MICRO ORGANISMS IN FOODS 2

Sampling for microbiological analysis: Principles and specific applications

Second edition

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Introduction: The application and use of criteria

This chapter provides background information on the considerations which have led the Commission to propose microbiological criteria for some commodities and not others. It also indicates how the criteria should be interpreted and applied.

A. FORMAT OF COMMODITY CHAPTERS

In the first edition of this book foods were grouped on the basis of commodities (e.g., milk and milk products) or processes (e.g., frozen foods, dried foods). In this edition the commodities grouping used in *Microbial Ecology of Foods,* vol. 2 (ICMSF 1980), has been followed with two exceptions. These are formulated foods, comprising raw materials from several commodity groups, and low-acid canned products. Criteria for formulated foods will depend on conditions of manufacture, the types of raw materials used, the process, the intended distribution system, and shelf-life. Hence criteria are proposed only where a need has been demonstrated and such information is available (see Chapter 24, Formulated Foods). Sampling plans and microbiological tests are not relevant to the safety of shelf-stable canned foods and are therefore not proposed. Extensive cross-reference is made to the volume referred to above (ICMSF 1980) so that information pertaining to the need for criteria and the appropriate cases may readily be found.

Each chapter discusses the microbiological hazards associated with a commodity group and, based on a consideration of their relevance, may propose criteria. General sampling procedures are dealt with in Chapter 9, but if a commodity requires special sampling procedures

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these are detailed within the chapter. For methods, reference is made to ICMSF, 1978, or to other appropriate sources.

B. SELECTION AND APPLICATION OF CRITERIA

Ideally the control of microorganisms in foods is at the point of production, processing, or preparation for consumption (see Chapter 7, Control at Source). However, for much food in international trade there is no knowledge of control at source or of the conditions used during processing and handling. Therefore, there remains a need for criteria to assess the acceptability of foods at port of entry.

Before recommending a criterion for a product, the Commission decided that each product must meet the following conditions:

1 The product must be in international trade.
2 There must be good epidemiological evidence that the product, or product group, has been implicated in food-borne disease and/or may have an inadequate shelf-life if Good Commercial Practice (GCP) has not been followed.
3 There must be good evidence, or good reason to believe, that the application of a criterion will reduce the health risk in food and/ or effectively assess adherence to MCP.

It is important to consider some of the difficulties in expecting a microbiological examination to portray the true microbiological condition of food. For example, a relatively few sample units may not accurately reflect the true microbiological status of a consignment, particularly if the consignment is composed of several batches of food. In this case, if only one of the batches is defective, sampling a portion of the consignment may not reveal the unacceptable part. The resulting decision, if applied to the whole consignment, would be quite unsatisfactory. The sampling plans proposed in this book should whenever possible be applied to identifiable lots (see definition of a lot, Chapter 3, Section A).

If at port of entry the intention is to safeguard against staphylococcal intoxication, a criterion for *Staphylococcus aureus* would not detect the hazard if the viable cells have died. If epidemiological evidence shows that this product constitutes a hazard, a more direct criterion would then be needed, such as one for thermonuclease or enterotoxin. If a criterion is applied to a finished product it may be inadequate if a destructive process has been applied (e.g., destruction of *Staph. aureus*, but with enterotoxin persisting).

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The microbiological status of some foods changes continually during storage and distribution and is taken into account when selecting the 'case'. Thus, control at the source is the ideal, and criteria applied at the port of entry will never be as effective, though there are many instances where their application is effective (e.g., *Salmonella* in dried milk).

C. CHOICE OF ORGANISMS

The ICMSF has attempted to include in criteria those microbes most important in respect of hazard and/or non-compliance with GCP. This choice was based on epidemiological evidence and expert opinion, recognizing limitations of current methodologies.

D. SELECTION OF LIMITS

For 3–class attributes plans it was necessary to establish *m* values (associated with GCP) as well as *M* values (related to the safety/'quality' limit). The latter are based on expert opinion as to the acceptable limit, but the former should be based on firm data obtained from producers and retailers operating according to Good Manufacturing Practice (GMP) and GCP.

Although ICMSF has collected a large amount of data, there is not sufficient information for certain commodities to establish *m* values on this basis. When information was not available, expert opinion was relied upon to establish *m* values for 3–class plans. Where such values could not be derived, 2–class plans are adopted. Appropriate values of *m* for 3–class plans must await the collection of further microbiological data.

The ICMSF has now implemented a pilot computer-based collection and retrieval system for microbiological data and is anxious to obtain data to expand this data base. Readers having relevant data are invited to contact the secretary of the ICMSF, who will be pleased to provide printed forms for confidentially recording data in a format suitable for the system.

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Sampling plans for raw meats

World trade in raw meats, both chilled and frozen, continues to be significant in terms of volume despite their potential hazard to health. Total world red meat production in 1979 was more than 78 million tonnes, with about 9% being traded internationally.

A. REASONS FOR SAMPLING

Raw meats are important sources of *Salmonella* and *Clostridium perfringens,* which are often incriminated in outbreaks of food-borne disease (ICMSF, 1980, p. 354). They are also sources of *Staphylococcus aureus, Campylobacterfetus* subsp. *jejuni,* and *Yersinia enterocolitica.* Salmonellae are found more often in pork and in veal from young calves than in other meats (ICMSF, 1980, p. 347). In the first edition of this book criteria for salmonellae were included as guidelines to assist control of this organism in raw meat. It was hoped that such guidelines would lead to a reduction in the contamination rate and a drop in the incidence of food-borne disease. However, outbreaks of salmonellosis due to meat have continued with little evidence of diminution. In some instances salmonellosis has been caused by the consumption of raw or inadequately cooked meat products but a more common hazard arises through cross-contamination from raw to cooked meat or other foods, and subsequent time-temperature abuse.

Clostridium perfringens is ubiquitous, and although it occurs on carcass meat, usually in low numbers, it cannot be controlled by any known means. The majority of outbreaks of *C. perfringens* gastroenteritis attributable to meat result from inadequate storage of the cooked product. Prevention involves attention to the time/temperature conditions

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of cooking and, more important, to hot holding, cooling, and reheating before consumption.

Staphylococcus aureus may occur on raw meat although usually only in low numbers. Contamination by animal strains of *Staph. aureus*, which are thought to have a low enterotoxin-forming potential, is probably of less consequence than contamination from human sources. *Staphylococcus aureus* competes poorly with the normal microbial flora of raw meat and constitutes a health hazard only when this competing flora is restricted and there is temperature abuse of the product.

Campylobacter fetus subsp. *jejuni* is often present in the intestinal flora of healthy animals used for food production (Blaser, 1982). However, the numbers present on red meat are generally low and the organism has only a limited potential for growth or survival on refrigerated or cooked meat. Food-borne outbreaks of *Campylobacter* enteritis appear to arise almost exclusively from eating raw or undercooked food (Blaser, 1982).

The use of microbiological criteria as a control measure for reducing the incidence of disease arising from the consumption of meat carrying *Salmonella*, *C. perfringens*, and *Staph. aureus* was considered by a FAO/WHO Working Group (FAO/WHO, 1979) in light of the Codex 'General Principles for the Establishment and Application of Microbiological Criteria for Foods' (Codex, 1981). According to these principles a microbiological criterion should be established and applied only where there is a definite need and where it is both practical and likely to be effective. Such criteria should form part of, or be related to, a Codex Code of Practice for the particular product. The presence of *Salmonella* and *C. perfringens* on raw meats is generally more a reflection of their incidence in the live animal than of a breakdown of hygiene. In present circumstances the occurrence of salmonellae and the other pathogens in raw meat cannot be entirely prevented by the application of codes of hygienic practice (ICMSF, 1980, p. 347). Also, the extreme variability of distribution of pathogens such as salmonellae in meats prevents the establishment of practical sampling plans which would ensure with any degree of confidence the absence of salmonellae in meats. Consequently the Working Group concluded that the application of limits for pathogenic microorganisms at port of entry was not appropriate in terms of the Codex 'General Principles'.

In view of the importance of *Salmonella* as a cause of food-borne disease and meat as a vehicle for its transmission there is much to be gained from regular in-plant monitoring to establish the extent of the problem in particular areas and to detect increases in prevalence.

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Products that have a history of being implicated as causes of salmonellosis should be sampled at appropriate points during production and distribution to determine the prevalence of contamination and to trace its source on the farm as well as to look at the effects of travel, holding, and processing of stock. The aim is to collect information that will help to motivate the producer to eliminate salmonellae in the live animal and thus control the problem at its source.

The ultimate solution to the *Salmonella problem* lies in producing *Salmonella*-free animals. The provision of *Salmonella*-free foodstuffs for animals would be a major advance towards this aim (see Chapter 14, Feeds of Animal Origin and Pet Foods). Until this is achieved treatments that destroy salmonellae in the product (e.g., irradiation) may provide some protection for the consumer. In the absence of effective control measures, environmental and line sampling are more useful than testing the end-products. Although improvement of slaughter hygiene is incapable of eliminating contamination with salmonellae, neglect of hygiene can make the situation much worse. A comprehensive monitoring program in the processing plant can provide a check on the prevalence of infection in livestock and the effectiveness of measures of hygiene in controlling spread of contamination.

The FAO/WHO Working Group (FAO/WHO, 1979) concluded also that the number of indicator organisms in meat neither reflects adherence to a code of hygienic practice nor indicates presence or absence of pathogens. Hence criteria based on indicator organisms were not justified for raw meat. These conclusions, and those concerned with pathogens, have been

B. SAMPLING PLANS

In the virtual absence of studies relating health risks to surface counts of aerobic organisms on carcass meats it is not appropriate to reject consignments of product for public health reasons solely on the basis of high aerobic plate counts. However, aerobic plate counts (APC) can be used to monitor Good Commercial Practice (GCP), and criteria based on such examinations are a valuable aid to in-plant quality control. Microbiological quality control of meat-processing involves development and use of processing methods designed to keep microbial numbers low by reducing contamination and preventing growth. Microbiological monitoring of product and plant at critical control points can be used

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TABLE 19

Sampling plans and recommended microbiological limits for raw meat[a]

Product[b]	Test	Method reference[c]	Case	Plan class	*n*	*c*	Limit per cm² or gram *m*	*M*
Carcass meat, before chilling	APC	118	1	3	5	3	10^5	10^6
Carcass meat, chilled	APC	118	1	3	5	3	10^6	10^7
Edible offal, chilled	APC	118	1	3	5	3	10^6	10^7
Carcass meat, frozen	APC	118	1	3	5	3	$5x10^5$	10^7
Boneless meat, frozen (beef, veal, pork, mutton)	APC	118	1	3	5	3	$5x10^5$	10^7
Comminuted meat, frozen	APC	118	1	3	5	3	10^6	10^7
Edible offal, frozen	APC	118	1	3	5	3	$5x10^5$	10^7

a **Not for use at port of entry** but for in-plant quality control. Refer to Section B for appropriate applications.

b Unfrozen carcasses and primal cuts, swab counts per cm²; other meats and meat products, per gram.

c Refers to page number in ICMSF 1978.

to assess the effectiveness of sanitation factors in limiting microbial growth. These counts are compared with APC criteria established for particular products prepared under specific conditions. Sampling plans and microbiological criteria for certain raw meat commodities are listed in Table 19.

