

Sampling plans for poultry and poultry products

There has been a steady increase in trade on the world poultry market. In 1980, over 1.4 million tonnes of poultry were exported from 45 poultry-producing countries (FAO, 1981). Imports by the industrialized and non-industrialized countries were nearly equal. Most of the product is frozen; however, there is some trade in fresh poultry between closely situated countries. There is also some trade in heat-processed and dried poultry products.

A. REASONS FOR SAMPLING

Raw poultry products are frequently contaminated by food-borne pathogens (e.g., *Salmonella*, *Staphylococcus aureus*, *Clostridium perfringens*, *Campylobacter fetus* subsp. *jejuni*, and *Yersinia enterocolitica*; ICMSF, 1980, pp. 419–50). Disease surveillance reports frequently identify poultry as vehicles in outbreaks of salmonellosis, staphylococcal food-poisoning, *C. perfringens* enteritis, and other enteric illnesses (Bryan, 1980; Hepner, 1980; Horwitz and Gangarosa, 1976; Todd, 1978; FAO/WHO, 1979). The prevalence of salmonellae in raw fresh and frozen poultry is of great concern and approaches 80% in some countries.

Contaminated chickens and turkeys bring salmonellae into kitchens and give rise to human salmonellosis, either directly or through cross-contamination to other foods (ICMSF, 1980, pp. 838–61; Bryan, 1981). Such outbreaks commonly occur from foods served in food-service establishments, hospitals, and other institutions. Deaths are infrequent, but they do occur in debilitated persons and infants.

Programs for decreasing *Salmonella* infection to low levels in fowl have been known for many decades; these are control of *Salmonella*

in breeding flocks, prompt fumigation of eggs for hatching, rigid sanitary control in hatcheries, and provision of *Salmonella*-free feed. Where practised, these measures can greatly reduce the prevalence of *Salmonella*-contaminated carcasses, leading to low incidence of human infection from poultry. Most countries, however, have few or ineffective programs to combat the *Salmonella* problem, increased cost of poultry products being the most common reason given. An effective, but at present commercially unavailable, treatment could be the irradiation of packaged poultry products (ICMSF, 1980, pp. 455–457).

Spores of *C. perfringens* survive in some heat-processed poultry products. Non-spore-forming pathogens may survive if heat-processing is inadequate, and they may also survive curing, smoking, or drying processes. Poultry products are subject to post-processing contamination from staphylococci when sliced, wrapped, or otherwise handled by workers in processing plants and food-service establishments, and by persons who prepare foods in homes.

B. SAMPLING PLANS

(a) *Fresh and frozen raw poultry*

At the time of preparing the previous edition of this book, it was thought that the sampling plan for *Salmonella* in poultry of case 10, $n = 5$, $c = 0$, $m = 0$ was not feasible; hence the c value was set at 1 (ICMSF, 1978b, pp. 137–139). Even this has proved to be unrealistic under current conditions of intensive poultry production and processing in most countries (FAO/WHO, 1979; ICMSF, 1980, pp. 438–442).

The proportion of salmonellae-contaminated poultry carcasses is determined mainly by the quantity of incoming infected or contaminated live fowl and by subsequent spread during defeathering and other processing (ICMSF, 1980, pp. 410–50). Unfortunately, even good manufacturing practices in a plant do not appreciably affect the situation (FAO/WHO, 1979). Until changes are made to prevent or minimize the infection and contamination of fowl on farms, or until a method of decontamination (e.g., irradiation of packaged carcasses or treatment with an acceptable chemical) is routinely applied, it is questionable whether practicable port-of-entry microbiological criteria can be set for *Salmonella* on frozen raw poultry without risk of eliminating poultry as a food. Educational programs are needed to maintain food safety in kitchens. Training must emphasize thorough cooking, prevention of cross-contamination, rapid cooling of cooked products that are not

to be eaten immediately, and thorough reheating, particularly for poultry being prepared in medical care and geriatric institutions.

Investigational sampling plans (see Chapter 5, Section N) may be used to (1) determine the extent of contamination of a feed supply or a product with salmonellae; (2) identify or monitor critical control points of hatchery, farm, or processing operations; (3) trace a source of contamination; or (4) evaluate effectiveness of control measures. Such data may help to convince producers and others that a serious problem exists and that steps should be taken towards the elimination of *Salmonella* in live fowl, thereby controlling the problem at source. Initially, a lenient sampling plan for *Salmonella* will be needed (e.g., $n = 5$, $c = 3$, $m = 0$); but as control measures (such as the use of *Salmonella*-free chicks and feeds) are initiated and maintained, the plan should be made more stringent by reducing c .

Although *Campylobacter fetus* subsp. *jejuni* and *Y. enterocolitica* are frequent contaminants of raw poultry products (deBoer et al., 1982; Leistner et al., 1975; Norberg, 1981; Simmons and Gibbs, 1979), and poultry-associated outbreaks of campylobacteriosis have been reported (Cunningham, 1982), criteria are not considered necessary. Thorough cooking, care to avoid cross-contamination, and proper storage of cooked products will minimize the risks associated with these bacteria.

Clostridium perfringens is a part of the normal intestinal flora of poultry and is shed in their faeces, and is widespread in their environment. Its presence on poultry carcasses cannot be avoided, but it will not multiply on chilled carcasses. While cooking destroys vegetative cells of *C. perfringens*, spores survive and may even be activated. Also the redox potential of carcasses is lowered by cooking. Temperature abuse of cooked poultry can promote spore germination and multiplication of the resulting vegetative cells. A criterion for *C. perfringens* in or on raw poultry for port-of-entry sampling is not recommended. Prevention of illness must therefore be based on time-temperature control after cooking.

Staphylococcus aureus is a part of the normal flora of poultry, and even adherence to good processing practices cannot totally prevent carcass contamination. However, staphylococci do not compete well with normal spoilage flora of poultry and are not likely to multiply on chilled carcasses. They become a problem only if cooked poultry is contaminated and subsequently subjected to time-temperature abuse. A criterion for *Staph. aureus* in raw poultry is not recommended for port-of-entry sampling. Poultry eaten shortly after cooking or not handled after cooking and rapidly cooled is not associated with staphylococcal food-poisoning.

A sampling plan for *Staph. aureus* is useful to evaluate carcass contamination from picking (plucking) machines in which staphylococci can accumulate particularly and contaminate carcasses passing through. A sampling plan of case 4 (staphylococci as an indicator of a source of contamination), $n = 5$, $c = 3$, $m = 10^3$, $M = 10^4$ per cm^2 of swabbed surface might be initially considered for in-plant quality control.

It is also considered unnecessary to have microbiological criteria for other indicator organisms (Enterobacteriaceae, coliforms, faecal coliforms, *Escherichia coli*, and enterococci) because they are part of the normal intestinal flora of poultry. Some will be spread among carcasses even under good processing practices (FAO/WHO, 1979). Some strains of species that fall into the indicator organisms categories (such as certain coliforms and *Enterobacter*) are psychrotrophic and can multiply on refrigerated raw poultry carcasses and products.

Microorganisms responsible for spoilage of poultry multiply during refrigeration, even at recommended temperatures of 1 to 4°C (ICMSF, 1980, p. 443). A commonly recommended microbiological method for estimating the shelf-life of chilled poultry, an aerobic plate count (APC) at 0 to 5°C, is impracticable for use at port-of-entry because it requires incubation for 10 to 14 days.

In the previous edition of this book, a criterion for APC (20°C) of case 1, $n = 5$, $c = 3$, $m = 5 \times 10^5$, $M = 10^7$, was suggested (ICMSF, 1978b, p. 138). This criterion is achievable for processed chickens, but not necessarily turkeys, in modern processing plants that follow good processing practices (CEC, 1979) and is a useful guideline. It is not recommended, however, as a port-of-entry microbiological criterion or as a basis for estimating shelf-life.

Irradiation could eliminate *Salmonella* from processed raw and frozen poultry (ICMSF, 1980, p. 455). A criterion of case 10, $n = 5$, $c = 0$, $m = 0$, would be applicable to *Salmonella* in raw or frozen irradiated poultry. It is premature to propose other microbiological criteria for irradiated poultry before these products enter international trade, and until additional microbiological data and experience become available.

