 organisms per 100 g., the total counts were 455,000 per g. at 22° C. and 705,000 at 37° C.; *Cl. welchii* was absent. As the amount of powder in the ice-cream was small compared with the amount of milk (being about 8 oz. to 1 gal.) the initial bacterial content of the food largely depended on the purity of the milk from which it had been prepared.

In the first experiment with pasteurized milk the *coli*, enterococcus and *welchii* content was approximately the same in the milk and in the ice-cream, but the total counts were slightly higher in the finished product.

In the second and third experiments with fresh milk the same thing happened, but in the third test there was an increase in the enterococcus figure, as well as an appreciable increase in the total counts. This increase may of course have been due to imperfectly washed vessels as well as to the growth of organisms during the thickening period.

https://doi.org/10.1017/S0022172400011396 Published online by Cambridge University Press

When sterile milk was used *coli* was absent from the ice-cream but enterococcus was present in 0·01 c.c. and was due to contamination from the powder which showed an excessive enterococcus count. The total counts which were approximately 30,000 in the thickened mixture rose to 35,000 in the ice-cream.

It is obvious that in using cold-mix ice-cream powder it is important to add only the best grades of milk or to sterilize the milk before use.

Three samples of ice-cream which had been produced in factories from cold-mix powder were tested at the laboratory. The first, made from graded milk, gave *coli* and enterococcus in 0·01 c.c. and total counts of approximately 350,000 per c.c.

The other two samples were collected from the same factory, one in September and one in December. The September sample gave total counts between 7,000,000 and 8,000,000 organisms per c.c., and *coli* and enterococcus were both found in the 1 in 1,000,000 dilution with McCrady figures of 90,000,000 organisms per 100 c.c. The December sample gave satisfactory *coli* and enterococcus counts, but the total counts were 9,000,000 organisms per c.c. Thus it appears that even in the cold autumn and winter months ice-cream made in this way may be grossly polluted if prepared from contaminated substances.

Examination of ice-cream powder

Six different ice-cream powders were tested for bacterial content (Table XI). None of them was sterile. The total counts at 22° C. ranged from 350 to 450,000 organisms per g. and at 37° C. from 230 to 705,000 organisms per g.

Table XI. *Examination of powder used in making ice-cream*

Sample no.	Coliform: Found in (g.)	Coliform: McCrady	Enterococcus: Found in (g.)	Enterococcus: McCrady	*Cl. welchii* found in (g.)	Total counts per g.: At 22° C.	Total counts per g.: At 37° C.
1	—	—	—	—	—	1,150	613
2	1	250	1	250	50	12,867	33,000
3	50	1	—	—	0·1	1,180	230
4	50	1	0·0001	1,800,000	—	455,000	705,000
5	—	—	10	7	1	350	590
6	—	—	10	50	10	7,200	8,450

Three powders were free from *coli* in 100 g., two yielded *coli* in very small numbers, approximately 1 per 100 g., and one yielded an appreciable coliform figure of 250 organisms per 100 g. or *coli* present in 1 g., absent in 0·1 g.

Four of the powders contained enterococcus, usually in comparatively small numbers, though one had the enormous enterococcus count of 1,800,000 organisms per 100 g. Four powders also yielded *Cl. welchii*, in one case the spores were present in 0·1 g.

Generally speaking, only a very small quantity of the powder is used compared with the amount of milk, and the bacterial content of the powder has therefore less effect on the final product than the bacterial content of the milk. Since, however, it should be a simple matter to have these powders sterilized, it would be well to insist on sterility whenever possible especially in the case of cold-mix powders.

THE EFFECT OF STORING AT LOW TEMPERATURE

Four samples made in the laboratory were kept at low temperature for varying lengths of time (Table XII). The first three contained few bacteria, but the last sample was made from cold-mix powder and was polluted.

In the first experiment the ice-cream was tested immediately on opening the freezer, was kept at 8° C. for 5 hr. and then again tested. The second sampling showed a marked rise in total counts, but the *coli* and enterococcus figures showed no increase.