The proposals for raw meats are based on limited data collected mainly from production and under a restricted range of circumstances. The criteria have been established as guidelines and some tolerance must be allowed in their application; for example, meat marginally acceptable, or even rejected, by these guidelines could be used in heated products, but would have very limited shelf-life as fresh meat, even under good refrigeration. Meat prepared according to the *Recommended International Code of Hygienic Practice for Fresh Meat* (CAC/RCP 11-1976, FAO, Rome) may on occasion exceed these limits, which should not be used as legal standards. No attempt has been made to distinguish between meat from different species although it has been noted that sheep carcasses normally have a slightly higher level of initial

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contamination than those of beef and numbers of bacteria tend to be higher again on pig carcasses (ICMSF, 1980, p. 342).

Although there are considerable quantities of horse meat in international trade, criteria have not been included because of lack of data.

The FAO/WHO Working Group (FAO/WHO, 1979) considered that the only point at which APC could be used for chilled meat, to evaluate the hygienic conditions under which it was produced, was at the slaughterhouse, and because of its perishable nature it would not be possible to set APC limits for use in guidelines or specifications for chilled meats at port of entry. This consideration applies to meat that is vacuum-packaged in an oxygen-impermeable plastic film as well as to chilled meat packaged in other ways. Vacuum-packaging and modified-atmosphere packaging bring about changes in the bacterial flora, and the storage life depends more on the nature of the flora that develops than on the numbers of bacteria present after processing.

Case 1 with a 3–class plan for the APC is appropriate for utility tests for general contamination of a product that is normally cooked before consumption. The values for *m* reflect current commercial attainment based on the examination of meat from different sources, mainly at point of production, and are supported by experience that meat with plate counts lower than the value set for *m* usually has not been subject to excessive contamination or undue faulty handling. A normal shelf-life would be anticipated. Overall data from a large number of samples have been analysed but not all products have been tested to a similar degree.

The *M* values for APC are based on experience that meat with APC values in excess of 10^7 is either grossly contaminated or has been exposed to conditions permitting microbial growth to a level not far short of the point at which incipient spoilage can be detected. Immediately after slaughter an *M* value of 10^6 is justified on the basis that counts below this level can be readily achieved under GCP. At this level some tolerance is allowed for further processing which could involve slight increase in microbial numbers.

Microbiological criteria applied to frozen meat should be similar to those applied to chilled meat. The criteria proposed for frozen carcass meat and frozen cuts assume that the meat is sampled in the frozen state and thawed quickly before examination of samples. Spoilage occurs at about the same level; hence the *M* values are the same. Reflecting the small decrease in numbers due to freezing and storage *m* values are 5 x 10^5 instead of 10^6. Commercially thawed meat usually has a

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higher count than the frozen product, and the criteria for chilled carcass meat could apply.

The microbiological quality of comminuted meat reflects the situation in the meat from which it is prepared. Counts in general are 10– to 100–fold greater in commercial minced meat than on the equivalent carcass (ICMSF, 1980, p. 370). If scrap meat and trimmings from the outer surface of carcasses are used, the counts in the mince will be higher than if whole cuts are minced. Mechanically deboned meat derived from good-quality raw material can be microbiologically equivalent to or better than minced, manually boned meat provided strict temperature control is exercised, but the process needs careful control in respect to the material being boned and sanitation of the equipment. There have been many proposals for microbiological criteria for minced meat but there is no evidence that a criterion has any relevance to health hazard. Accordingly criteria similar to those for carcass meat are proposed.

There is little information available on the microbiology of edible offals but the considerations are similar to those for carcass meat and the criteria proposed are the same.

C. SAMPLING PROCEDURES

(a) *Carcasses and primal cuts*

For chilled or frozen carcass meat and large bone-in or boneless cuts, as $n = 5$ for all criteria suggested, take subsamples from 5 individual carcasses or cartons of cuts (sample units). If the lot is distributed in several shiploads or freightcars take the sample units from more than one transportation unit.

Because contamination of meat is often very uneven, a number of subsamples should be taken from different parts of the carcass or cut, including areas known to be subject to contamination or particularly favourable for microbial growth. The subsamples from a carcass or carton may be pooled and thoroughly mixed to form a composite analytical unit, or each may be treated as a separate analytical unit.

Bacterial contamination of carcasses or cuts is generally on the surface, and this should be recognized in the collection of subsamples. Because defacing of carcasses or cuts is often economically undesirable, the surface swab technique is recommended for the APC on unfrozen meat. Samples should be taken at sites likely to be contaminated (see,

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e.g., Roberts *et al*., 1980). Lamb and pork carcasses should be swabbed at at least two points (leg and brisket), and beef and horse at three (leg, flank, and neck). Sampling of cuts should include the original exterior surface and a cut surface.

For a comparison of swabbing and excision methods see ICMSF, 1980, p. 359.

(b) *Boneless bulk cuts, comminuted meat, and edible offals*

Except for the offals it is usually not possible to identify the original surface. From each of 5 cartons or packages remove a sample unit of about 200 g of tissue, which should comprise subsamples from a number of different parts of the pack. This composite sample unit should be mixed thoroughly and the appropriate analytical unit withdrawn.

(c) Retail packages

These include chilled or frozen consumer-portion packed cuts and offals as well as comminuted meat. The sampling procedure entails the examination of 5 packages from the lot.

D. SAMPLE COLLECTION

Remove any wrappings from the carcass or package carefully without handling the meat. Use sterile swabs and templates for taking surface swab samples. Mark the sampling area with sterile metal guides (e.g., 5, 50, or 100 cm^2). Use two sterile swabs to sample each area. Moisten the first swab with sterile peptone water and rub firmly across the exposed area several times in all directions. Use the second swab dry and rub over the same area. Introduce both swabs into a bottle containing 3 or 4 glass beads and an appropriate known volume of diluent (e.g., 0.1 % peptone, 0.9% NaCl). Shake vigorously.

To avoid cross-contamination when removing portions of meat, use sterile instruments (knives for unfrozen meat; saws, cleavers, and special drills for frozen meat), and transfer the piece aseptically to sample containers.

Samples of frozen meat should remain frozen during transport to the laboratory. Samples from chilled carcasses should not be frozen.

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E. TEST PROCEDURES

(a) *Aerobic plate counts*

Comminute, blend, or stomach the various portions of meat composing the sample unit and withdraw the analytical unit. Combine the swabs to form an analytical unit, or treat each separately. Prepare dilutions and perform the APC test according to the directions given in ICMSF, 1978, p. 118. Spread plates are preferable to pour plates when examining raw meats.

The temperature used for incubation of plates should be related to the material being examined. To estimate numbers of mesophilic contaminants on freshly dressed carcasses it is advisable to incubate plates at 35 or 37°C. For meat that has been held under chilled conditions, plates should be incubated at 25°C to include psychrotrophs and thus give a better indication of the microbial growth that has occurred. Counts at 20°C give similar results after a longer incubation time.

(b) *Salmonella*

The test for salmonellae requires an analytical unit of 25 g and is based on positive or negative results using the method described in ICMSF, 1978, p. 163. For frozen products use a non-selective enrichment before selective enrichment and plating on selective agar media. Sample units should be taken from appropriate parts of carcasses, cuts of meat, and offals.

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Sampling plans for processed meats

Processed meats comprise a number of meat products, manufactured by many different technologies, including heat-processing, curing, drying, fermenting, acidifying, and packaging (ICMSF, 1980).

Processing changes the eating quality of meat. It also changes the composition of the microflora, and some processes kill or prevent growth of pathogens. Basically processing is intended to prolong the shelf-life of the products. The increase in shelf-life may range from a few days to several years. Processed meats with only a slight increase of shelf-life would not be candidates for international trade, while those given some degree of heat-treatment or curing combined with an adequate storage temperature move in international trade.

According to the FAO (1981), international trade during 1980 of processed meat and meat preparations amounted to about 1 million tonnes, of which sausages comprised about 115,000 tonnes. These FAO figures relate mainly to three groups of products: (a) shelf-stable cured canned meats; (b) perishable cured canned meats; and (c) cooked or uncooked, fermented, dried, or semi-dried sausages.

A. REASONS FOR SAMPLING

In the following section processed meats will be treated according to the outline in ICMSF, 1980, pp. 378-409. Discussion will deal with hazards (public health or spoilage), international trade of the products, and the possibility and feasibility of instituting means of control other than by microbiological criteria.

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TABLE 20
Sampling plans and recommended microbiological limits for processed meats[a]

Product	Test	Method reference[b]	Case	Plan class	*n*	*c*	Limit per gram *m*	Limit per gram *M*
Dried blood, plasma, and gelatin	*Staph. aureus*	220	8	3	5	1	10^2	10^4
	C. perfringens	264	8	3	5	1	10^2	10^4
	Salmonella	163[c]	11	2	10	0	0	
'Roast' beef[d]	*Salmonella*	163[c]	12	2	20	0	0	
Pâté	*Salmonella*	163[c]	12	2	20	0	0	

***a* Refer to text for appropriate applications.**
b Refers to page numbers in ICMSF, 1978.
c See also ISO 6579 (1981).
d Includes beef cooked in water baths; see Section B(f).

B. SAMPLING PLANS

(a) *Dried meats and dried animal products*

Dried meats like jerky, charqui, and biltong are mainly produced and consumed locally. Most dried meats found in international trade are intended for further processing. Further processing would normally consist of dry-blending with other food ingredients (e.g., dried soups) or rehydration and canning. In both cases the dried meats are subjected to conditions that either would not change or would reduce the hazards.

In international trade no evidence of hazard has been demonstrated, and no microbiological criteria are proposed.

Dried animal products such as dried whole blood, blood plasma, and gelatin are used as ingredients for formulated foods. When these ingredients are used in further processed products that are not subjected to adequate heat-treatment it is proposed to include sampling plans for *Staphylococcus aureus, Clostridium perfringens,* and *Salmonella* (Table 20).

For *Staph. aureus* and *C. perfringens* 3–class sampling plans, case 8 with $n = 5$, $c = 1$, $m = 10^2$, $M = 10^4$, are proposed. *Salmonella* should be considered in case 11 with a 2–class sampling plan with $n = 10$, $c = 0$.

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(b) *Raw cured meats, high a_w*

High a_w meat products are defined as those with an a_w above 0.92, such as occurs in bacon produced by a Wiltshire or similar process (ICMSF, 1980, pp. 383-388).

In Wiltshire curing sides or parts of sides of pork, with or without bones, are injected with brine before either tank- or dry-curing. Smoke may be applied, but the product is not heated. There are many other types of manufacture of bacon, but this is the most common for bacon in international trade.

The microbiology of bacon is characterized by a rapid change from the initial gram-negative flora to gram-positive salt-tolerant micrococci and lactic acid bacteria. Only where curing is inadequate or the pH of the meat (usually the collar) is high will putrefactive spoilage dominate. Otherwise, spoilage is characterized by sliming.

Staphylococci could be considered to be of concern but are not able to compete with the normal saprophytic flora. Applying microbiological criteria at manufacture can make little contribution to the control of spoilage.

Salmonella is not a major concern because the product is cooked immediately before consumption.

(c) Raw, cured, or salted meats and natural casings, low a_w

Low a_w meat products are defined (ICMSF, 1980) as those having a water activity of 0.92 or below. This group includes whole cuts of meat (e.g., Bundener Fleisch, Parma ham, Westphalian ham, Smithfield ham, Prosciutti), non-fermented dried sausages, and natural casings, all of which are in international commerce.