(b) *Frozen, heat-processed poultry products*

There is increasing international trade in heat-processed poultry products (i.e., turkey rolls and entrée items such as fried chicken, chicken à la king, and pot pies). (See Chapter 20, Cereals and Cereal Products, for suggested microbiological criteria and further discussion of pot pies

TABLE 22
Sampling plans and recommended microbiological limits for poultry and poultry products

Product	Test	Method reference ^a	Case	Plan class	n	c	Limit per gram	
							m	M
Cooked poultry meat, frozen; to be reheated before eating (e.g., prepared frozen meals)	<i>Staph. aureus</i> ^b	218	8	3	5	1	10 ³	10 ⁴
	<i>Salmonella</i>	163	10	2	5	0	0	
Cooked poultry meat, frozen, ready-to-eat (e.g., turkey rolls)	<i>Staph. aureus</i> ^b	218	8 ^c	3	5	1	10 ³	10 ⁴
			9 ^c	3	10	1	10 ³	10 ⁴
	<i>Salmonella</i>	163	11	2	10	0	0	
Cured and/or smoked poultry meat	<i>Staph. aureus</i> ^b	218	9	3	10	1	10 ³	10 ⁴
	<i>Salmonella</i>	163	11	2	10	0	0	
Dehydrated poultry products	<i>Salmonella</i>	163	11	2	10	0	0	
Raw chicken (fresh or frozen), during processing	APC	118 (20°C)	1	3	5	3	5 × 10 ⁵	10 ^{7d}

^a Refers to page numbers in ICMSF 1978a, where the methods are described. Use analytical unit sizes recommended in the methods, except where otherwise indicated.

^b If either packaged or repackaged after processing; not for products processed in packages that are kept closed until time of final preparation.

^c Case depends on whether (case 9) or not (case 8) subsequent time-temperature abuse of the products is likely.

^d **Not for port-of-entry sampling**; for use to evaluate production and operations and critical control points in processing plants only.

and other blended products with pastry shells or covers.) Presence of *Salmonella* may be due to failure of heat processes or cross-contamination from the raw to the cooked product (ICMSF, 1980, pp. 850–859). A microbiological criterion for *Salmonella* in heat-processed poultry products is given in Table 22.

Staphylococci and faecal indicators are useful monitors of contamination of frozen cooked poultry products. Such monitoring is unnecessary when products are adequately cooked in plastic casings or bags, but it is essential when these products are handled, cut, repackaged after heat-processing, or otherwise subjected to any operation that may permit contamination. Although the data base is limited, microbiological criteria for *Staph. aureus* are recommended. Case 8 applies, because enterotoxins of *Staph. aureus* withstand the time-temperature exposure of reheating. If there is likelihood of time-temperature abuse, case 9 would apply (see Table 22). Staphylococci survive freezing better than the Gram-negative organisms (such as *E. coli*) and can be a useful indicator of post-heat-process contamination.

(c) *Smoked, cured, and dried poultry products*

Although there is limited international trade in smoked, cured, and dried poultry products other than dried eggs, and the need for microbiological criteria has not clearly been established, these products should be free from *Salmonella*. The presence of *Salmonella* constitutes a health hazard and case 11 applies (Table 22). Dried products are shelf-stable, as long as packages are intact, until rehydrated.

Monitoring for APC, *E. coli*, and *Staph. aureus* would give some indication of inadequate processing (heating, curing, smoking), post-process contamination, or storage at temperatures above those recommended for frozen or refrigerated distribution and storage. These products are likely to become contaminated during slicing or wrapping. The a_w level may inhibit common spoilage organisms but not necessarily *Staph. aureus*.

Because only limited microbiological data on these commodities are available, criteria for APC or *E. coli* are not recommended. However, a criterion is suggested for *Staph. aureus* on smoked or cured poultry products (Table 22). Case 9 is applicable because these products are frequently distributed or stored at temperatures conducive to multiplication of staphylococci.

C. SAMPLING PROCEDURES

Take 5 or 10 sample units, as appropriate, from each lot. Sample units of fresh or frozen raw poultry should be taken from more than one carton where possible. Sterile disposable plastic gloves are useful for holding carcasses. Use sterile instruments to cut portions, if necessary, and to collect sample units (usually 200 g) of comminuted, cooked, dehydrated, smoked, or cured poultry, and aseptically transfer them to sterile containers. (See Chapter 9, Collecting and Handling Sample and Analytical Units, for more information on aseptic sampling, identifying samples, and transporting samples to the laboratory.)

Use the shake and rinse procedure for sampling fresh or thawed raw poultry carcasses to be tested for *Salmonella*. In this procedure, the carcass is placed aseptically into a heavy-duty, plastic bag (which has not been previously used) containing 300 ml buffered peptone water. The bag is closed, and the enclosed carcass shaken vigorously, vertically and horizontally, for 30 seconds. The entire surface and cavity of the carcass should come into contact with the water during the shaking. Pour the rinse into a sterile container and keep it at 4°C until it is used in the laboratory. If raw turkey carcasses are to be tested, use 500 ml buffered peptone water. This procedure is difficult to use for large turkeys because they are heavy and it is difficult to shake them effectively. Swabbing is a suitable alternative method (see below).

The shake and rinse samples are not usually practicable for 'on-the-line' sampling. In such situations, rub a swab (which has been moistened in sterile peptone water) over the skin and, if practicable, internal regions of the carcass. Include both neck and vent in the swabbed areas. Alternatively aseptically cut portions of neck skin and take interior scrapings. When these procedures are used, however, the likelihood of detecting salmonellae may be less than with the shake and rinse procedure.

The recommended procedure for sampling raw carcasses for APC is to collect samples of neck skin. Use sterile instruments to grasp the carcass and to cut sample units from carcasses and aseptically put them into a sterile container.

D. TEST PROCEDURES

Carcass-rinse and swab sample units become the analytical units for the *Salmonella* or other test. Thaw ('drip') water from raw poultry can

also be used as analytical units. Rinse and thaw waters can also be used for tests for other organisms. Incubate each as prescribed in ICMSF, 1978a, p. 163. When testing skin sample units for *Salmonella*, put cut portions of skin into pre-enrichment broth according to procedures in ICMSF, 1978a, p. 164. For APC, weigh 20–50 g of neck skins and homogenize this analytical unit with 0.1% peptone saline (ratio 1:9) in a stomacher or blender.

Sample units from frozen poultry should be thawed at 1 to 5°C, preferably within 18 hours. Commminute and blend a sample unit, and from this composite weigh out analytical units for the APC, *Staph. aureus*, and other counts and for the *Salmonella* test according to directions given in ICMSF, 1978a.

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Sampling plans for milk and milk products

For purposes of applying microbiological criteria, dairy products may be divided into two broad groups: (a) the more perishable ('fresh') products such as milk, cream, flavoured milk and skim milk drinks, fresh cheese (cottage cheese), and fermented milks; and (b) the relatively stable products having extended shelf-life under appropriate conditions of storage, such as hard cheese, butter, dried milk products, ice cream mixes, evaporated (canned) milk, and sterilized or ultra-high-temperature (UHT) milk (for fluid consumption).

A. RELATIVELY PERISHABLE PRODUCTS

It is impracticable to apply end-product microbiological criteria to detect defective products in this category, which will have been widely distributed and probably consumed before microbiological examinations have been completed. However, periodic analyses (see below) are commonly made by local authorities to decide whether or not to continue to accept a product. Judgment is based on the performance record of the producer or processor over a period of time, such as the 3 out of 5 rate of compliance with the standard as specified in the U.S. *Grade A Pasteurized Milk Ordinance* (USPHS, FDA, 1978).

Health problems to be anticipated from pasteurized fluid milk and related products are likely to be of local concern, and control is normally in the hands of public health authorities. Pasteurized fluid milk rarely crosses frontiers, so agreements between exporting and importing countries appear as the best solution to the problem of control. Therefore, microbiological criteria are not proposed for pasteurized milk in international trade. The report of the joint FAO/WHO Expert Committee on Milk Hygiene (FAO/WHO, 1970) and ICMSF (1980b, pp. 482–486)

provide useful information on milk control practices and disease hazards. Hygienic codes and methods of sampling and analysis are described in publications of the International Dairy Federation (IDF, 1973, 1980b, 1980c) and the American Public Health Association (APHA, 1978), and in the Grade A Pasteurized Milk Ordinance (USPHS, FDA, 1978).