Table XII. *The effect of storing ice-cream at low temperatures*

	Coliform		Enterococcus		*Cl. welchii* found in	Total counts at	
Experiment	Found in c.c.	McCrady	Found in c.c.	McCrady	c.c.	22° C.	37° C.
Ice-cream 1st sampling	1	80	0·1	900	—	90,000	5,975
2nd sampling 5 hr. later after storage at low temperature	1	80	0·1	900	—	115,500	11,600
Ice-cream 1st sampling	50	1	10	25	10	2,500	1,500
2nd sampling 18 hr. later after storage at 8° C.	50	1	10	25	10	2,800	1,486
3rd sampling 6 days later after storage at 8° C.	50	1	1	250	10	6,200	1,600
Ice-cream 1st sampling	—	—	100	3	10	770	110
2nd sampling after intermittent exposure to the air during a whole day	—	—	100	5	10	1,605	900
3rd sampling after exposure in a dusty room for 20 min.	50	1	10	25	10	91,000	58,000
4th sampling after further exposure in the street in a wind for about 10 min.	0·01	60,000	0·1	1,800	10	940,000	160,000
Ice-cream 1st sampling	0·1	1,800	0·01	18,000	10	222,250	65,000
2nd sampling after 2 days at 8° C.	0·01	9,000	0·01	18,000	10	450,000	67,000
3rd sampling after 7 days at 8° C.	0·001	160,000	0·01	18,000	10	13,400,000	74,500

In the second experiment the ice-cream was sampled, kept at 8° C. for 18 hr. and then resampled. The two results were very similar, but when kept for a further 6 days the counts showed an increase and the enterococcus figure rose from 25 to 250, i.e. from occurrence in 10 c.c. to occurrence in 1 c.c.

The third experiment consisted in sampling the food (*a*) immediately on opening the freezer, (*b*) after exposure to the air at intervals during a whole day, (*c*) after exposure in a dusty room for 20 min., (*d*) after further exposure in the street in a high wind for about 10 min. The results showed a decided rise in bacterial content after each exposure. The initial counts at 22 and at 37° C. were 770 and 110 organisms per c.c. After the series of intermittent exposures they rose to 1605 and 900, after exposure in a dusty room they were 91,000 and 58,000, but after exposure near the road in a wind they reached 940,000 and 160,000 organisms per c.c. The ice-cream was prepared free from coliform bacilli, but these organisms were found in small numbers after the freezer had been opened in a dusty room and in appreciable numbers after exposure in the wind. The enterococcus figure showed a similar increase.

The fourth experiment was carried out on a "cold-mix" ice-cream with a *coli* count of 1800 and an enterococcus count of 18,000 organisms per 100 c.c., and with total counts of 222,250 at 22° C. and 65,000 at 37° C. After storage for 2 days at 8° C. the *coli* had increased to 9000 and after 7 days to 160,000 organisms per 100 c.c. The enterococcus figures did not alter, but the total counts at 22° C. were 450,000 after 2 days and 13,400,000 per c.c. after 7 days. The counts at 37° C. were 67,000 after 2 days and 74,500 after 7 days.

These results seem to show that if the ice-cream is not heavily polluted and is protected from dust quite satisfactory results may be obtained after storage at low temperatures for at least as long as 2 days, but if the food is polluted to begin with, storage even at a low temperature may allow the organisms to grow and thus raise the bacterial content very appreciably.

Strains isolated from ice-cream

For sixty-five samples of ice-cream, 7 samples of milk used in making ice-cream in the laboratory and 3 samples of ice-cream powder the highest dilution giving acid and gas in MacConkey's medium was plated out and a number of colonies, usually three, were picked off and tested in pure culture. In this way 251 strains were isolated from ice-cream, 51 from milk and 13 from the powders. They were classified according to Wilson's method (1935) on the basis of indole production, M.R. and V.P. reactions, growth in citrate, production of acid and gas in MacConkey broth at 44° C. and liquefaction of gelatin (Table XIII).

Table XIII. *Classification of strains of coliform bacilli isolated from ice-cream and from milk and powder used in the manufacture of ice-cream*

Type of organism	Ice-cream	Milk	Ice-cream powder
Bact. coli I	17	10	0
Bact. coli II	18	6	0
Intermediate type I	90	26	1
Intermediate type II	0	0	0
Bact. lactis aerogenes I	88	4	11
Bact. lactis aerogenes II	27	0	0
Bact. cloacae	1	0	0
Irregular strains	10	5	1
Total strains	251	51	13