Although some of the raw cured or salted meats have been implicated in outbreaks of food-borne illness, particularly botulism, the incidents have usually involved home-prepared products. Commercial products have had a very good record of safety. Growth of non-pathogenic bacteria occurs during the process of salting, curing, and drying of many of these products and can contribute to their flavour characteristics. Hence, microbiological criteria need not be applied to these commercial products. Cured, dried non-fermented sausages are in international trade but the only spoilage problem appears to be mould formation on the casing. No public hazards have been identified, and no microbiological criteria are proposed.

Natural casings from international commerce are generally packed

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in salt and have not been identified as a vehicle of food-borne illness. Survival and growth of some salt-tolerant bacteria occurs in salted casings, but *Salmonella* indigenous to the casings does not survive for more than two or three weeks after salting. Microbiological criteria are unnecessary after salting.

(d) *Fermented sausages*

Cured fermented sausages include both semi-dry and dry types. Both types undergo a fermentation process which should rapidly lower the product pH to <5.5. At this point semi-dry products may or may not be heated but if the pork used contains *Trichina* heating to 58.3°C may be mandatory. Other semi-dry sausages may only receive light heating (e.g., Thuringer). Dry sausage is placed in "dry rooms" with controlled humidity for an extended period after fermentation. During this ageing process, the product loses moisture and the a_w falls to c. 0.88, which is sufficiently low to make the heat-treatment to control *Trichina* unnecessary.

In both semi-dry and dry fermented sausages the main hazard is the growth of *Staph. aureus* during the initial stages of the fermentation before the pH of the mix falls sufficiently to inhibit its growth.

It has been suggested that microbiological criteria for *Staph. aureus* (not more than 100,000 per gram) be applied at the conclusion of the fermentation process or at the beginning of the drying process. More benefit could be obtained by a controlled acidulation to below pH 5.4 (using starter cultures or chemical acidulants such as glucono-delta-lactone) than by microbiological testing. *Salmonella* derived from the raw ingredients may survive processing but is unlikely to multiply. Total counts criteria are inappropriate because of growth of fermenting organisms.

The American Meat Institute has recently formulated Good Manufacturing Practices in connection with the production of fermented sausages (AMI, 1982). A maximum pH of 5.3 is prescribed in both dry and semi-dry fermented sausages.

(e) *Cured meats packed under vacuum*

Some of these products support the growth of *Clostridium botulinum* (Pivnick and Barnett, 1965) and *Salmonella* (Davidson and Webb, 1972) at abuse temperatures. The developing flora in vacuum-packaged cured meats is composed almost entirely of lactic acid bacteria. Large

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numbers of these organisms may develop without objectionable organoleptic changes. Despite the fact that these products are often abused through inadequate refrigeration, outbreaks of staphylococcal foodpoisoning are seldom traced to them, probably because staphylococci fail to grow in competition with other microorganisms. Salmonellae are capable of survival during protracted storage of these products at 5°C and can grow when the meats are incubated at ambient temperature (Goepfert and Chung, 1970), but these products have not proved to be a significant *Salmonella* hazard.

It is assumed that sliced bacon will be heated prior to consumption and, in addition, that the growth of lactic acid bacteria will afford protection against growth of *Staph. aureus.* There is no evidence of food-borne disease from sliced bacon and thus criteria are not proposed.

Ham is sometimes involved in food-borne illness due to *Staph. aureus,* usually as a consequence of contamination during slicing or handling, followed by temperature abuse. Vacuum-packed sliced hams are rarely implicated. During storage of vacuum-packed meats, including hams, under refrigeration a large population of lactic acid bacteria develops in whose presence staphylococci fail to grow. There is no evidence that criteria at manufacture or at port of entry would reduce the hazard.

Frankfurters and bologna have also been implicated occasionally in food-borne illness, but control of the production process affords greater assurance of safety than would the application of criteria.

(f) *Cooked uncured meats*

Cooked uncured meats (e.g., roast-beef joints and cuts) are increasingly important in international trade. Their involvement in food-borne salmonellosis could be avoided by proper heat-processing, prevention of post-process contamination, rapid cooling, and subsequent appropriate time-temperature control in the home or food-service establishment. A criterion for *Salmonella* is proposed: case 12, $n = 20$, $c = 0$ (Table 20). There is no evidence that aerobic plate count (APC) limits would serve a useful purpose.

Considerable international trade exists in pasteurized comminuted meat products such as pates. At port of entry they frequently have high APCs and are occasionally associated with food-borne illness. Control is by adequate heat-processing, prevention of recontamination, and appropriate chill storage. Microbiological data are inadequate, but these products may contain *Salmonella.* If there is reason for concern,

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the appropriate sampling plan for *Salmonella* would be case 12, $n = 20$, $c = 0$ (Table 20).

(g) *Perishable cured canned meats*

The most important meat products of this kind in international trade are pasteurized canned cured hams and pork shoulders. A high proportion of these products are heated to centre temperatures of only 65 to 75°C. They should be transported under refrigeration, and should be labelled 'perishable, keep under refrigeration' or with words to similar effect. While the importance of testing such products is recognized, the high price of canned hams and similar foods is a serious obstacle to intensive examination. For these products, data on thermal processing, water supply, seam inspection, and chemical composition should be available, together with records of temperature during shipping. If all such data are satisfactory, no testing is necessary.

The Codex Alimentarius Committee for Processed Meat and Poultry Products is currently considering sampling plans for this category of products. Until the results become available the following is offered for guidance.

1. Sampling procedure at port of entry. If adequate production records are not available, sampling should be done according to the following scheme (see also Table 21).

a Measure the air temperature in the space between containers, preferably with an electronic measuring device.

b Take 10 containers at random from the lot. Identification of individual containers at this point is unnecessary. Examine the 10 containers for 'swells' and defects (see footnote, Table 21).

c If the temperature does not exceed 10°C or any specified lower temperature and if no swells or defective containers are found accept the lot.

If one or more defective or swollen containers are found, hold the lot and determine the cause by more extensive sampling (see Chapter 5, Section N, Investigational Sampling).

If the temperature exceeds 10°C or any specified lower temperature, or if there is reason to suspect that these temperatures have been exceeded during transport, proceed to step *d*.

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TABLE 21
Sampling plans for perishable canned cured meats (to be used when processing data are not available or are unsatisfactory)

	Test	Case	Plan class	*n*	*c*	Limit per gram *m*	*M*	Acceptance
Step 1	Visual inspection for swells and defective containers*	–	2	10	0	–	–	Reject if 1 or more defective cans are found. Proceed to step 2 if no defective cans are found.
Step 2	Measurement of air temperature between cans	–	2	10	0	–	–	Accept if temperature is below 10°C. If higher proceed to step 3.
Step 3	Determination of aerobic plate count in centre and periphery	–	3	5	2	10^3	10^4	See Section G of this chapter for interpretation of the presence of microorganisms.

* A defective container is a container which is of faulty manufacture, is improperly closed, or has been damaged in such a manner that may permit contamination of the contents following the heat-process (post-processing contamination).

d Take 5 containers from the warmer places in the lot and hold the lot. Proceed to step *e.*

e Identify the 5 containers selected under *d* and send them to a laboratory for microbiological examination. Transport should take place under refrigeration, at 10°C or less.

2. Laboratory analysis

a In the laboratory draw sample units from the 5 containers aseptically so as to obtain one unit from the centre and one from the periphery of each container.

b Examine for APC (ICMSF, 1978, p. 118). A direct Gram stain and an anaerobic count may sometimes be helpful. Rarely, the only bacteria present in large numbers are anaerobes.

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c Reject if an analytical unit from one or more of the 5 containers has a viable count exceeding 10^4 per gram. Also reject if one or two analytical units from 3 or more containers show a viable count higher than 10^3 per gram. Otherwise, accept.

d When rejection is indicated an investigation into the cause of the problem might be appropriate and the supplier informed of the results. It should be emphasized that the values for *m* and *M* in this context do not necessarily reflect any spoilage or health hazard, but merely indicate degrees of undesirable commercial practice. The limits have been established from data derived from very large numbers of analyses over several years and are known to be attainable under GCP. It has been found that at export counts are normally less than 10^2 per gram. If the lot does not meet this criterion, the decision on the action to be taken should be one of the options given in Chapter 2.

(h) *Shelf-stable cured or uncured canned meats*

Shelf-stable meats have undergone a treatment sufficient to guarantee the subsequent safety and stability of the product at normal ambient temperatures. Canned cured and canned uncured meat products are considered with other low-acid canned foods in Chapter 26.

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13

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*

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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

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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 timetemperature 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.

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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 entree 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

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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*	Limit per gram *M*
Cooked poultry meat, frozen; to be reheated before eating (e.g., prepared frozen meals)	*Staph. aureus*[b]	218	8	3	5	1	10^3	10^4
	Salmonella	163	10	2	5	0	0	
Cooked oultry meat, frozen, ready-to-eat (e.g., turkey rolls)	*Staph. aureus*[b]	218	8[c]	3	5	1	10^3	10^4
			9[c]	3	10	1	10^3	10^4
	Salmonella	163	11	2	10	0	0	
Cured and/or smoked poultry meat	*Staph. aureus*[b]	218	9	3	10	1	10^3	10^4
	Salmonella	163	11	2	10	0	0	
Dehydrated poultry products	*Salmonella*	163	11	2	10	0	0	
Raw chicken (fresh or frozen), during processing	APC	118 (20°C)	1	3	5	3	$5x10^5$	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.

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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

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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. Comminute 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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14

Sampling plans for feeds of animal origin and pet foods

Large quantities of feeds destined for animals and increasing quantities of dehydrated pet foods are in international trade. Feeds and pet foods have constituents of vegetable and animal origin, both of which can be contaminated with many kinds of microorganisms or their metabolites, including those of animal or human health significance. Although the absence of other pathogens in feeds cannot be guaranteed, the main organisms of concern are salmonellae. Fungal metabolites are potentially hazardous to animals and to human health through residues in foods of animal origin (ICMSF, 1978).

A. FEEDS

Animals ingesting feeds contaminated with *Salmonella* may contract clinical disease or subclinical infections and excrete salmonellae for long periods. This fact has become important with the current increase in world trade of feeds, and fish meal and meat-bone meal have been responsible for the spread of certain *Salmonella* serotypes, such as *Salmonella agona*, world-wide (Clark *et al*., 1973).

Animals excreting salmonellae may endanger public health either by direct contact (e.g., turtles in the home) or indirectly when foods of animal origin are contaminated as in slaughtering or milking. For discussions of factors in addition to contaminated feeds, such as the environment, see ICMSF, 1980, pp. 459-469; Riemann and Bryan, 1979; WHO, 1981; Silliker, 1980.

It is accepted that elimination of *Salmonella* from feeds will not guarantee the absence of *Salmonella* in animals. However, to assist in the reduction of the hazard, efforts should be made to reduce the contamination of animal feeds either by producing *Salmonella*-free

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feed components of animal and vegetable origin or by subjecting the compounded feed to a decontaminating treatment like certain methods of pelleting, heating, or irradiation (Statutory Instruments, 1981a, b; van Schothorst and Brooymans, 1982). However, criteria for livestock feed have not been proposed because they are unlikely to reduce the prevalence of *Salmonella* in animals effectively unless other measures are taken at the same time. Feed subjected to heating, irradiation, and some pelleting processes should be free from *Salmonella.* For such feeds a sampling plan $n = 10$, $c = 1$, or more desirably $c = 0$, is recommended. Feeds subjected to other pelleting processes, not primarily intended to destroy salmonellae, cannot be expected to meet this criterion.