The microbiological tests most often used are the aerobic plate count (APC) and the coliform test. In addition, the phosphatase test is used world-wide. If this enzyme is detected in allegedly pasteurized milk, it is almost certain either that the milk was underpasteurized or that raw milk had somehow entered the product.

B. RELATIVELY STABLE PRODUCTS

Milk products of this group are usually subject to test before being distributed and consumed. Those that are currently common in international trade and are of concern because of microbiological hazards they may present are dried milk and ripened cheese. While others may be important in international trade, either they are unlikely to present a microbiological health hazard or their safety and quality are not amenable to measurement by end-product microbiological criteria. For example, butter is an important commodity in international trade, but modern methods of manufacture ensure a relatively long shelf-life under normal commercial practice, and only rarely has illness been attributed to butter (see ICMSF, 1980b, pp. 775–776). The safety of UHT milk, as of other low-acid canned foods (including evaporated milk), depends primarily upon ensuring that the heat-processing is adequate and that container integrity is maintained. Routine sampling and testing of end-product will not provide adequate assurance of safety (see Chapter 10 and ICMSF, 1980a, pp. 32–36). However, it is recommended that industry monitor microbiologically, wherever applicable, critical control points in processing operations. To some extent ice-cream mixes occur in international trade. Ice cream itself is generally subject to control by local authorities. If such controls do not exist it is recommended that the WHO proposals (Christian, 1983, pp. 14–16) be followed for monitoring purposes.

(a) *Dried Milk*

Several types and grades of dried milk occur in commerce. Few outbreaks of food-borne disease due to dried milk have been reported in

recent years. This fact undoubtedly reflects the effectiveness of (a) the extensive *Salmonella* testing of dried milk and subsequent action to prevent distribution of contaminated product and (b) the critical control measures now commonly practised in the industry (see ICMSF, 1980b, pp. 492–494; and Chapter 7, Control at Source) introduced following the outbreaks of staphylococcal food-poisoning and salmonellosis that spanned the years 1953–56 and 1964–65, respectively (Anderson and Stone, 1955; Armijo et al., 1957; Collins et al., 1968). Nevertheless, considering its intended use, dried milk is a sensitive product and may present serious health hazards if not manufactured and used according to good hygienic practices.

The various cases for concern are given in Tables 6 and 10 (pp. 43 and 74). The m and M values are given in Table 24. Case 2 for APC and case 5 refer to indicator measurements and are recommended in association with 3-class plans (see Table 10). Coliform organisms in dried milk may die out during storage. They should, nevertheless, be absent or present only in low numbers in dried milk, even when the product is freshly made. Thus, control of coliforms is necessary but their absence is not sufficient to qualify a product as acceptable. Ideally the m value for coliforms should be low (c. 1 per g) and, indeed, an m value of <3 was suggested in the previous edition of this book. Since the current method (3-tube MPN) is imprecise in estimating such a low number, a different value is indicated. It was desired to change this previous plan to one having essentially the same consumer's and producer's risks but using a higher m value. The adjustment can best be achieved by decreasing c from 2 to 1 but increasing m to 10. n remains at 5 and M at 100 (see Table 24). It is anticipated that this case 5 sampling plan for coliforms in dried milk will provide essentially the same assurance as the plan previously proposed ($n = 5$, $c = 2$, $m = <3$, $M = 100$).

Enterotoxigenic *Staphylococcus aureus* is a potential hazard in dried milk and should be absent or be present only in very low numbers. The hazard of enterotoxin being present in the dried product is likely only when deviations from Good Manufacturing Practice (GMP) provide opportunities for sufficient growth of the organism either in the milk before condensing or in the condensed milk prior to drying. The likelihood that conditions would permit such extensive growth is remote in modern dry-milk manufacture because of industry's awareness of the problem and of the measures taken to prevent it. There are, however, situations where the product might become contaminated after drying. Thus, if reconstituted milk is exposed to times and temperatures

TABLE 24

Sampling plans and recommended microbiological limits for dried milk and cheese

Product	Test ^d	Method reference ^a	Case	Plan	n	c	Limit per gram	
							m	M
Dried milk	APC	118	2	3	5	2	3×10^4	3×10^5
	Coliforms	126–131	5	3	5	1	10	10^2
	<i>Salmonella</i> , ^e normal routine	163	10	2	5	0	0	
			11	2	10	0	0	
			12	2	20	0	0	
	<i>Salmonella</i> , ^e for high-risk population	163	10	2	15	0	0	–
			11	2	30	0	0	–
			12	2	60	0	0	–
	Cheese, 'hard' and 'semi-soft' types ^{b, c}	<i>Staph. aureus</i> 220	8	2 ^f	5	0	10^4	–

^a Refers to page number in ICMSF, 1978, where the method is described. Use sample unit sizes recommended in the methods, except where otherwise indicated.

^b In addition, such cheese should be aged for 60 days at not less than 4.4°C (40°F) when made from unpasteurized or unheated milk or curd (see Section B(b) 1).

^c See also discussion on thermonuclease, Section B(b) 3.

^d APC, see also IDF, 1970; coliforms, see also IDF, 1971; *Salmonella*, see also ISO, 1981.

^e The case for *Salmonella* is to be chosen in accordance with whether the expected use of the product will reduce, cause no change in, or increase concern (see Table 10, p. 74)

^f See Section B(b) 3 for discussion of sampling plan.

sufficient to permit growth of staphylococci – for example, temperature abuse during mass feeding operations in hospitals, schools, institutions, or in relief or emergency conditions – the hazard of enterotoxin could exist. For discussion of sampling plans for products used in this manner, see Chapter 24, Formulated Foods.

The presence of *Salmonella* in dried milk is a serious hazard. While current GMPs are effective in excluding *Salmonella* from dried milk, contaminated lots are occasionally found (see ICMSF, 1980b, p. 494). End-product testing for *Salmonella* is widely practised and is an important factor in identifying contaminated lots and in preventing them from

entering trade. For routine sampling, in accordance with expected use of the product cases 10, 11, and 12 would apply (see Table 10, p. 74). Where the intended use is not known case 12 also would apply. More stringent sampling plans for cases 10, 11, and 12 are indicated when the product is intended for consumer groups of increased susceptibility, such as infants, the aged, and infirm (see Table 8, p. 55; Chapter 6, Section B; and Table 12, p. 78). The 2-class plans which are recommended are given in Table 24.

Recently, the Codex Committee on Food Hygiene proposed end-product specifications to be included in a Code of Hygienic Practice for Dried Milk (Codex 1981, 1983). Their proposed criteria are intended to apply only when the product is used for other than high-risk populations and are similar to those shown in Table 24 except that only one specification for *Salmonella* ($n = 15$, $c = 0$, $m = 0$) is recommended regardless of product use.

Microbiological guidelines were not recommended for inclusion in the Code of Hygienic Practice for Dried Milk. It was concluded that guidelines should be formulated by manufacturers taking into account the need to meet limits prescribed in end-product criteria. ICMSF believes this conclusion was prudent. It leaves the mechanism of compliance with the code flexible and at the option of producers or manufacturers.

Foods prepared especially for infants and children may consist wholly or in part of dried milks. These products are included in the Codex 'Code of Hygienic Practices for Foods for Infants and Children' (Codex, 1979) and are considered in Chapter 24 (Formulated Foods).

(b) Cheese

Throughout the world diverse procedures are used to produce various types of cheese from milk, which may be raw, pasteurized (phosphatase-negative), or heat-treated. For many types of cheese data on their bacteriological quality, from the hygienic point of view, are few and often have not been related to GMP. More data are necessary before meaningful bacteriological limits can be recommended for every cheese variety. Nevertheless, for some cheeses common in international trade, present knowledge and experience are sufficient to warrant proposals for microbiological criteria.

Although cheese has been the vehicle in outbreaks of food-borne illness, epidemiological evidence places it among the relatively infrequent vehicles of transmission.

1. Aged cheese made from raw milk

Many varieties of cheese are made from milk which has not been heated before the cheese-making process begins. Certain types, however, require heat-treatment of the curd at some point during the cheese-making process, such as during cooking.