Bact. coli I (indole + MR + VP_0 $citrate_0$ 44° C. + Gel_0) is the dominant organism in faeces. It was never found in the powders and only 17 (6·77 %) of the ice-cream and 10 (19·61 %) of the milk strains were of this type. *Bact. coli* II ($indole_0$ MR + VP_0 $citrate_0$ 44° $C._0$ Gel_0) formed 7·17 % of the strains from ice-cream and 11·76 % of those from milk. Wilson frequently found this organism in raw milk and on hay and straw but not often in faeces. The intermediate-*aerogenes-cloacae* group includes all those strains which are able to grow in citrate. None of them produce acid and gas in MacConkey broth at 44° C., and few are able to produce indole. These bacilli are generally believed to be comparatively rare in faeces, but they are the dominant coliform types in infected urine (Burke-Gaffney, 1933), in soil, in dust, on grain and in certain foodstuffs. Many workers consider them to be more resistant than the faecal type, so that their presence in food or water in the absence of *coli* I may indicate excretal contamination of indirect or remote origin. 206 (82·07 %) of the strains from ice-cream, 30 (58·82 %) from milk and 12 (92·31 %) from ice-cream powder belonged to the intermediate-*aerogenes-cloacae* group. These results agree with other findings. Bardsley (1934) isolated 365 coliform bacilli from ice-cream of which 314 (86 %) were able to grow in citrate. Wilson found that the citrate utilizers formed 69·3 % of 245 coliform strains from raw milk, 56·5 % of 46 strains from pasteurized milk and 66·2 % of 80 strains from certain foodstuffs. The prevalence of these bacilli in ice-cream is probably the result of infected ingredients and imperfectly washed vessels as well as blowing dust and indirect faecal pollution.

STANDARDS FOR JUDGING THE PURITY OF ICE-CREAM

If in the present series of 237 examinations of ice-cream samples the geometric mean figures are taken as representing the *coli*, enterococcus and total bacterial content in the average sample, then it follows that the average sample would in all probability just satisfy the graded milk standard having no *coli* in three out of five tubes inoculated with 0·01 c.c. and a total count of less than 200,000 organisms per c.c.

The samples prepared in the laboratory by either of the heating methods were well within this limit. In fact it was very difficult indeed to produce ice-cream with an appreciable *coli* content though on a large scale when cooling periods are longer and utensils and vessels more difficult to handle and clean it is probably only too easy to get contamination. When cold-mix powder was used the bacterial content of the finished product depended on the state of the milk and to a lesser extent on the state of the powder. When the milk was polluted the ice-cream also was polluted, and it was impossible to make an ice-cream conforming to the above standard unless a graded or sterilized milk was used.

If the former Grade A milk standard were adopted in the present series as far as the count and *coli* figures were concerned there would be 94 samples (39·66 %) conforming both to *coli* and to count, 18 samples (7·59 %) failing in the *coli* test but satisfying the count, 38 (16·03 %) satisfying the *coli* but failing in the count and 87 (36·71 %) failing in both count and *coli* (Table XIV).

Benson's standard using 100,000 as the limiting number of bacteria per c.c. and condemning samples which have coliform bacilli in 0·1 c.c. would not in the present series allow the average sample to be regarded as satisfactory. Only 60 samples (25·32 %) conform to this standard. It is true that ice-cream which is heated during manufacture might be expected to conform to the standard for heated milk as far as count is concerned, except that after heating

Table XIV. *A comparison of different standards applied to the* 237 *samples of ice-cream examined in the laboratory*

Standard	No. of samples conforming to count and to *coli*		No. failing in *coli* test		No. failing in count		No. failing in count and *coli*	
	No.	%	No.	%	No.	%	No.	%
Standard A (Thierens, 1930)	94	39·66	18	7·59	38	16·03	87	36·71
Benson (1933)	60	25·32	34	14·35	28	11·81	115	48·52
Buchan (1910)	111	46·84	47	19·83	20	8·44	59	24·89
Standard B	134	56·54	7	2·95	43	18·14	53	22·36

ice-cream is exposed to further contamination and is not, like milk, immediately filled into sealed containers. Most ice-cream on the market is not even sold in wrapped portions or in sealed cartons. Finally, there seems to be no point in fixing a standard which is higher than that applied to graded milk.

If Buchan's standard were used the acceptance of total counts of less than 1,000,000 per c.c. at 22 and at 37° C. would allow 17 samples which failed by the graded milk standard to be reported satisfactory. The *coli* content then becomes the limiting factor, for nearly 20% of the samples contain coliform organisms in less than 0·1 c.c., although the counts are within the prescribed limit. It would appear therefore that Buchan's standard is somewhat high in the *coli* test but lenient as far as total bacteria are concerned. Table XIV gives the percentage of samples satisfying a further possible standard when *coli* may not be found in less than 0·01 c.c., and the count must not exceed 500,000 organisms per c.c. It will be noticed that there are approximately the same number of samples failing to conform to both count and *coli* tests by this and by Buchan's standard, which probably means that there are certain samples showing gross pollution which could not reasonably be accepted as suitable food however leniently they may be judged.