Heating during the fat-extraction stage of production of proteinrich feed components of animal origin (e.g., fish meal) or of vegetable origin (e.g., soya flour, cotton-seed meal) yields products free from *Salmonella* and other heat-sensitive contaminants. However, post-process contamination frequently occurs, sometimes reintroducing *Salmonella* by cross-contamination with raw material and from common machinery as well as aerosols from grinders, dust, flies, and excrement of birds and rodents.

During processing good hygienic housekeeping, keeping dry areas of the factory dry, completely separating raw material and finished product, and preventing recontamination are the most important measures to ensure *Salmonella*-free products. Environment and line sampling are more effective in *Salmonella control* than testing end-products (ICMSF, 1980, pp. 459-469; van Schothorst and Oosterom, 1984). At port of entry, testing for the presence of *Salmonella* may identify contaminated consignments.

B. PET FOODS

Dehydrated, extruded, pelleted, and intermediate-moisture pet foods can be produced free from *Salmonella* even though some of the raw materials contain it. In contrast pet foods produced by blending such raw ingredients cannot be produced free from *Salmonella* because no bactericidal treatment is applied (ICMSF, 1980; van Schothorst and Brooymans, 1982). Irradiation might be considered in this case (WHO, 1981).

Canned pet foods are not dealt with in this chapter because methods for control do not differ in principal from those described in Chapter 26 (Shelf-Stable Canned Foods).

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TABLE 23

Sampling plans and recommended microbiological limits for pet foods[a]

Product	Test	Method Reference[b]	Case	Plan	*n*	*c*	*m*	*M*
Intermediate moisture pet foods	*Salmonella*	163–172	11	2	10	0	0	–
Dry pet foods not to be reconstituted	*Salmonella*	163–172	11	2	10	0	0	–
Dehydrated pet food to be reconstituted	*Salmonella*	163–172	12	2	20	0	0	–

a Case 11 could also be used for monitoring animal feeds for *Salmonella* (see this chapter).

b Refers to page numbers in ICMSF, 1978; see also ISO 6579 (1981).

Frozen raw meat of origins not covered in Chapter 11 (e.g., horse and kangaroo meat) and meat offals are shipped as pet food to many countries of the world. These pet foods may contain *Salmonella* and may be a source of contamination of the kitchen as well as sources of infection for animals. These pet foods are occasionally eaten by humans, though this may be regarded as misuse of the product. Taking into account the present status of the *Salmonella* problem (FAO/WHO, 1979), criteria for these pet foods at the port of entry are not warranted (WHO, 1981; cf. Chapter 11, Raw Meats; Chapter 13, Poultry). However, monitoring of product for investigational purposes would provide information on the magnitude of possible problems.

C. CHOICE OF MICROBIOLOGICAL CRITERIA

If the same considerations are applicable to the selection of cases for *Salmonella* in animal feeds as for human foods, case 11 and case 12 would be chosen for the various categories of feeds or pet foods. This choice is reasonable because the ranges of minimal infective doses which provoke clinical disease in humans and in animals are similar.

Case 11 was previously chosen for animal feeds and dry and intermediate-moisture pet foods, because their low levels of water activity prevent multiplication of salmonellae (ICMSF, 1980, p. 469). For dehydrated pet foods which are reconstituted and left at room temperature, case 12 applies (ICMSF, 1980, p. 469) (Table 23). Feed components of animal origin are not included.

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The criterion for *Salmonella* in animal feeds should therefore, in principle, be case 11, $n = 10$, $c = 0$, $m = 0$. However, many feeds in international trade cannot meet this criterion. Decontamination (e.g., by irradiation, heating, or pelleting) would increase costs; and adequate facilities for these processes are not yet widely available. Therefore, it is recommended that consignments be monitored for the presence of *Salmonella* for epidemiological or investigational purposes.

In Chapter 9, guidance on sample collection, preparation, and analysis is given. The method of ICMSF, 1978, pp. 163-172, is recommended. It is, in principle, in agreement with ISO Standard No. 6579.

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15

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 ultrahigh-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)

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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 icecream 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

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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

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TABLE 24

Sampling plans and recommended microbiological limits for dried milk and cheese

Product	Test[d]	Method reference[a]	Case	Plan class	*n*	*c*	Limit per gram *m*	Limit per gram *M*
Dried milk	APC	118	2	3	5	2	$3x10^4$	$3x10^5$
	Coliforms	126–131	5	3	5	1	10	10^2
	Salmonella,[e] normal routine	163	10	2	5	0	0	
			11	2	10	0	0	
			12	2	20	0	0	
	Salmonella,[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]	*Staph. aureus*	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.

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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 cheesemaking 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

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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

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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 Schouwenberg-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 Schouewenburg-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* (entero-pathogenic, 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

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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 cheesemaking 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 BairdParker (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

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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

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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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16

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 (I) 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-

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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 heatresistant 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

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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 highsalt 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 a-amylase in whole-egg melange, are practised with good effect in some countries and in particular in the United Kingdom.

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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*	Limit per gram *M*
APC	118	2	3	5	2	$5x10^4$	10^6
Coliforms	126–131	5	3	5	2	10^1	10^3
Salmonella,[d]	163	10	2	5	0	0	–
normal routine		11	2	10	0	0	–
		12	2	20	0	0	–
Salmonella,[d]	163	10	2	15	0	0	–
for high-risk		11	2	30	0	0	–
population		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.

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(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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17

Sampling plans for fish and shellfish

Fish and shellfish are taken in nearly all the salt and fresh waters of the world. The annual catch is estimated at about 71 million tonnes, of which 72% is for direct human consumption (FAO, 1979). The catch is made up of a great diversity of species (800 species are listed by FAO), which range over several phyla from primitive molluscs to marine mammals. A large proportion of the fish catch enters international trade, moving world-wide and involving products from both developing and industrialized countries. This is exemplified by the fact that 162 countries in 1978 exported 32% of their total production of 17.63 million tonnes of seafood (FAO, 1979).

A. PUBLIC HEALTH ASPECTS OF FISH AS HUMAN FOOD

Despite the fact that fishery products are of diverse origin and include many species of animals, the composition of the naturally occurring (autochthonous) microflora is similar in most cases. The microorganisms most commonly encountered are typical of the free-living forms found in water and sediment and rarely include any species of mammalian pathogens (ICMSF, 1980, pp. 575-578). Hence fish caught in waters not polluted by human or animal wastes are free from intrinsic microbiological hazard when handled according to good commercial practice.

Indeed fish and other free-swimming marine animals do not usually carry those organisms generally considered to be typical of the mammalian microflora, including *Escherichia coli*, the 'faecal coliforms', and enterococci. The presence of human enteric organisms on marine food products is clear evidence of contamination from a terrigenous source.

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Fish and shellfish products are a minor source of bacterial food-borne disease in North America, the United Kingdom, and Australia (Todd, 1978), but there is a continuing high relative incidence of bacterial food-borne disease from fish products in Japan and probably in Southeast Asian countries where fish are commonly eaten raw or with little cooking (Sakazaki, 1979). In most cases the aetiologic agent is a vibrio though some botulism due to improperly prepared products also occurs in Japan, Poland, and the USSR and in other North Pacific rim countries including northern Canada and the USA (Hobbs, 1976).

A number of different *Vibrio* species have been implicated in food-borne illness resulting from eating seafood. These include *V. parahaemolyticus*, *V. vulnificus*, *V. cholerae*, *V. fluvialis*, and *V. alginolyticus*. Only *V. alginolyticus* occurs consistently on the living animals, and the actual involvement of this organism in human gastroenteritis is somewhat doubtful. *Vibrio parahaemolyticus* is relatively common in warm-water areas and is a cause of gastroenteritis in coastal regions of India and in Japan and countries of Southeast Asia. Disease due to *Vibrio* species is usually associated with situations where large populations of living *Vibrio* species are present on Seafoods, either in the raw state where fish and shellfish are eaten uncooked or in recontaminated cooked products which are stored at temperatures that permit growth. However, infective population levels of vibrios can occur on freshly harvested marine animals, particularly where water quality is poor and temperatures are high. Experiments in the USA in connection with the investigation of cholera from ingestion of cooked crabs suggest that normal cooking practices may not always destroy vibrios (CDC, 1981).

Non-proteolytic strains of *Clostridium botulinum* types E, B, and F occur in small numbers in the intestines of fish and may be transferred to flesh during evisceration or other primary processing. Botulism has been caused by ingestion of botulinal toxin produced in improperly fermented products in Japan, in marine products in arctic North America, in hot smoked fish held at improper temperatures, and in imperfectly canned seafoods (Sakaguchi, 1979).

Raw or processed seafoods are in general excellent substrates for the growth of most common bacterial agents of food-borne disease if held at improper temperatures. It is important therefore to avoid contamination of these foods during preparation and storage and to hold them at chill temperatures.

There is a particular problem in the case of sessile molluscs such as oysters, mussels, and clams which grow in and are harvested from

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inshore waters most likely to be contaminated by human or animal wastes. These organisms feed by passing large volumes of water over selective mouthparts which strain out small particles from the water. They select and concentrate bacteria from the environment and when held in polluted waters may become dangerous vehicles of both pathogenic bacteria and viruses. Contaminated shellfish may be a source of hepatitis A virus as well as of more generally recognized bacterial enteric pathogens (ICMSF, 1978a, p. 65).

At present the only practical control measure is through bacteriological testing of growing-area waters for coliforms and *E. coli,* supplemented by testing of harvested shellfish for aerobic plate count (APC), coliforms, and *E. coli;* shellfish harvesting is closed when bacterial counts rise unusually (FAO/WHO, 1978). This system has proved to be very effective in countries that use it; and food-borne disease, including viral hepatitis, caused by eating shellfish is almost invariably due to animals taken from unregulated or closed beds. However, poor correlation has been reported between virus incidence in shellfish and indicator bacteria counts in water, and a direct test method for hepatitis A virus would be desirable. In cases where the level of contamination is low, relaying in clean waters or active depuration will effectively clean bivalves of undesirable bacteria although virus and some vibrios may persist. The most effective control is mandatory heat-treatment of bivalve molluscs to destroy bacteria and viruses, but this is often impracticable. In any case shellfish in commerce should be tested for enteric indicator bacteria as an index of bacterial quality although this will not assure safety if the animals are collected from unregulated beds since the test method cannot be expected to give reliable indications of the presence or absence of viruses.

Fish and shellfish poisonings are due to toxins in the flesh of animals either intrinsically present or derived from the food they eat. This is true for puffer fish poisoning (caused by tetradotoxin), ciguatera (caused by ciguatoxin and possibly other toxins), and paralytic shellfish poisoning (PSP) (caused by saxitoxin and other toxins). Scombroid toxin is an exception and is apparently due to products of bacterial growth on tunas, mackerels, and related genera after their death. Histamine is most commonly identified as the toxin. These conditions generally require chemical or biological analysis for toxic substances, and therefore no recommendations for criteria are included. However, individuals and agencies concerned with the safety of seafoods in international commerce should be aware of the potential hazard of the toxicants and should include toxin tests where appropriate.