Several pathogenic bacteria, including *Salmonella*, *Campylobacter*, and *Listeria*, can survive in cheeses made from raw milk. Some protection against the transmission of infectious disease, especially brucellosis, is achieved by requiring cheese made from other than pasteurized milk to be aged for at least 60 days at a temperature of not less than 4.4°C.

2. Aged cheese made from milk heated during the cheese-making process

Some types of cheese (e.g., Gruyere, Emmental, and Grana) are made from raw milk, but the curd undergoes a heat-treatment at approximately 53–55°C for about 1 hour during the cheese-making process. These heating processes reduce the bacteriological risk in such cheese.

3. Aged cheese made from pasteurized or 'heat-treated' milk

Cheese is made also from pasteurized milk (phosphatase-negative) or milk heated to a degree which may not provide a negative phosphatase test but does substantially reduce the number of microorganisms. The latter process is sometimes referred to as 'heat-treating' or 'thermization' of milk; the heat-treatment approximates 65.5°C for 15 to 20 seconds. Such heat-treatments destroy or greatly reduce the number of heat-labile organisms, including staphylococci and other pathogens (Zottola and Jezeski, 1969; Zottola et al., 1969). The presence of excessive numbers of such organisms in the final product results either from contamination of the milk after heating and subsequent growth or from some faulty practice or accident during the cheese-making process (e.g., starter failure).

The most serious health risk presented by cheese shipped in international trade is from staphylococcal enterotoxin. Available information about staphylococci in cheese and factors influencing their growth and production of enterotoxins is limited primarily to hard (Cheddar and similar types, and Swiss) and semi-soft (Roquefort, Blue, Gouda, Brick, and Mozzarella) varieties (ICMSF, 1980b, pp. 508–510; Devoyod et al., 1968, 1969). The maximum growth of staphylococci occurs when whey drainage is complete and generally prior to salting or brining. Later, during the ageing period, they die slowly and only

a relatively small proportion of the earlier numbers will be recovered when cultured. However, any enterotoxins produced will remain.

Growth of enterotoxigenic staphylococci to c. 1–5 million per gram of cheese during cheese-making is necessary to produce amounts of enterotoxin detectable by current assay methods (Tatini et al., 1971a, 1971b, 1973). Heat-treatments less severe than those indicated above (65.5°C for 15 to 20 seconds), when applied to milk before or after the cheese-making process begins, may leave a residual population of coagulase-positive staphylococci. In addition, insanitary equipment may recontaminate the milk after heat-treatment. However, the most important factor permitting the growth of staphylococci, and of many other undesirable bacteria, during cheese-making is failure of the lactic acid starter culture to develop normally ('starter failure'). This usually results from infection of the starter culture by bacteriophage, or from the presence of small amounts of antibiotics or other inhibitory substances in the milk. Staphylococci found in ripened cheese probably represent only a fraction of the population present in the unripened cheese. Thus, the reliability of criteria for staphylococci in ripened cheese is limited, and this fact must be taken into account when test results are interpreted.

The varieties of cheese that have been associated with outbreaks of staphylococcal food-poisoning are Cheddar and similar types; recently Swiss-type cheese was involved in a Canadian outbreak (Todd et al., 1981). Outbreaks have been infrequent since 1965, perhaps because of a better understanding of the behaviour of *Staph. aureus* in milk before and during cheese-making, which has led to the application of effective preventive measures (ICMSF, 1980b, pp. 510–513).

A viable count of 10,000 *Staph. aureus* per gram of cheese appears to be well within technological achievement, and numbers below that level in ripened cheese in international trade are likely to indicate that numbers had not reached a level associated with detectable toxin (see above comment on reliability). The sampling plan indicated for case 8 would seem to apply (see Table 10, p. 74). However, because of the different rates of die-off of staphylococci in different cheese varieties during ripening, specifying an *m* value in a 3-class plan, which would assure GMP, is not possible. Accordingly, a 2-class plan is recommended for case 8 as shown in Table 24.

The thermonuclease (TNase) test for assessment of staphylococcal growth and possible presence of enterotoxins in cheese is recommended. The utility of this test has been shown by many authors (Cords and Tatini, 1973; Tatini et al., 1975, 1976; Park et al., 1978; van

Schouwenburg-van Foeken et al., 1978; Stadhouders et al., 1980; Todd et al., 1981; Ibrahim and Baldock, 1981). In some instances, however, cheese containing enterotoxin did not contain detectable TNase (Todd et al., 1981), which prompted concern whether cheese suspected of containing enterotoxins, or of having contained large numbers of *Staph. aureus*, should be released for consumption if TNase is not detected. However, Ibrahim and Baldock (1981) and van Schouwenburg-van Foeken et al. (1978) always detected TNase in extracts of cheese containing enterotoxin. Further, Ibrahim and Baldock (1981) detected TNase in cheese whenever the count of *Staph. aureus* reached c. 10^6 per gram.

Although the reasons for the above differences are not clear, the TNase assay used by Ibrahim and Baldock (1981) was more sensitive than that used by others (perhaps as much as 30 times greater than the procedure used by Todd et al., 1981). Differences in sensitivity of enterotoxin assay procedures used and other factors may also contribute. Nevertheless Ibrahim and Baldock (1981) concluded that TNase-positive cheese should be considered unfit for further processing for human consumption unless proved to be free from staphylococcal enterotoxins, and that TNase-negative cheese containing *Staph. aureus* may be processed for human consumption. Similarly, van Schouwenburg-van Foeken et al. (1978) previously concluded that the presence of enterotoxin is unlikely in cheese negative for TNase and that it can be declared safe for consumption. However, cheese positive for TNase may not always contain detectable enterotoxin. Therefore, TNase-positive cheeses must always be subjected to enterotoxin assay before they are declared unsafe for consumption. For further information on staphylococci in cheese and measures for their control, reference to the following should be helpful: IDF, 1973, 1980a; NCI, 1980.

During the latter part of 1971 and early 1972 numerous outbreaks of gastroenteritis occurred in the United States caused by imported soft cheese which contained pathogenic strains of *Escherichia coli* (enteropathogenic, enteroinvasive, or enterotoxigenic) (Marier et al., 1973). A few earlier outbreaks in other countries had been reported (Costin et al., 1964; Gaines et al., 1964). While the coliform problem in cheese is well known, presence of these organisms in many cheese varieties is extremely difficult to prevent completely. With some varieties, if coliforms are present initially, it is virtually impossible to prevent their growth during manufacture or during the ripening period. In several types of cheese *E. coli* can even be considered characteristic. With the

exception of some strains of *E. coli* high populations of coliforms are unlikely to present a health hazard. There is ample evidence that if pathogenic strains of *E. coli* (PEC) are present early in the cheese-making process their numbers may increase to hazardous levels (Mourgues et al., 1977; Frank et al., 1977, 1978; Park et al., 1978). However, in view of the scarcity of evidence of recurring outbreaks due to PEC in cheese, and the high cost of routine testing, it is doubtful that establishment of end-product criteria for either coliforms or *E. coli* would be justified. Accordingly, no sampling plan is proposed.

Nevertheless, every effort should be made to adhere to manufacturing practices that will prevent coliform, especially *E. coli*, contamination and subsequent growth (see ICMSF, 1980b, pp. 507, 510, 511). Toward this end the direct plate method (DPM) of Anderson and Baird-Parker (1975) for the enumeration of *E. coli* and as modified by Holbrook et al. (1980) to include a resuscitation step should be useful. With this method results are obtained in 24 hours rather than the 4 days by traditional procedures. Thus, it would be feasible to examine on a routine basis soft and semi-soft cheese for *E. coli* should subsequent developments deem it necessary. Certainly, the application of guidelines for *E. coli* at critical control points of cheese manufacture is advisable, and industry should be encouraged to establish and use them as part of an internal microbiological safety and quality assurance system.

C. SAMPLING PROCEDURES

For general instructions on collecting and handling field samples, see Chapter 10. For specific procedures on sampling milk and milk products consult the IDF Standard 50A proposed by the International Dairy Federation (IDF, 1980c). IDF procedures not included in the general procedures in Chapter 10 are outlined below, but it is recommended that the full IDF text be studied by all persons who collect samples of dairy foods.

(a) *Dried milk*

- 1 Where feasible, take original unopened packages.
- 2 When sampling bulk containers in the field or packages in the laboratory, use the precautions described in Chapter 9, Section B (c), when opening the containers. Take the sample from a point near the

centre of the container if possible. First remove the surface layer of powder with a sterile instrument (e.g., a broad-bladed knife) and then draw the sample with a sterile spoon or probe.