It would be possible to go on multiplying suggestions for standards by trying various limiting values for both count and *coli*, but there seems to be no useful purpose in making a standard to suit the results obtained. It is obvious that in spite of interest taken by public authorities, inspection of factories and general improvement in conditions since Buchan published, there is still a great deal of polluted ice-cream offered for sale. It would be advisable wherever possible to restrict the manufacture of this food to large factories built especially for the purpose and which may easily be controlled. Also it would be an advantage to have ice-cream sent out in wrapped portions or sealed cartons which would obviate the possibility of contamination with blown dust. Produced under these conditions there is no reason why samples should not conform to the graded milk standard suggested by Thierens, but while the food still continues to be made on unsatisfactory premises with no accommodation for steaming or sterilizing the vessels, and while it is sold loose from the refrigerator and afterwards repeatedly refrozen and exposed for sale, one cannot expect to reach this standard. It is perhaps advisable when reporting on the examination of ice-cream at the present time to keep two standards in view. These have been formed after reviewing the results reported in this paper and are designated A and B. In order to satisfy "standard A" the sample must contain no *Bact. coli* in less than 0·1 c.c., the total counts must not exceed 200,000 organisms per c.c. at 22 or at 37° C., enterococcus must be absent in less than 0·01 c.c. and spores of *Cl. welchii* must be absent in 10 c.c. In order to satisfy "standard B" the sample must contain no *coli* in less than 0·01 c.c., the total counts must not exceed 500,000 organisms per c.c. at 22 or at 37° C., enterococcus must be absent in less than 0·001 c.c. and spores of *Cl. welchii* must be absent in less than 10 c.c.

When dealing with several samples from the same factory it sometimes happened that although most samples were satisfactory an occasional sample would be heavily polluted and an increase in the arithmetic mean for the season would result. This is the reason why figures for factories (p. 535) are so much higher than for samples. The possibility of an occasional lapse should be kept in mind when assessing the general cleanliness of a particular factory.

CONCLUSIONS

There appears to be no reason why ice-cream should not be manufactured in a clean and safe manner providing the producer is willing to exercise the utmost care. Ordinary cleanliness is probably not sufficient on the large scale, although it seems to be effective in laboratory manufacture. The producer should therefore aim at biological cleanliness by sterilizing all the vessels and utensils which come into contact with the food. In order to do this the premises should be specially constructed for the purpose of manufacture and be fitted with the necessary steamers, boilers and water supply. The construction of the building should be such that cleaning is easy and there should be no question of making the food on enclosed domestic premises or in sheds imperfectly constructed with mud floors and uneven wood or plaster walls.

Experiment has shown that either of the two heating methods should give satisfactory results, but if cold-mix powder is used the milk should be sterilized.

The food should not be kept longer than 2 days after boiling, as some organisms appear to be capable of growth and reproduction even at low temperature.

Ice-cream powders should be free from bacteria, particularly the spores of *Cl. welchii*.

"Standard A" is the one to aim for in assessing the purity of ice-cream. This means that samples of ice-cream should contain

(i) No coliform organisms in less than 0·1 c.c.

(ii) Total counts of not more than 200,000 organisms per c.c. on Yeastrel milk agar after 3 days' incubation at 22° C. or after 2 days' incubation at 37° C.

(iii) No enterococcus in less than 0·01 c.c.

(iv) No spores of *Cl. welchii* in 10 c.c.

Samples which satisfy this standard may be considered "good".

"Standard B" may be used to designate samples which contain

(i) No coliform organisms in less than 0·01 c.c.

(ii) Total counts of not more than 500,000 organisms per c.c. on Yeastrel milk agar after 3 days' incubation at 22° C. or after 2 days' incubation at 37° C.

(iii) No enterococcus in less than 0·001 c.c.

(iv) No spores of *Cl. welchii* in less than 10 c.c.

Ice-creams which satisfy this standard may be accepted although they can hardly be considered very satisfactory.

Samples may then be reported as follows:

This sample satisfies "standard A" and may be considered good.

This sample satisfies "standard B" but not "standard A".

This sample fails to satisfy "standard B" and is unsatisfactory.

Acknowledgements

I should like to take this opportunity of thanking Prof. H. B. Maitland for his interest and help during the course of the investigation, and I am also greatly indebted to Mr R. C. Locke, M.R.C.V.S., D.V.S.M., and to Inspector E. C. Hemming for arranging several visits to ice-cream factories in Manchester.

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(*MS. received for publication* 11. xi. 37.—Ed.)