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B. THE BASIS FOR SPECIFIC SAMPLING PROPOSALS

The number of outbreaks of food-borne disease from consumption of seafood during the years since the first volume of the book was published has, apart from chemical fish poisoning, shown little change. A higher incidence of bacterial and viral disease from fish and shellfish continues to occur in India, Japan and Southeast Asian countries than in Europe or North or South America. The microorganisms of principal concern on a world-wide basis appear to be vibrios and *C. botulinum,* with hepatitis A virus and enteric bacterial pathogens important in molluscan shellfish. The more common food-borne pathogens such as *Salmonella, Staphylococcus aureus,* and *C. perfringens,* which have been reported in recent years as causes of fish-borne disease, are not typical environmental contaminants but generally gain access to the food during processing or food-service operations.

Most seafood products are handled and stored under conditions which preclude growth and toxin production by *C. botulinum.* Botulism has been particularly associated with improperly processed canned fish, hot smoked fish, and certain pickled and fermented products (Hobbs, 1976). The increasing use of modified-atmosphere storage to extend the shelf-life of fish may increase the risk of growth of C. *botulinum* (Eklund, 1982). In the case of products at risk, proper processing (e.g., heat-treatments to destroy the organism) and control of storage conditions to prevent germination and outgrowth of *C. botulinum* spores are necessary to ensure safety of the product. The types of *C. botulinum* of greatest concern are the non-proteolytic psychrotrophic types B, E, and F. While *C. botulinum* is recognized to be a significant pathogen associated with fish, no criteria are proposed. The detection of *C. botulinum* on a food does not signal an immediate hazard but only indicates potential risk. Microbial counts or simple determination of the presence of *C. botulinum* spores serve no useful purpose. Even in botulism outbreaks toxin tests, not counts of cells or spores, are necessary to confirm involvement of the suspect food.

For the purposes of applying microbiological criteria, seafood products are grouped as follows:

(a) *Fresh, frozen, and cold smoked fish,* including fresh and frozen (including shipboard frozen) marine and freshwater fish, fish blocks, comminuted fish blocks, scallops, cold smoked fish.
(b) *Precooked breaded fish,* including breaded fillets, portions, fish sticks, and fish cakes.

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(c) *Frozen raw crustaceans,* including frozen raw shrimp, prawns, and lobster tails.
(d) *Frozen cooked crustaceans,* including frozen cooked shrimp (peeled or unpeeled), prawns, and lobster tails.
(e) *Cooked crabmeat:* cooked or pasteurized, chilled or frozen, whole crab, or picked meat.
(f) *Molluscs*: fresh and frozen mussels, clams, oysters in shell or shucked.

Note that seafoods not included in these categories are omitted because insufficient microbiological data are available. This grouping differs from that in the previous edition of this book (ICMSF, 1978b) in that some product groups have been combined because the criteria were the same. *Escherichia coli* has been accepted as a better criterion of potentially hazardous contamination than 'faecal coliforms', and is now used here for fish and shellfish. This change conforms with criteria used for other commodities and makes possible the use of rapid and more objective analytical methods based on membranes (Holbrook *et al*. 1980; Sharpe *et al*. 1981).

Vibrio parahaemolyticus, Salmonella, and *Staphylococcus* in (a), (b) and (c) have been made optional criteria to be applied in special circumstances, and their appropriate use is indicated in footnotes to Tables 26 and 27 below.

In general there has been little change in the assignment to cases within the new groupings except that *V. parahaemolyticus* is allocated to case 7, 8, or 9 rather than to case 10, 11, or 12 in accordance with the revision on Table 6 (p. 43). A 3–class plan is now proposed for *V. parahaemolyticus.*

Neither hot smoked fish nor semi-conserved fish products have been included. They have been associated with a number of outbreaks of food-borne illness including botulism, staphylococcal intoxication, and salmonellosis, and in the case of hot-smoked mackerel scombroid poisoning, but there is insufficient evidence that microbiological criteria would contribute significantly to preventing outbreaks attributed to those products.

Dried fish are important to world trade. These are produced in both temperate and tropical regions of the world, often under conditions of poor hygiene, and are frequently shipped with minimum protection against casual contamination. Unfortunately, little has been published on the microbiology of dried fish or their involvement in food-borne illness. Therefore, microbiological criteria are not proposed.

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The selection of criteria for seafoods has been made with due attention to variability factors such as goegraphical source and handling, storage, and processing practices (ICMSF, 1980, pp. 567-605; FAO/WHO, 1976, 1978; WHO, 1974) and with consideration of existing national and international criteria (Codex, 1977).

Microbiological criteria for molluscs are dependent on proper control of the growing waters and harvesting process. A list of microbiological criteria used in a number of countries is presented in the Codex Alimentarius Commission's *Recommended International Code of Hygienic Practice for Molluscan Shellfish* (FAO/WHO, 1978), which includes the U.S. standards.*

In selecting criteria in Table 27 we have been guided by actual levels of counts reported in seafood products in international trade – particularly products moving in significant quantity – and the reported occurrence of food-borne disease from various fish products.

A realistic sampling plan should allow the inspecting agency to make the necessary examinations without excessive expenditure of manpower and expense. Concern with expenditure is particularly important with fish and fishery products where there is a large and continuing flow of yearly shipments with a great diversity in the size, weight, and type of package. Often the history of handling, processing, packaging, storage, and transportation is unknown. In addition, the perishability of many fish products requires that tests be completed quickly in order to minimize delay within the usual trade channels. With fish and fishery products, counts at 20 to 25°C are probably the most useful indicators of incipient spoilage (utility) because of the psychrotrophic nature of the spoilage flora. Hence we recommend that the APC be carried out at 25°C. The APC at 35°C is frequently of the order of one-tenth of the count at 25°C (Liston, 1957; Shewan, 1977).

* Maximum APC levels are 100,000 per gram in Denmark and 500,000 per gram in the USA; there are no APC criteria in France, Italy, Netherlands, and the UK.

DENMARK APC (20°C): n = 10, m = 100,000 per gram (2–class); *E. coli* I: n = 10, absent in 1/5 ml; *Salmonella*: n = 10, absent in 1/5 ml.

FRANCE *E. coli*: n = 5–10, absent in 1 ml macerated shellfish or up to 2 *E. coli* per ml for shellfish consumed cooked; *Salmonella*: n = 5–10, 25 g tested, no *Salmonella* in 25 g.

ITALY *E. coli*: n = 10, <6 *E. coli* per gram (by MPN).

NETHERLANDS *E. coli*: n = 10, for hold pending investigational sampling, m = 4 per ml, for rejection m = 15 per ml.

UK A commonly operated guideline <2 *E: coli* per ml.

USA APC (35°C): <500,000 per gram; faecal coliform: <2.3 per gram.

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Although in certain countries Good Commercial Practice (GCP) is commonly attained by plants processing Seafoods, no information is available for a significant proportion of producers whose products are in international trade. Although the sampling procedures that are recommended attempt to take cognizance of this fact, the first consideration has been the safety of the consumer. It is, however, important that safety tests (e.g., for *Salmonella)* should not be confused with indications of shelf-life or quality such as the APC. It should be recognized that the bacterial counts on raw fish vary very widely both geographically and because of differences in the duration of shipboard storage, which greatly affects counts on processed products and makes the application of criteria with unreasonably low counts with little scope for variation undesirable. Several items of fish and fishery products in international trade have high *APC*s, but there is no evidence that they have given rise to any corresponding health hazard. Accordingly, the recommended APC limits reflect present accepted practice in the fish industry. The faecal coliforms and *E. coli* are particularly useful as indicators of contamination and mishandling of seafoods since the organisms are absent from fish and crustaceans at the time of capture (except in grossly polluted waters). Moreover, fish and shellfish should be held at temperatures below those which support growth of *E. coli* or faecal coliforms when GCP is followed (see FAO/WHO, 1976, 1978). Thus the presence of detectable numbers of these organisms indicates contamination while relatively large numbers suggest temperature abuse in product handling.

In view of the increasing contamination of inshore marine waters with domestic sewage and hence of the increased probability of contamination of fish and crustaceans taken in such waters, examination for enteric viruses might be as important as for *Salmonella* (Clarke and Chang, 1959; Murphy *et al.*, 1979; Bryan, 1980). However, the lack of appropriate methodology prevents this (Cliver, 1979).

C. SAMPLING PLANS

The sampling plans selected for seafood products are outlined in Table 26. Criteria for APC and *E. coli* counts are recommended in all instances. Staphylococcal criteria are recommended for crabmeat and frozen cooked crustaceans because staphylococcal food-poisoning is a recognized hazard in these products. Additional criteria for *V. parahaemolyticus, Salmonella,* and *Staphylococcus* are recommended for breaded or precooked products, warm-water products, and molluscs.

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This procedure is recommended to avoid unnecessary routine application of expensive and time-consuming tests except where the known or suspected origin of the seafood products suggests a hazard may exist.

For economic reasons the number of sample units to be examined, *n*, is limited to five except for *Salmonella* and, in one instance, for *V. parahaemolyticus*. Even five samples would tax the laboratory facilities for routine testing available in many areas which import large quantities of fish. Testing for salmonellae is recommended for some crustaceans and fish harvested from warm-water areas, but should be applied on a selective basis taking into account the origin of and processing applied to the products.

Similarly, while *V. parahaemolyticus* is of widespread occurrence in inshore marine waters, sediments, and marine animals, it is usually present in significant numbers only on seafoods taken from tropical or subtropical waters or from temperate-zone waters in summertime. Routine testing for *V. parahaemolyticus* enumerates both non-pathogenic and enteropathogenic strains, but it is unusual, even in foods implicated in outbreaks of food-borne illness, to find high numbers of the pathogenic serotype.

Except for crabmeat, testing for *Staph. aureus* is also recommended only in special circumstances, because most seafood products carry few staphylococci. Where testing is indicated the recommendations are based on demonstrated hazard for that category of food under the conditions specified. It should be noted that counts on selective media for both staphylococci and *V. parahaemolyticus* are often erratic below, or near, 10^2 per gram (see Table 27, footnote *f*). Reproducibility improves at higher numbers.

An aerobic plate count is recommended for all products because of its usefulness as an indicator of utility and the condition and length of storage of products prior to stabilizing processes such as freezing. Most aquatic animals at the point of harvest have counts in the region of 10^2–10^5 organisms per gram. There are exceptions including some tropical shrimp, molluscs, and freshwater fish. An increase in APC to levels in excess of 10^6 per gram is usually indicative of long storage at chilling temperatures or temperature abuse prior to freezing. Thus APC is indicative of general quality and to a lesser extent of handling and storage procedures.