3 In the event of dispute concerning the bacteriological condition of the surface powder in a package, take a sample of the product from this area.

4 Preferably use brown glass sample containers, to exclude light.

(b) *Cheese*

1 Use one of the following three techniques, depending on the shape, weight, and type of cheese: (a) sampling by cutting out a sector; (b) sampling by means of a trier; (c) taking a complete cheese as a sample unit. When a choice must be made between (a) and (b), method (b) is often more practicable, especially with hard cheese of large size.

(i) *Sampling by cutting out a sector*. Using a knife with a pointed blade, make two cuts radiating from the centre of the cheese. The size of the sector thus obtained should be such that after removal of any inedible surface layer, the remaining edible portion is more than twice as much as will be required for analysis. Use this method for Edam and Gouda cheese. It can also be used for semi-hard and soft cheese, and in general for all cheese when sampling with a trier is impracticable.

(ii) *Sampling by means of a trier*. The trier may be inserted obliquely towards the centre of the cheese once or several times into one of the flat surfaces at a point not less than 10 to 20 cm from the edge. From the plug or plugs thus obtained cut off not less than 2 cm of the extremity containing the rind and use this piece to close the hole made in the cheese. The remainder of the plug or plugs constitutes the sample unit.

To prevent avoidable loss of product close the plug holes with great care, especially with a large cheese, and if possible seal over with an approved compound. This method is most suitable for hard and semi-hard cheese, such as Emmental and Cheddar.

The trier may be inserted perpendicularly into one face and passed through the centre of the cheese to reach the opposite face. This method is suitable for Provolone, Caciocavallo, and similar varieties.

The trier may be inserted horizontally into the vertical face of the cheese, midway between the two plane faces, towards the centre of the cheese. This method is suitable for cheese such as Tilsit, Cantal, Roquefort, Pecorino, and Romano.

In the case of cheese transported in barrels, boxes, or other bulk

containers, or which is formed into large compact blocks, sampling may be performed by inserting a 10 to 15 inch trier obliquely through the contents of the container from the top toward the base. This method is suitable for processed cheese and cheese foods.

(iii) *Sampling by taking an entire or substantial portion of cheese.* Use this method for fresh cheese (for example, cottage, cream, or double cream cheese), for soft cheese of small size, and for wrapped portions of cheese packaged in small containers (e.g., some processed cheese and various soft cheeses).

2 Immediately after sampling, place each sample unit (plug, sector, entire small cheese) in a sterile container of suitable size and shape, and seal. The sample unit may be cut into pieces for insertion into the container, but do not compress or grind it.

3 Send or transport the sample units immediately to the laboratory, and conduct the analysis as soon as possible, preferably on the same day. If either dispatch or analysis must be delayed, place the containers in a refrigerator at a temperature of 5 to 8°C. This is especially important with perishable cheese, such as soft cheese.

Precaution. If bacteriological, chemical, and/or organoleptic analyses are to be made on the same sample unit, always remove the analytical units for the bacteriological tests first.

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Sampling plans for eggs and egg products

Eggs and egg products are important commodities in international trade. Exports of shell eggs exceed 20 million boxes per annum, and exports of egg products approach 100,000 tonnes. Of the latter, 85–90% is in liquid or frozen form and of this some 60% is whole egg (IEC, 1980). For the purposes of this chapter, eggs and egg products are subdivided into (1) shell eggs and (2) liquid, frozen, and dried eggs. Mention is also made of foods containing eggs.

The introduction to the subject in ICMSF, 1980, p. 521, describes the properties of the whole egg in the shell and the methods of preservation for the three categories of egg products. The whole egg in the shell is self-protective unless abused by excessive exposure to contamination at the laying stage and gross temperature and humidity changes during storage. Immediately after the shell is broken, by whatever means, the liquid egg is exposed to contamination from hands, equipment, and the external surface of the shell itself.

The most important pathogen likely to be present in liquid, frozen, and dried eggs is *Salmonella*. This organism may occasionally be present within the egg at the time of laying, but much more commonly it contaminates the liquid egg from the external surface of the shell during breaking of the shell. There are then the dangers of (a) eating the contaminated raw product, (b) cross-contamination from raw or unpasteurized egg products to foods prepared in the bakery and confectionery trades or in food-service kitchens, (c) direct contamination when an unpasteurized dried-egg product is incorporated into blended foods, and (d) inadequate heating of egg products (e.g., those used for infant feeding) before consumption.

Mandatory pasteurization of liquid egg should eliminate these hazards. However, any salmonellae surviving pasteurization or contami-

nating the product subsequently may be expected to survive freezing or drying. Thus, salmonellae remain a potential hazard in egg products, because of the opportunities for multiplication that may arise from time/temperature abuse after thawing or rehydration.

Manufactured foods containing egg, such as certain pastas, puddings, and frostings, may also constitute a *Salmonella* hazard. Although prepared with egg of acceptable bacteriological quality, a poorly controlled process may permit substantial multiplication of contaminating salmonellae before the a_w falls below the level limiting their multiplication.

Pasta may be a vehicle for the growth of staphylococci and the formation of enterotoxin. Growth may occur during mixing if wet spots develop and are allowed to persist in the mixer, resulting in detectable levels of enterotoxin in commercial products.

A. SAMPLING PLANS

(a) *Shell eggs*

Sampling plans are not proposed for shell eggs.

(b) *Liquid, frozen, and dried eggs*

The cases for the sampling plans are discussed in ICMSF, 1980, with respect to liquid and frozen eggs (p. 560) and dried eggs (p. 565).

The aerobic plate count (APC) may be regarded as an indicator of the quality of the raw material before processing and also of the possibility of contamination after processing; it may be usefully applied at port-of-entry, manufacturing, and retail levels. In the criteria proposed, the APC is omitted for dried egg albumen because (a) there is always extensive growth of Group D streptococci during desugaring, even when the enzymic method is used; these bacteria are more heat-resistant than organisms of concern such as *Salmonella* and will initiate growth at the pH of egg albumen (c. 9.3); and (b) the pasteurization process that can be applied to liquid albumen must be less severe than that used for liquid whole egg and yolk.

The criteria for APC listed in Table 25 differ slightly from those in ICMSF, 1978, pp. 111–115, 120. A major report (FAO/WHO, 1975), which included a survey of national criteria for egg products, concluded that there was no justification for setting different plans and limits for

dried and frozen egg and that the ICMSF (1978) m value of 10^4 was too strict. The APC criteria in Table 25 conform with the recommendation of the above report, which is now part of the Code of Hygienic Practice for Egg Products (Codex, 1978), and with the best estimate based on the data available.

The use of coliform counts is intended for evidence of (a) insufficient heat-treatment or (b) post-pasteurization contamination. This test is more applicable at the point of manufacture than at port of entry, particularly when there is no record of manufacturing procedures. In ICMSF, 1978, coliform tests were included in criteria for dried (p. 111), but not for frozen (p. 120), egg products. They have been included in Table 25 for both products at the level suggested previously for dried egg, as they serve the same purpose in both types of product. This is consistent with Codex, 1978.

The *Salmonella* criteria suggested in ICMSF, 1978, were stricter for frozen egg whites (case 11) (p. 120) than for frozen whole egg (case 10) (p. 120) because the former are often not adequately cooked to destroy *Salmonella*. However, all of the egg products considered in this chapter may have a range of food-ingredient applications that will influence the degree of concern in different ways. Thus the case chosen should be carefully considered in relation to the use intended for each product. For normal routine sampling for *Salmonella* in accordance with intended use, case 10, 11, or 12 would apply (see Table 10, p. 74). The age and condition of the intended consumer should also be considered. The very young, the elderly, and the infirm are more susceptible to infection so more stringent sampling plans should be applied to egg products destined for such customers (see Table 12, p. 78).

The criteria suggested in Table 25 taking the *Salmonella* sampling plan as case 11 are consistent with those of the Codex Alimentarius Commission (Codex, 1978).

Occasionally small numbers of staphylococci may be found in salted yolks, presumably because of the selective nature of the high-salt menstruum. However, no criterion is suggested for staphylococci in this product because there is no evidence of hazard. Few countries include staphylococcal counts in their criteria for egg products (FAO/WHO, 1975).