Escherichia coli testing is also included in all cases, and it is noted that faecal coliform counts may be used instead where this is the preferred method. The usefulness of this test for seafoods was indicated earlier and is based on the fact that these organisms should be absent

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Allocation of fish and shellfish products to case

Product	Condition	Test	Case
Fresh and frozen fish[a]	Usually cooked before eating; hazard reduced or low	APC *E. coli*	1 4
Precooked breaded fish products[b]	Usually heated before eating but heating may be insufficient to destroy pathogens; low health hazard	APC *E. coli*	2 5
Frozen raw crustaceans[c]	Usually cooked prior to eating; hazard reduced	APC *E. coli*	1 4
Frozen cooked crustsceans[d]	Little or no cooking before eating; frequently held under conditions after thawing which cause increased moderate hazard	APC *E. coli* *Staph. aureus*	2 5 8
Cooked, chilled, frozen crabmeat[e]	Not cooked prior to consumption; known health hazard; hazard increased	APC *E. coli* *Staph. aureus*	3 6 9
Fresh and frozen bivalve molluscs[f]	Often eaten raw or with light cooking only	APC *E. coli*	3 6

a For fish known to derive from inshore or inland waters of doubtful bacteriological quality, particularly in warm-water areas and where fish are to be eaten raw, it may be desirable to test for *Salmonella* by case 10 and *V. parahaemolytlcus* by case 7. Smoked fish may be tested for *Staph. aureus* by case 7.

b Staph. aureus may also be tested for by case 8, for cooked fish products likely to be mishandled.

c Staph. aureus may be tested in breaded products using case 7. *Salmonella* and *V. parahaemolyticus* tests may be applied to products from warm-water areas and *V. parahaemolyticus* tests in products from temperate regions in summer using case 8, if likely to be eaten raw.

d In view of the storage conditions cases 3, 6, and 9 should apply. However such a load of microbiological testing would be impracticable for most fish-testing laboratories. Also, some survey data indicate that cases 3, 6, and 9 would reject an unacceptably large proportion of the product in international trade. Experience suggests that the criteria recommended above would assist in maintaining hygienic standards. In the case of cooked crustaceans originally harvested from waters and processed in regions of known high environmental hazard, *Salmonella* and *V. parahaemolyticus* may be tested for according to cases 11 and 8 respectively.

e Crabmeat from animals harvested from waters above 15°C may be tested for *V. parahaemolyticus* using case 9.

f I. These criteria are to be used only for molluscs from approved harvesting areas where waters are free from enteric bacteria or virus contamination and there is no significant contamination by toxic metals or toxic and carcinogenic chemicals which may be accumulated by animals. Molluscs from non-approved areas should enter trade only after processing by a treatment to destroy enteric bacteria and viruses and may be tested for dangerous chemicals before distribution.

II. *Salmonella* should be tested for when there is concern for bacteriological safety using case 12, and *V. parahaemolyticus* should be tested for in molluscs from endemic areas and harvested from warm waters using case 9.

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TABLE 27
Sampling plans and recommended microbiological limits for seafoods

Product	Test[a]	Method reference[b]	Case	Plan	*n*	*c*	Limit per gram or per cm^2 *m*	*M*
Fresh and frozen fish and cold-smoked	APC	115	1	3	5	3	$5x10^5$	10^7
	E. coli	133[e]	4	3	5	3	11	500
Precooked breaded fish	APC	115	2	3	5	2	$5x10^5$	10^7
	E. coli	133[e]	5	3	5	2	11	500
Frozen raw crustaceans	APC	115	1	3	5	3	10^6	10^7
	E. coli	133[e]	4	3	5	3	11	500
Frozen cooked crustaceans[c]	APC	115	2	3	5	2	$5x10^5$	10^7
	E. coli	133[e]	5	3	5	2	11	500
	Staph. aureus	220	8	2[f]	5	0	10^3	–
Cooked, chilled, and frozen crabmeat [d]	APC	115	2[g]	3	5	2	10^5	10^6
	E. coli	133[e]	6	3	5	1	11	500
	Staph. aureus	220	9	2[f]	5	0	10^3	–
Fresh and frozen bivalve molluscs	APC	115	3	2	5	0	$5x10^5$	–
	E. coli	133[e]	6	2	5	0	16	–

a **For additional tests, conditions of election are given in footnotes to Table 26, p. 189. These tests are described in Table 27 (supplement), p. 191.**

b Refers to page numbers in ICMSF, 1978a.

c Note that although *Staph. aureus* is a moderate hazard, it is infrequently found at high levels and a 2–class plan $n = 5$, $c = 0$ is recommended to reduce analytical work.

d Refer to note *c* for explanation of *Staph. aureus* case assignment. APC for frozen products only.

e See text.

f A 3–class plan would require *m*–values of about 10^1, which are not detectable with current methodologies.

g In this product APC is not related to hazard.

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TABLE 27 (supplement)

Additional tests to be carried out when appropriate (refer to footnotes, Table 26, and see text)

Product	Test	Method reference	Case	Plan	*n*	*c*	Limit per gram or per cm^2 *m*	*M*
Fresh and frozen fish and cold-smoked	*Salmonella*	163	10	2	5	0	0	–
	V. parahaemolyticus	202	7	3	5	2	10^2	10^3
	Staph. aureus	220	7	3	5	2	10^3	10^4
Precooked breaded fish	*Staph. aureus*	220	8	3	5	1	10^3	10^4
Frozen raw crustaceans	*Salmonella*	163	10	2	5	0	0	–
	V. parahaemolyticus	202	8	3	5	1	10^2	10^3
	Staph. aureus	220	7	3	5	2	10^3	10^4
Frozen cooked crustaceans	*Salmonella*	163	11	2	10	0	0	–
	V. parahaemolyticus	202	8	3	5	1	10^2	10^3
Cooked, chilled, and frozen crabmeat	*V. parahaemolyticus*	202	9	3	10	1	10^2	10^3
Fresh and frozen bivalve molluscs	*Salmonella*	163	12	2	20	0	0	–
	V. parahaemolyticus	202	9	3	10	1	10^2	10^3

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from the freshly caught raw material. They also provide inferential evidence of temperature abuse in handling of the product.

Attention is drawn to the warning footnote in Table 26 concerning standards for molluscs. The hazard associated with these seafoods when unprocessed and of unknown origin is unpredictable and yet potentially so high that no simple set of criteria can ensure a safe product for the consumer. It is essential that the condition of the growing waters from which the animals are harvested be known before hazard can be assessed. Also be aware of the possibilities of paralytic shellfish poisoning and poisoning from other shellfish toxins in certain areas of the world.

The sampling plans and bacteriological criteria are listed in Table 27. For fresh and frozen fish, *m* for APC has been reduced to 500,000 per gram from 1,000,000 per gram because this value is well within the reach of GCP products in most situations. In a few cases where fish are taken in tropical waters or inshore waters heavily contaminated with bacteria this level may not be attainable. For such situations it is recommended that, if the other bacterial safety indicators are within limits, a high *m* may be accepted, since this criterion relates primarily to utility. The *m* for cooked fish has also been set at 500,000 per gram and it is recognized that this is slightly high since most regulatory agencies set their limit for cooked products at 100,000 per gram. Nevertheless, this figure recognizes the actual situation in international trade, and it has not been shown that products with higher counts are associated with food-borne illness. The *m* for APC for raw crustaceans is set at 1,000,000 per gram, recognizing the high count on much of the shrimp in international trade. However, small pink shrimp from cold northern and southern waters generally have much lower counts, and an *m* of 500,000 per gram might be applicable for them. Cooked crustaceans have been allocated an *m* for arc of 500,000 per gram. Again this is somewhat high for a cooked product but more representative of the world trade situation than 100,000 per gram or less. This and other considerations have led to cases 2, 5, and 8 being proposed (see Table 26, footnote d). The values for molluscs represent a compromise among the standards listed for various countries in the *Recommended International Code of Hygienic Practice for Molluscan Shellfish* (FAO/WHO, 1978). They are probably excessively strict for products to be cooked before eating but, since molluscs are often eaten raw or lightly cooked, the more stringent view has been accepted.

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D. SAMPLING PROCEDURES

For general directions on collecting sample units and handling analytical units, see Chapter 9. In routine analysis, collect 5 sample units (see Table 27; 10 are needed in certain cases) from each lot (or consignment) and in each case take at least twice as much food as will be required for the laboratory analysis. Use 0.1% peptone water with salt (ICMSF, 1978a, p. 320) as the diluent for all tests; for fish and fish products, experience has shown that this is the best diluent.

(a) *Iced or chilled raw fish*

1 Whole fish in international trade is normally destined for further processing and is not usually tested bacteriologically. Where whole fish are to enter directly into the retail market, bacteriological tests are normally directed at obtaining estimates of bacterial populations on skin surface for large fish and total bacterial count per unit weight for small fish. Sample units usually consist of single fish in the case of large fish and one or more fish in the case of small fish. Surface samples may consist of swabs taken over a prescribed surface area (e.g., 200 cm^2), or of an aseptically excised area of skin including a minimum of underlying tissues. Shake the swab or skin tissue thoroughly in 10 ml of peptone diluent and proceed with analyses as described in ICMSF, 1978a, p. 115 or 118. Express results in counts per unit area (e.g., per cm^2).

2 For chilled or iced fillets of small fish, take a whole fillet for each sample unit; for large fish such as halibut take a representative portion (e.g., three sections from different parts of the fillet). In the laboratory weigh out an analytical unit of 100 g from each sample unit and proceed with blending, dilution, and analysis as in 1. The initial dilution in blending can range from 1:3 to 1:10.

3 For scallops in ice, collect one sample unit from each of five containers. Weigh out 25 g analytical units from each sample unit and proceed with blending, dilution, and analysis as in 1.

(b) *Frozen fish*

Frozen fin fish products are normally shipped as blocks of whole gutted fish, fillet blocks, comminuted fish blocks, or consumer packages of fillets or other prepared material.

1 Large fish such as salmon, tuna, swordfish, or halibut may be

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shipped as frozen whole fish (usually gutted) with or without heads. Sampling and bacteriological testing of such products is difficult and generally is not done. In certain cases imported products of this type are detained until examination of a sample from subsequent processing (e.g., steaking operation) has been shown to be satisfactory. Examination of the processed sample follows the methods given in 2 below. 2 Sample units of wholesale lots of frozen fish may consist of individual fish or entire blocks, cartons, or institutional packages; they may be pieces cut, sawn, or drilled from these larger units (preferably during a primary processing operation in the receiving plant). In the laboratory, thaw sample units for 3 hours at room temperature (20-25°C), overnight in a chilled room (2-5°C), or in sealed plastic pouches under running water, and weigh out 100 g analytical units. Whenever possible the analytical unit should be blended with diluent before becoming completely defrosted. The temperature of the product should not rise above 5°C prior to analysis. Conduct blending, dilution, and analysis as in 1. The initial dilution can range from 1:3 to 1:10.

3 For sampling retail packages of frozen fish, use 100 g analytical units, and treat as in 2 above.

4 Sample and analyse frozen raw or cooked shrimp, prawns, and lobster tails, and frozen raw breaded shrimp (items 3, 4, 5 in Table 27) as described in 2 and 3 above.

(c) *Cooked and cold smoked fish*

Sample and analyse cold smoked fish as described for raw (fresh or frozen) fish above. Sample cooked fish by cutting portions and blending at least 100 g with diluent. Then proceed as for raw fish.

(d) *Molluscs*

For shellstock. Clean off the surface using a stiff brush and running water of potable quality. Open the shell using a sterile instrument to cut the adductor muscle and transfer the shell contents (liquor and tissue) to a sterile container using aseptic technique. Dilute with an equal weight of peptone diluent (ICMSF, 1978a, p. 320) and proceed according to ICMSF, 1978a, p. 115. For the *E. coli* count follow the procedures in ICMSF, 1978a, p. 126 et seq.

For shucked meats. Dilute with an equal weight of peptone diluent and proceed as described above.

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18

Sampling plans for vegetables, fruits, and nuts

Vegetables and fruits are important in international commerce mainly as fresh, frozen, dried, or canned products. Ground and tree nuts are also shipped internationally. The range of products is enormous, and with the continued introduction of new varieties it is impossible to discuss all hazards presented by them and all requirements of Good Manufacturing Practice (GMP). Therefore only the broad classes of these commodities are considered, together with yeasts, and single-cell proteins. For further information on these commodities and a detailed discussion of their microbiology see ICMSF, 1980, pp. 606-642.

A. RAW VEGETABLES AND FRUITS

Many vegetables and fruits are not cooked before eating. There is an increasing market for fresh vegetables which are chopped, packaged, then chilled or frozen, and intended to be consumed uncooked in the form of salads. Such preparations are subject to more contamination than are unchopped raw vegetables.

Human and animal enteric pathogens (except soil-borne spore-formers such as *Clostridium perfringens* and *Bacillus cereus*) are usually absent from fresh vegetables at harvest, unless they have been fertilized with human or animal wastes or irrigated with water containing such wastes. A few outbreaks of human disease as a result of eating raw vegetables contaminated by waste water have been reported (Bryan, 1977). Recently an outbreak of listeriosis was caused by contaminated cole-slaw (cabbage) (HWC, 1981). Unclean containers may cause additional contamination as demonstrated by the detection of salmonellae on produce packed in wooden crates formerly used to ship

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iced poultry (NCDC, 1968). Further details on the pathogens of concern and the products involved are given by ICMSF, 1980.

Raw vegetables intended to be cooked prior to eating are less hazardous, because the pathogens contaminating them, other than bacterial spores, are mostly heat-sensitive. Spores are of little concern as a health hazard in the raw product but may become a problem in the cooked product if it is subjected to time-temperature abuse. Nevertheless, raw vegetables and fruits may introduce pathogens into processing plants and kitchen environments, and contaminate other foods.

Good agricultural practices in growing crops, combined with acceptable hygienic methods during harvesting, packing, and transporting of vegetables and fruits, are far more important than microbiological testing. Therefore, adequate knowledge of fertilization, irrigation, and harvesting and washing practices in the production area is essential.

Vegetables likely to be eaten raw should not be obtained from areas where these practices are known, or suspected, to be unhygienic. While the use of potable water to wash and freshen the harvested vegetables is useful both in commercial practice and in the home, the effects of a water rinse on the level of contamination should not be overestimated. Even thorough commercial washing can remove only c. 90% of the surface flora, which means that 10^5 organisms per gram or cm^2 will often remain on the surface of produce after washing. Residual water may support extensive microbial growth during further storage.

Several appropriate codes of hygienic practice and good manufacturing procedure are available (e.g., Codex, 1983; FDA, 1969).

Routine microbiological examination of raw vegetables and fruits is unlikely to reduce hazards to any great extent and is not recommended. However, tightened inspection may be appropriate on lots suspected of being implicated in food-borne illness, or of having been produced under hygienically unsatisfactory conditions (see Chapter 5 Section O: Tightened Inspection).

B. FROZEN VEGETABLES AND FRUITS

Prepacked frozen vegetables and fruits continue to be popular convenience foods and are in international trade. These products have a commendable microbiological safety record. Blanching, a commonly used procedure, intended primarily to inactivate degradative enzymes, usually kills any vegetative form of pathogens contaminating surfaces of vegetables and fruits. Therefore, the bacteriological condition of such products is primarily a reflection of the hygienic practices employed

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after blanching. In many instances, however, recontamination of the blanched products occurs.

Although sampling plans for aerobic plate counts (APC) and *Escherichia coli* were recommended in the first edition of this book, the Commission no longer supports the need for the APC recommendation. Without information on the history of a product and its processing conditions, it is impossible to interpret the results of an APC performed at a port of entry. If this information is available (e.g., to industry) appropriate limits can be established and case 1 or 2 would be applicable depending on whether or not the product is to be cooked.

Escherichia coli is usually an indicator of faecal contamination so a criterion (case 5) is proposed for both blanched and unblanched products having a pH value greater than 4.5 (Table 28). Although blanching may diminish the level of contamination, recontamination can occur during subsequent handling. Products having a pH value of 4.5 or less are unlikely to cause problems because the low pH accelerates die-off during frozen storage and inhibits growth in thawed products. A sampling plan for *Salmonella* is not recommended because there is neither epidemiological nor laboratory evidence that *Salmonella* occurs in these frozen products.

C. CANNED VEGETABLES AND FRUITS

Both acid and low-acid canned vegetables and fruits are shelf-stable products whose safety is ensured by a heat process, providing that all canning procedures are in accordance with GMP. No sampling plans are proposed for these products (see Chapter 26: Shelf-Stable Canned Foods).

D. DRIED VEGETABLES AND FRUITS

Information on the microbiology of dried vegetables and fruits is scarce (ICMSF, 1980, pp. 606-642). The flora depends largely on the conditions of growing and harvesting of the particular commodity (see Section A of this chapter) and the processing prior to drying (washing), or other cleaning and blanching. Several products, mainly fruits, such as apricots and prunes, are treated with sulphur dioxide. Although such gassing, spraying, or dipping is primarily intended to stabilize colour, it also considerably reduces the microbial load. The method of drying affects the microflora of products but cannot be relied on to eliminate all pathogenic organisms. Drying of vegetables and fruits is mostly

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TABLE 28
Sampling plans and recommended microbiological limits for vegetables, fruits, nuts, and yeast

Product	Test	Method reference[a]	Case	Plan	*n*	*c*	Limit per gram *m*	Limit per gram *M*
Frozen vegetables and fruits (pH > 4.5)[b]	*E. coli*	126/131	5	3	5	2	10^{2c}	10^3
Dried vegetables	*E. coli*	126/131	5	3	5	2	10^{2c}	10^3
Coconut(desicated)	*Salmonella*	160[d]						
Growth not anticipated			11	2	10	0	0	–
Growth anticipated			12	2	20	0	0	–
Yeast	*Salmonella*	160[d]	12	2	20	0	0	–

a Refers to page number in ICMSF, 1978, where methods are described. Use analytical unit sizes recommended in the methods.
b pH measured at the time of sampling. Commodities with pH 4.5 or less are not likely to represent a major hazard and criteria are not recommended.
c In the absence of systematic data *m* values are estimates.
d See also ISO 6579 (1981).

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combined with heating, but the internal temperature of the product during drying rarely exceeds 35 to 40°C because of evaporation of water. Microbial growth can occur during drying in those internal tissues which still contain sufficient moisture. In the final dried product the water activity is usually below 0.70, and only some moulds and osmotolerant yeasts can multiply.

For the reasons explained in Section B, the Commission recommends that a criterion (case 5) for *E. coli* be applied to dried vegetables and fruits, primarily to monitor GMP (Table 28).

To the best of our knowledge dried vegetables and dried fruits have not caused salmonellosis. Therefore a criterion for *Salmonella* is not recommended. If routine monitoring detects *E. coli*, there may be reason to monitor for *Salmonella.*

E. YEASTS AND SINGLE-CELL PROTEINS

Dried yeast and yeast extract have caused outbreaks of salmonellosis. These products are used as ingredients for dietary foods which are often eaten without prior heating. Therefore limits are recommended for *Salmonella;* case 12 applies (Table 28).

Single-cell proteins – that is, proteins derived from microorganisms – are potentially important replacements of traditional proteins in manufactured foods. However, because their usage is limited, criteria are not recommended. If international trade becomes significant the same criteria as for dried yeasts would apply.

F. NUTS

(a) *Ground nuts*

Peanuts are the only important commodity in this category. The only hazard is the presence of mycotoxins. This is controlled by sorting out damaged nuts by visual inspection or electronic devices. Lots are checked for aflatoxins by chemical procedures or ultraviolet light. No microbiological criteria are suggested. Peanut butter is dealt with in Chapter 22 (Fats and Oils).

(b) *Tree nuts*

Though many varieties of tree nuts move in international trade, only coconut gives cause for concern. The hazard is contamination with

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Salmonella. Case 11 is appropriate for products where multiplication of *Salmonella* does not occur; in products where growth may occur case 12 is recommended (Table 28). For other tree nuts, testing for *E. coli* has proved an effective monitor of GMP.

G. SAMPLING PROCEDURES

Appropriate methods for sampling frozen or dried foods are given in Chapter 9 (Collecting and Handling Sample and Analytical Units). For sampling most raw fruits and vegetables a rinse technique should be used as the organisms of concern will be present mainly on the surface. Make a 1/10 dilution (w/v) of the commodity in 0.1 % peptone water containing 1 % tergitol; shake for 1 minute. Care should be taken not to break the skin of fruits.

If acid fruits are blended, the acids released may destroy the organisms being sought. Counts should be expressed on a per gram basis recognizing that data obtained using the rinse technique for different-sized fruits (e.g., watermelon and red currant) are not comparable because of the large difference in the surface/volume ratios.

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Sampling plans for soft drinks, fruit juices, concentrates, and fruit preserves

The microbiological safety and stability of this diverse group of products depend on their formulation (including the use of chemical preservatives, carbonation, low pH values, and pasteurization). For details of preservation requirements see ICMSF, 1980, pp. 643-668. Control should be by application of the HACCP system to raw materials, formulations, processing conditions, and packaging (see Chapter 7: Control at Source).

Examples of such controls would be: (a) measurement of temperature during heat-treatment of pasteurized products; (b) measurement of carbonation levels by routine monitoring of $C0_2$ pressures in carbonated products; (c) measurement of pH, a_w, product viscosity, and preservative levels in products preserved by the use of chemical preservatives in combination with other formulation parameters; (d) maintenance of good hygienic practices to prevent the build-up of spoilage organisms on the plant equipment and in the production areas.

Most microbiological problems arise because of poor quality of raw materials (e.g., fruit concentrates, sugars, and syrups) and poor process hygiene, which lead to overcoming of the preservation system applied during manufacture by the spoilage organisms. Thus there is a need for routine sampling of raw materials, preventive maintenance of equipment, and monitoring of those parts of the process where microbiological contamination can occur (e.g., during filling and in holding tanks). For the routine monitoring of raw materials simple microbiological tests such as the use of the direct microscopic count, a yeast and mould count, or a standard plate count (using a medium capable of supporting lactic acid bacteria) are most generally applicable. For certain materials more specialized tests, such as the determination of diacetyl and acetylmethylcarbinol in citrus and apple juices

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and their concentrates, may give a better index of microbiological quality (Murdock, 1968).

It is impossible to recommend specifications for specific types of microorganisms in raw materials as these will very much depend on how they are processed and how they are to be used in the final product. For instance, for a pasteurized juice it is important to control levels of heat-resistant moulds in raw materials whereas in a product preserved by the use of chemical preservatives it is more important to know the level of preservative-resistant microbes. Thus, *Gluconobacter* and related bacteria such as *Acetobacter* are important spoilage organisms in products that contain benzoate and/or sorbate and are distributed in plastic containers. If the differentiation of these organisms is important, the method described by Cirigliano (1982) may be used. A case 1 sampling plan would be appropriate for the examination of materials.

Although routine microbiological testing of end-products is not useful for the direct control of manufacturing operations it may provide useful trend data on the performance of a particular operation over a period of time. For this purpose, once a production line has been established to perform to within commercially acceptable spoilage levels, the numbers of containers that should be examined from a production plant on a daily basis should be between 0.01 and 0.1 per cent of the production units taken from production lines.

Unless prior knowledge indicates that sampling of containers should be concentrated on particular parts of a production period (e.g., during start-up, change in product type, or adjustment of packaging equipment), containers should be sampled at random.