Physical measurements of efficacy of pasteurization, such as the destruction of α -amylase in whole-egg mélange, are practised with good effect in some countries and in particular in the United Kingdom.

TABLE 25

Sampling plans and recommended microbiological limits for pasteurized liquid, frozen, and dried egg products

Test ^a	Method reference ^b	Case	Plan class	n	c	Limit per gram	
						m	M
APC ^c	118	2	3	5	2	5×10^4	10^6
Coliforms	126-131	5	3	5	2	10^1	10^3
<i>Salmonella</i> , ^d normal routine	163	10	2	5	0	0	—
		11	2	10	0	0	—
		12	2	20	0	0	—
<i>Salmonella</i> , ^d for high-risk population ^e	163	10	2	15	0	0	—
		11	2	30	0	0	—
		12	2	60	0	0	—

NOTE: SAMPLING PLANS AND RECOMMENDED LIMITS FOR FOODS CONTAINING EGG:

Sampling plans for indicator organisms need to take account of microbial contributions of non-egg ingredients. Where salmonellae are considered a hazard, the above criteria for salmonellae are appropriate.

a APC, see also ISO 4833 (1978c); coliforms, see also ISO 4831, 4832 (1978a, b); *Salmonella*, see also ISO 6579 (1981).

b Refers to page numbers in ICMSF, 1978.

c Omit APC for dried egg white.

d The case for *Salmonella* is to be chosen in accordance with whether the expected use of the product will reduce, cause no change in, or increase concern (see Table 10 on p. 74 and ICMSF, 1980, Chapter 19, pp. 521-566).

e For food intended for high-risk populations, the case remains as indicated in Table 10, but n is increased to increase stringency.

However, it should be emphasized that, for the amylase test to be a reliable indicator of the adequacy of the process, the pasteurization temperature must reach 64.5°C (for 2.5 minutes) or higher (UK Ministry of Health, 1963).

Some unpasteurized eggs are shipped between countries, and represent a serious hazard as vehicles for the dissemination of salmonellae. The international shipment of unpasteurized egg products should be discouraged and their sale prohibited. No recommended limits are proposed for these products.

(c) *Foods containing egg*

There is a wide range of manufactured foods which contain egg products (ICMSF, 1980, p. 524). It is not possible to generalize in terms of sampling plans for indicator organisms, because of the major contributions that non-egg ingredients may make to these populations. The most important pathogens in such foods are likely to be salmonellae and, where they are considered to be a hazard, the criteria for salmonellae in Table 25 are appropriate, with due regard being given to the case. *Streptococcus pyogenes* and *Staphylococcus aureus* are also of concern, particularly in foods prepared in food-service establishments and homes (Riemann and Bryan, 1979).

B. SAMPLING PROCEDURES

Reference should be made to Chapter 9, and in particular to those sections dealing with the sampling of frozen, liquid, and powdered products. The sample will usually consist of unopened containers, such as hermetically sealed cans or consumer packs (e.g., dehydrated egg products). Select the required number of sample units, which may be individual or composited sample units from individual cans or packs.

(a) *Frozen egg*

Drill diagonally through the frozen material in the can after opening with aseptic precautions. Use a drill or brace and sterile bit, and scrape about 50 g of the drillings into a sterile container with a sterile spoon; then close container and can. Alternatively a modified plastic funnel may be used with the electric drill to collect samples (Adams and Busta, 1970). Thaw the sample unit for not more than 90 minutes at room temperature, or place the required amount of analytical unit directly into an homogenizer with pre-enrichment medium in the case of *Salmonella* testing, or into appropriate diluent for other tests.

(b) *Dried egg*

Open the original container or laboratory container (waxed carton or plastic bag) taking aseptic precautions, and spoon the appropriate amount of analytical unit directly into pre-enrichment medium in the case of *Salmonella* testing, or into appropriate diluent for other tests.

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Sampling plans for formulated foods

Commercially prepared ready-to-cook or ready-to-eat foods have been widely accepted throughout the world. When such a product consists of major ingredients from more than one commodity category, it is termed a formulated food. Examples of the multitude of such products are meat and vegetable pot pies, seafood and meat salads, ice cream, infant formulae, dried soups, dessert pies, and certain ethnic dishes including certain pasta products, enchilada, and eggrolls. New products are being developed and marketed, and others discontinued. Consequently it is not possible to provide a meaningful list of all formulated foods.

A wide range of processes are used to prepare these foods, which may be offered for sale to the public as perishable, semi-preserved, or shelf-stable products. Relatively minor changes in formulation, especially the post-processing addition of condiments such as grated cheese, sesame seed, ground spices, or chocolate frosting, may alter the microflora of the product to a degree that different microbiological criteria would be needed for apparently similar products.

For the reasons noted above, the ICMSF makes no pretence of covering the entire range of formulated foods. The purpose of this chapter is to provide guidance and examples for those food industries and regulatory agencies that perceive the need to apply microbiological criteria to particular formulated foods. For information about the basic concepts and principles involved, reference should be made to Part I of this book.

Several commodity chapters in Part II of this book contain examples of formulated foods that are traditionally associated with a particular commodity which is a major ingredient of the product (e.g., ice cream with milk products, pasta with cereal products). Some of the formulated

foods mentioned in the first edition of this book have been omitted from this revision because there is insufficient information to determine whether microbiological criteria are useful. Accordingly the types of products included in this chapter are dried soup and gravy mixes, meat pies, salads, selected precooked frozen foods and certain dietetic foods, and foods specifically for infants and children. Other products such as salads and pies are described in ICMSF, 1980, pp. 822–833. For most of those foods the quality of the raw materials is of paramount importance for the quality and safety of the end-product. In such cases, setting criteria for the end-products for the purpose of reducing the eventual hazard to the consumer will be less effective than testing the raw ingredients.

For many formulated foods, the number of microorganisms as determined by the aerobic plate count (APC) is not indicative of adherence to Good Manufacturing Practice (GMP). Moreover, the food will be cooked just prior to eating, thus killing most microorganisms, or will be eaten shortly after preparation, thus avoiding any extended opportunity for microbial growth. For example, certain dried soups and ready-to-cook foods, which will be cooked before eating, contain raw ingredients that may have unavoidably large numbers of microorganisms including coliforms and occasionally *Salmonella*. As the process of manufacture does not always reduce the number of microorganisms in the raw material, their presence in retail samples does not indicate a lack of GMP.

A few commercially processed formulated foods, such as meat pies that are eaten cold, have been incriminated in outbreaks of food-borne disease due to lack of GMP in the factory. Most outbreaks have occurred because of post-processing time-temperature abuse, improper storage, or mishandling by the preparer before serving. Commercial food preparation introduces no hazard that is not present in the domestic situation. However, the magnitude of the hazard is much greater because of the greater number of persons exposed to the lot of the commercial product.

In general the need to establish microbiological criteria for most formulated foods has not been demonstrated. Indeed, in the case of dried soups, the Codex Alimentarius Committee on Food Hygiene decided specifically that such criteria are not justified. However, there are some products that require special attention either because they may contain pathogenic organisms that are not killed before eating, or because they are typically eaten by highly susceptible persons such as those debilitated by age or illness. The latter include foods for infants

and children who are in the high-risk category, and the costs involved in controlling the microbial populations mentioned below seem justified. Microbiological end-product specifications for these products were established by the Codex Alimentarius Commission (Codex, 1981).

A. FOODS FOR INFANTS AND CHILDREN

Commercially processed foods for infants and children were not considered in detail in ICMSF, 1980, and some comments concerning their manufacture are necessary before considering sampling plans and limits. Canned baby foods, however, will not be discussed here because they must meet the same requirements for safety as other shelf-stable, sterilized products (see Chapter 26, Shelf-Stable Canned Foods, for discussion of these foods). Three other types of foods are dealt with in the Codex Alimentarius Commission's microbiological specifications for foods for infants and children (Codex, 1979). These are dried biscuit-type products, dried instant products, and dried products requiring heating before consumption.

Plain biscuit-type foods are usually thoroughly baked, and no hazard should exist for these because of the heat process and their low a_w . Those with a coating or a filling require more sophisticated manufacturing practices, but the prior heat-treatment of fillings and coatings should be such that the numbers of vegetative forms of microorganisms are sufficiently reduced. The a_w of the product prevents microbiological multiplication during storage and distribution; moreover, since these products are not reconstituted in water or milk before consumption, risks of mishandling of product are minimal.

Dried instant products range from simple milk powders to very complex mixtures. The milk, with or without additional ingredients, is heat-treated (but not sterilized), concentrated, and then spray-dried, or roller-dried when cereals have been added. The heat-treatment is as mild as possible to maintain high nutritive quality. Dried instant foods include certain dry-blended products which are not heated after blending.

Good manufacturing practices should include careful selection of raw materials, hygienic design of processing equipment, adequate control of the critical points during manufacture, and, of course, continuous good housekeeping. Adherence to these good manufacturing practices will normally lead to products containing very few vegetative microorganisms. The APC may, however, be 10^3 to 10^4 per gram,

consisting usually of spores that have survived heat-treatments applied during preparation of the product or its ingredients.

Bacillus cereus, a normal contaminant of milk, can be expected to be present in low numbers. Its mere presence does not constitute a hazard, but if the product is sufficiently abused to permit growth to high numbers, a significant hazard could result.

In principle, there should be very little risk that the product contains heat-sensitive pathogenic organisms. Nevertheless, contamination by *Salmonella* occasionally occurs, sometimes from the use of contaminated ingredients added during dry-mixing or from the environment of the production line. The severity of the hazard presented by these organisms depends on subsequent treatment of the product. If adequately heated the hazard from *Salmonella* is removed. If not heated, this hazard remains and will increase if subsequent abuse of the product occurs.

Mishandling – that is, leaving the reconstituted product at room temperature for periods of more than 1 to 2 hours – may present a risk to the young, old, or debilitated consumer. It should also be taken into account that uncontaminated infant formula may become contaminated during preparation. If prepared in advance, such products should be cooled rapidly in order to prevent bacterial growth.

A third category of foods for infants and children is dried foods which require heat-treatment before consumption. The production of such foods by dry-mixing of non-heat-treated raw materials or with less severe heat processes means that most of these products contain higher numbers of microorganisms than do dry instant foods, and even some heat-sensitive bacteria may be present. However, the presence of these microorganisms in these products is accepted with the full knowledge that cooking will reduce their numbers to non-hazardous levels, making the food safe. Instructions for heating such products should appear on the label.

The advisory end-product specifications of Codex Alimentarius are too strict to be met by all the above categories of products in international commerce (Collins-Thompson et al., 1980). These criteria were established to give guidance on what is considered good microbiological quality. However, only at the factory, when the microbiology of raw material and ingredients and the influence of the process are known, will it be possible to judge if divergence from the end-product specifications truly represents some fault in GMP. Products are present on the market, made according to GMP, which contain higher

numbers of aerobic bacteria than stated in the Codex criteria (for dried and instant products, APC: $n = 5, c = 2, m = 10^3, M = 10^4$; coliforms: $n = 5, c = 1, m = 3, M = 20$). Such products may have been made with raw materials containing higher spore counts. Moreover, a few coliforms can sometimes be present without indicating a risk to the health of the consumer. Thus, in order to avoid unnecessary rejection of safe foods in retail markets, or at a point of entry where the history of the product is unknown, the criteria for many of these products will have to be more lenient than the end-product specifications of Codex Alimentarius.

B. SAMPLING PLANS FOR FOODS FOR INFANTS AND CHILDREN AND FOR CERTAIN CATEGORIES OF DIETETIC FOODS

The following considerations are applicable to foods for infants and children and certain categories of dietetic foods (e.g., those intended for consumption by debilitated, or otherwise sensitive, consumers).

The microorganisms to be considered are the mesophilic aerobes, coliforms, staphylococci, and salmonellae. The APC may be used to determine the number of bacterial spores in the raw materials, growth of bacteria at or between critical points, the effect of the applied heat-treatment, growth in accidentally rehydrated products during storage and distribution, and, in general, adherence to Good Commercial Practices (GCP). Since all coliforms should be killed during the heat-treatments applied, this group of microorganisms can be used as indicators for post-process contamination and growth; in short, they can be used for the same purposes as the APC to check adherence to GCP. In dry-mix processes, when no heat-treatment is applied, the coliform count is useful to indicate adherence to GCP in the production, storage, and distribution of the raw materials.

Staphylococci may occasionally be present, but usually only in low numbers, as a result of contamination after drying or during dry-blending (see Chapter 15, Milk and Milk Products). In the production of foods for infants and children rigid quality-assurance programs should be applied to meet the end-product specifications. A massive contamination with staphylococci without an alert during production or an increase in APC or coliforms is highly improbable, while their presence in low numbers does not indicate a direct public health hazard. Such a hazard would arise only after mishandling of the product after

reconstitution. In this case the situation is not different from that described above for *B. cereus*.

In the light of these considerations there is no need for routine examination for *Staphylococcus aureus* in these foods. A similar conclusion was reached in Codex, 1979. However, when excessive contamination with *Staph. aureus* is suspected, case 9 ($n = 10$, $c = 1$, $m = 10$, $M = 10^2$) would apply. When test results or other information indicate that a build-up of *Staph. aureus* has occurred, the product should be considered as unsuitable for the use intended. *Salmonella* is considered a hazard because the minimal infective dose might be low for the intended consumer group, and for products requiring reconstitution the hazard will be increased if subsequent mishandling occurs. For these products sampling plans for *Salmonella* are recommended (Table 33).

For plain dried biscuit no microbiological criteria are suggested because there is no hazard which can be limited by setting such criteria. For coated and filled biscuits, tests for coliforms and *Salmonella* would suffice. Since the condition of use of this product would not increase the hazard, case 5 is selected for the coliform criterion. Case 11 is chosen for *Salmonella* because of (a) the possible presence of an ingredient contaminated with *Salmonella* and (b) the conditions of use, which lead to no increased hazard.

For dried and instant products, a criterion for APC is recommended in addition to those for coliforms and *Salmonella*. Directions for use of these products do not include any microbial destructive treatment (e.g., heating) which can be relied on. As these products must be rehydrated, the possibility of subsequent bacterial growth due to mishandling cannot be excluded. Case 6 is selected for the APC and the coliform counts. For *Salmonella* case 12 remains but, because of the high-risk consumer group, n is increased to 60 (see Table 12, p. 78).

For dried products that require heating before consumption case 4 is chosen for APC and coliforms and case 10 for *Salmonella*, because cooking will reduce the hazard.

The limits are based on available published and unpublished data, and take into consideration that maintaining the nutritive quality of such products is more important than reducing the numbers of mesophilic bacteria of little public health significance. In the last two categories values of m and M have been modified from those in Codex, 1981, as a consequence of bacteriological surveys and commercial experience.

TABLE 33

Sampling plans and recommended microbiological limits for foods for infants and children and certain categories of dietetic foods

Product	Test	Method reference ^a	Case	Class plan	n	c	Limit per gram	
							m	M
Coated or filled, dried shelf-stable biscuits	Coliforms	126-131 (Method 3) ^b	5	3	5	2	10	10 ²
	<i>Salmonella</i> ^{e,f}	163-172 ^c	11	2	30	0	0	—
Dried and instant products requiring reconstitution	APC ^g	115-118 (Method 1) ^d	6	3	5	1	10 ⁴	10 ⁵
	Coliforms	126-131 (Method 3) ^b	6	3	5	1	10	10 ²
	<i>Salmonella</i> ^e	163-172 ^c	12	2	60	0	0	—
Dried products requiring heating to boiling before consumption	APC	115-118 (Method 1) ^d	4	3	5	3	10 ⁵	10 ⁶
	Coliforms	126-131 (Method 3) ^b	4	3	5	3	10	10 ²
	<i>Salmonella</i> ^e	163-172 ^c	10	2	15	0	0	—

^a Refers to page number in ICMSF, 1978.

^b See also ISO 4831 (1978a).

^c See also ISO 6579 (1981).

^d See also ISO 4833 (1978b).

^e Use 25 g analytical units which may be pooled.

^f Applies only to products containing a *Salmonella*-sensitive ingredient.

^g Not applicable to fermented products.

C. SAMPLING PROCEDURES

Reference should be made to those sections of Chapter 9 (Collecting and Handling Sample and Analytical Units) dealing with the sampling of dried and powdered products.

In the examination of dried products for *Salmonella*, rapid rehydration by shaking the powder with the pre-enrichment fluid should be avoided (van Schothorst et al., 1979; Andrews et al., 1983).

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Glossary

acceptance sampling The application of a predetermined sampling plan to decide whether or not the lot meets defined criteria of acceptance.

aerobic plate count (APC) The number of colony-forming units of aerobic mesophilic microorganisms present per gram or per ml in the analytical unit as determined by a standard method.

aliquot The portion of food that is inoculated into a container of bacteriological medium in accordance with a specified method. For example, from a 1 kg sample unit, a 25 g analytical unit may be blended with 225 ml of diluent to give a 1:10 dilution. A 1 ml portion of this suspension would be an aliquot of 0.1 g.

analytical unit That amount of product withdrawn from a sample unit for an analysis.

attributes plan A sampling plan in which each selected sample unit is classified according to the quality characteristics of the product and in which there are only two or three grades of quality: for example, acceptable, defective; absent, present; acceptable, marginally acceptable, defective; low count, medium count, high count.

c The maximum allowable number of defective sample units (2-class plan) or marginally acceptable sample units (3-class plan). When more than this number are found in the sample, the lot is rejected.

case A set of circumstances related to the hazard a food represents and to its anticipated subsequent treatment.

consignment A quantity, large or small, of food destined, in commerce, to a particular recipient, usually consisting of multiple containers of food from one or more lots.

consumer's risk The probability that a lot will be accepted on the basis of results of testing analytical samples when in reality the lot as a whole is substandard relative to the stated criteria of acceptability.

defective Not suitable for the intended purpose.

frame That portion of the lot from which the sample units are drawn. Ideally it should be the whole lot; in practice it may be the accessible portion of the lot.

Good Commercial Practice (GCP) A general term defining conditions of Good Manufacturing Practice combined with acceptable conditions of distribution and storage.

Good Manufacturing Practice (GMP) Those procedures in a food-processing plant which consistently yield products of acceptable quality. GMP is usually described in a code defining processes, equipment, plant layout, sanitation, hygiene, and laboratory tests; but, as used here, it does not apply to any particular code now in use.

hazard The potential to cause (i) disease when eaten or (ii) organoleptic spoilage in a period shorter than the normal shelf-life of the food.

Hazard Analysis Critical Control Point (HACCP) A system in which observations and/or tests are made to identify actual or potential hazards in operations and to identify critical control points in a process. Control measures are designed and implemented, and the control points are monitored to ensure that control is maintained.

indicator Historically, an organism itself non-pathogenic, but often associated with pathogens, used to portray a risk of the presence of pathogens for which feasible methods of detection were not generally available (sometimes called 'index organisms'). This usage is currently expanded to denote groups or species of organisms whose presence in a food reveals exposure to conditions that might introduce hazardous organisms and/or allow their growth. Specific indicators are now used to reveal excessively contaminated raw materials; unsanitary manufacturing practice; contamination from faecal, nasopharyngeal, or suppurative sources; unsuitable time-temperature conditions of storage; or failure of a process. The indicator groups and their methods of enumeration are described in ICMSF, *Microorganisms in Foods, 1. Their Significance and Methods of Enumeration* (1978).

lot In the commercial sense, a lot is a quantity of food supposedly produced under identical conditions, all packages of which would normally bear a lot number that identifies the production during a particular time interval, and usually from a particular 'line,' retort, or other critical processing unit (see Chapter 3, Section A). Statistically, a lot is considered as a collection of units of a product from which a sample is to be drawn to determine acceptability of the lot.

m A microbiological limit which, in a 2-class plan, separates good quality from defective quality or, in a 3-class plan, separates good quality from marginally acceptable quality. In general, values equal to *m*, or below, represent an acceptable product and values above it are either marginally acceptable or unacceptable.

M A microbiological limit which, in a 3-class plan, separates marginally acceptable quality from defective quality. Values above *M* are unacceptable.

microbiological criterion A criterion concerning the presence or quantity of microorganisms or their toxins per unit of mass, volume, or area, established by use of defined procedures and applied in acceptance sampling of foods (see Appendix 6: General Principles for the Establishment and Application of Microbiological Criteria for Foods).

microbiological guidelines A microbiological criterion used by a manufacturer or regulatory agency to monitor a food, ingredient, process, or system; often used also to describe a microbiological criterion where no standard has been prescribed. The Codex Alimentarius definition is more restrictive (see Appendix 6: General Principles for the Establishment and Application of Microbiological Criteria for Foods).

microbiological purchasing specification The microbiological criterion or criteria

conditional to acceptance of a specific food or food ingredient by a food manufacturer or other private or public purchasing agency.

microbiological standard A microbiological criterion in a law or regulation controlling foods produced, processed, or stored in the area of jurisdiction of a regulatory agency, or imported into the area.

moderate hazard The hazard associated with ingestion of a food containing a pathogen or toxin which can cause a short-term disease not critically severe in its manifestations and normally without sequelae: for example, salmonellosis, staphylococcal food-poisoning.

Most Probable Number (MPN) A statistically estimated number (per unit of mass or volume) of the test organism present in a sample unit, based upon its presence or absence in replicate aliquots (see also ICMSF, *Microorganisms in Foods, 1. Their Significance and Methods of Enumeration* [1978]).

n The number of sample units which are examined from a lot to satisfy the requirements of a particular sampling plan.

population (epidemiological) The number of people estimated to be at risk from a particular food.

population (microbiological) The number of microorganisms, total or of a species or group(s), dispersed within a defined quantity of food and detectable by the methods used. Hence, a total microbial population or a population of staphylococci, coliforms, etc.

population (statistical sampling) The aggregate of units about which an inference is to be made from analytical results. In this book, population usually relates to the totality of hypothetical individual portions within a lot, each identical in quantity with the defined sample unit.

probability of acceptance The likelihood that a lot of known quality would be accepted on the basis of results of testing analytical samples relative to the stated criteria.

producer's risk The probability that a lot of acceptable quality will be rejected on the basis of results of testing analytical samples relative to the stated criteria.

random sample of size *n* A collection of *n* sample units obtained in such a way that every sample of size *n* has an equal chance of selection, hence excluding bias. Reference to a table of random numbers is usually involved (see Chapter 3, Section C).

sample The total number (one or more) of individual sample units drawn from a lot (ideally at random) which will be tested in accordance with a specific sampling plan and method(s).

sample unit The individual portion or container of food taken as part of the sample and from which one or more analytical units may be drawn for analysis.

sampling plan Used synonymously in this text with *lot acceptance sampling plan*, and meaning a statement of the criteria of acceptance to be applied to a lot, based on examination of a required number of sample units by defined analytical methods.

severe hazard The hazard associated with the presence of a pathogen or toxin in a food which when ingested is likely to cause severe disease, either in a healthy population or in a particularly susceptible group to which the food in question is often destined. Severity of hazard is largely determined by clinical severity of the disease induced, but many ancillary factors are pertinent (see Chapter 5 and Tables 6 and 7). Pathogens of concern in this category include *Brucella* spp.,

Clostridium botulinum, *Cl. perfringens* type A, *Salmonella typhi*, *Shigella dysenteriae*, *Vibrio cholerae* O1 (Table 7); they are normally sought by application of investigational sampling (see Chapter 5, Section N).

shelf-life The period after processing during which a food remains in acceptable condition under appropriate conditions of storage.

stratification A technique for dividing consignments into logical groupings such as size of cans, grades, box, cases, which controls known sources of variation. It may be used where prior knowledge exists that the consignment is potentially not of uniform quality.

stringency The probability that a lot of food of defined microbiological quality will be rejected by a prescribed sampling plan, relative to other sampling plans.

subsample The several individual swabs or portions of food taken from a single carcass or carton and which make up a single sample unit.

variables plan A sampling plan in which the criteria employ statistics of a sample, such as the mean and standard deviation, rather than the segregation of sample units into attribute classes (*see* attributes plans). In the context of this book the variables plans referred to are based on the application of the mean ($\bar{x}$) and standard deviation (s) of the sample log counts. These parameters are combined with a constant or constants (k_1 , k_2) selected for the appropriate number of sample units (n) for comparison with a limit value (v or V).