Samples should be incubated appropriately and examined for evidence of microbial spoilage. In most products simple visual inspection for haze, sediment, gas, or mould clumps will suffice. If microbiological examinations are made, the methods used should be the most suitable for the likely spoilage organisms. Most spoilage problems result from the growth of yeasts (principally *Saccharomyces*, but often *Brettanomyces* in carbonated beverages) and/or acid-tolerant bacteria such as *Acetobacter*, *Gluconobacter*, *Lactobacillus*, and *Leuconostoc.* The complete identification of spoilage organisms is often useful in identifying the cause of a particular microbiological problem.

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20

Sampling plans for cereals and cereal products

Cereal grains and their products, such as flours, grits, and meal, are important commodities in international trade and form the staple foods of many communities. There are many cereal-based products and a wide variety of confectionery and bakery products with a cereal as a main component. Some of these products are potential microbial foodborne disease risks by virtue of their cereal components but many more may be risks because of the various animal and other vegetable components they contain. Those products for which the use of microbiological criteria would appear to be of value are listed in Table 29. The microbiology of cereals and their products is discussed in detail in ICMSF, 1980, pp. 669-730.

A. GRAINS AND FLOURS

Cereals include wheat, maize, barley, oats, rye, durum, rice, millet, and sorghum. Protein-rich oil seeds, such as soya beans, and the flours and protein isolates and concentrates derived from them are included in this chapter as their storage, handling, and use pose risks that are somewhat similar to those of cereal grains.

Grains, provided they are harvested when in good condition and rapidly dried to a water activity level preventing microbial growth, and then stored under conditions such that the excessive ingress or movement of water is avoided, have virtually no microbiological risks. However, in practice, these conditions are not always met and mould growth may result. The principal organisms of concern are moulds which produce mycotoxins. Many of the field and storage fungi found on grains and in flours derived from them are capable of producing

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mycotoxins in incorrectly dried or stored cereal products (see ICMSF, 1980, p. 685).

Their control should be principally at source, that is, by controlling conditions during and after harvesting. It is not recommended that these products be routinely monitored for mycotoxins because routine analytical methods for most mycotoxins are not available. However, where the presence of a mycotoxin of concern (e.g., aflatoxin) has been identified in a particular production area, extensive monitoring of the product may be indicated. For methods of analysis of products for mycotoxins, see Horwitz, 1980.

Levels of mould propagules found in food-quality grains and flours vary between approximately 10^2 and 10^5 per gram depending on the type of grain and the growing, harvesting, drying, and storage conditions (including time of storage). Grains and flours may be variously treated, and this circumstance will affect the levels of moulds found on examination. Thus grains may be washed in chlorinated waters and flours may be heat-treated to change functional properties or treated with a bleaching agent such as chlorine dioxide. All these treatments will reduce mould levels, although none will have a significant effect on mycotoxins, if present. Patent flours, made from part of the endosperm of the grain, will generally contain only low levels of moulds. Wholemeal flours will often contain much higher levels as these include the surface layers of the grain, which are those parts principally contaminated with moulds. Thus when deciding on an acceptable limit for moulds it is necessary to know the conditions to which the flour or grain has been subjected prior to sampling. Without such information, results of analyses cannot be properly interpreted. Providing the processors have this knowledge, mould counts of grains in flours may provide useful information concerning the suitability of these products in various formulations. For this purpose a 3–class sampling plan (case 5) with an M value of 10^5 mould propagules per gram would be applicable. In place of a single criterion, a range of m values is proposed because of the wide range of grains, flours, and related products involved for which neither data are available nor a single Good Commercial Practice (m value) would be applicable.

Salmonellae are recognized contaminants of oil seeds and flours, and isolates and concentrates derived from them (e.g., soya beans, cotton seed, and sunflower seeds). Although these commodities are invariably cooked, or heat-processed, before eating, they may be a source of contamination, and levels of salmonellae should thus be

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controlled. Because the major problem is with soya products a 2–class sampling plan (case 10) for *Salmonella* is recommended for them in addition to the 3–class plan for moulds (Table 29).

Bacillus cereus is common. It is a soil organism and an unavoidable contaminant of grains and flours. While levels of *B. cereus* can be controlled by correct storage and may be reduced somewhat by cleaning grains, it cannot be eliminated. Two cereal products, fried rice and custard powder, have been frequently associated with food-poisoning, although on occasion other cereal-based products have been incriminated (Gilbert, 1979). *Bacillus cereus* food-poisoning associated with fried rice is the consequence of holding cooked rice for several hours at warm (room) temperatures, or in large containers in refrigerators, prior to frying. The problem with custard also results from temperature abuse of the prepared custard prior to consumption. Such incidents are best prevented by education and training of food-handlers and informative labelling of products (e.g., 'refrigerate after preparation for consumption'). They will not be controlled by monitoring products for *B. cereus* contamination. Thus criteria for *B. cereus* are not recommended except for frozen entrées containing rice or cornflour as a main ingredient, where there is a risk of abuse during manufacture. For this purpose a 3–class sampling plan (case 8) is applicable but because of very limited data on a narrow range of products only a tentative *m* can be proposed. The *B. cereus* selective agar of Holbrook and Anderson (1980) is recommended.

Flours and particularly starches are frequently contaminated with thermophilic spore-forming bacteria which can cause spoilage of canned foods stored above c. 37°C. Thus it is recommended that levels of aerobic thermophilic spores in starches and other cereal products to be used in canned products should not exceed 150 spores per 10 g with an average of no more than 125 spores per 10 g based on the method of analysis specified by the National Food Processors Association (Maunder, 1976). This criterion is not applicable to these commodities when intended for other purposes.

Flours used in the manufacture of bread and pastry products may be a source of specific spoilage microorganisms. Of particular concern in breads are the rope-causing bacteria (mucoid variants of *B. subtilis* and *B. lichenformis).* Various microbiological methods have been proposed for monitoring flours for such organisms but none has been found to be useful in practice. Because the sensitivity of bread to rope formation depends on its formulation and expected storage condition it is useful to carry out a practical baking test to determine whether a

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TABLE 29

Sampling plans and recommended microbiological limits for cereals and cereal products

Product	Test	Method reference[a]	Case	Plan class	*n*	*c*	Limit per gram *m*	Limit per gram *M*
Cereals	Moulds	158	5	3	5	2	10^2-10^{4f}	10^5
Soya flours, concentrates, and isolates[b]	Moulds	158	5	3	5	2	10^2-10^{4f}	10^5
	Salmonella[c]	163[d]	10	2	5	0	0	–
Frozen bakery products (ready to eat) with low-acid or high-a_w fillings or toppings	*Staph. aureus*	220	9	3	5	1	10^{2g}	10^4
	Salmonella[c]	163[d]	12	2	20	0	0	–
Frozen bakery products (to be cooked) with low-acid or high-a_w fillings or toppings (e.g., meat pies, pizzas)	*Staph. aureus*	220	8	3	5	1	10^{2g}	10^4
	Salmonella,[c]	163[d]	10	2	5	0	0	–
Frozen entrées containing rice or corn flour as a main ingredient	*B. cereus*	274[e]	8	3	5	1	10^{3g}	10^4
Frozen and dried products	*Staph. aureus*	220	8	3	5	1	10^{2g}	10^4
	Salmonella,[c]	163[d]	10	2	5	0	0	–

a Refers to page number in ICMSF, 1978.
b Soya flours, concentrates, and isolates should in addition be examined for *Salmonella* (case 10; plan case 2; $n = 5$; $c = 0$; $m = 0$).
c 25 g sample units should be used; these may be pooled for analysis.
d See also ISO 6579 (1981).
e Preferably the method of Holbrook and Anderson (1980) should be used.
f Best estimate based on limited data. Value will depend on type of grain. In case of flours, value will depend on conditions used for their manufacture.
g Best estimate based on limited data; should be checked against products produced under GCP.

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particular flour is suitable for bread manufacture; that is, prepare a small batch of bread in the usual way, store the bread under expected conditions of storage, and check by visual inspection whether or not rope develops (see ICMSF, 1980, p. 717).

Refrigerated doughs and other raw pastry products may sour as a result of growth of lactic acid bacteria present in cereal components. Such organisms occur in flours used for making doughs and grow to high numbers on the dough-making equipment, which is difficult to clean. Microbiological methods have been proposed for checking levels of lactic acid bacteria in cereal products (Hesseltine *et al*., 1969). However, the potential for souring depends on the formulation and storage conditions, and the numbers of lactic acid bacteria that can be tolerated in particular products can be determined only by practical tests. Problems are avoided by strict attention to process hygiene.

B. FROZEN AND DRIED PASTA PRODUCTS

Pastas are raw dough products made from wheat flour, semolina, farina, water, and other ingredients (e.g., egg) to form a stiff dough usually containing about 30% moisture. The dough is extruded into various shapes and for certain types (e.g., ravioli) may be formed around a meat-containing ingredient. Because of ingredients used, conditions of storage prior to drying, temperatures used for drying, and the slow rate of drying which is necessary to prevent damage to certain types of pasta, these may contain salmonellae and *Staphylococcus aureus.* As these products are to be cooked prior to consumption, case 10 is recommended for testing for salmonellae, and case 8 for *Staph. aureus* (Table 29). A limit of $M = 10^4$ per gram is proposed for *Staph. aureus.* However, if *Staph. arueus* is found in pasta or pasta products in excess of 10^3 per gram additional tests such as the thermonuclease test (or ideally tests for enterotoxins) should be used as *Staph. aureus* may be destroyed during processing but any enterotoxins may not be inactivated. For a suitable method see ICMSF, 1978, p. 242).

Pasta may also be used in made-up dishes such as frozen lasagne, and for such products the same limits should apply.

C. FROZEN FILLED AND TOPPED BAKERY PRODUCTS

Most frozen bakery products are not a food-borne disease risk. Those of potential concern are filled or topped with sensitive ingredients such

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as products of animal origin (for example, meat pies, dairy-cream-filled doughnuts, pies and cakes, pizzas, and flans) or contain coconut. Abuse may occur after these products leave processing plants. For example, the incidents of botulism in California during 1975 and 1976 involving frozen pot pies resulted from temperature abuse to which the frozen products where subjected after thawing, such as the leaving of unheated pies in ovens overnight. Such gross mishandling of a product cannot be taken into account by any criterion. Limits for *Staph. aureus* and salmonellae are considered appropriate for certain types of frozen, filled, and topped bakery products (see Table 29).

The potential for *Staph. aureus* and salmonellae to grow in non-frozen bakery products depends on the formulation of the fillings and toppings. The presence of these organisms depends mainly on whether or not the sensitive ingredients have been pasteurized and hygienically handled prior to use. Of particular concern are those bakery products that contain unpasteurized ingredients, are ready to eat, and are eaten without heating. No criterion is proposed for products containing fillings and toppings that have water activity levels below 0.85, pH values below 4.5, or with a combination of a_w, pH, and preservatives that prevents growth of these organisms. Such products include jams, jellies, certain artifical creams, acid cheeses, spices, and certain fermented meat products.

The hazard also depends on conditions of use. For products likely to be abused after preparation, stricter limits should be applied (i.e., case 9 for *Staph. aureus* and case 12 for salmonellae).

D. SAMPLING PROCEDURES AND METHODS

Appropriate sampling procedures are described in Chapter 9 (Collecting and Handling Sample and Analytical Units), and the relevant microbiological methods are given in Table 29.

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Sampling plans for spices, condiments, and gums

Spices are dried plants or parts of plants which are used primarily for flavouring, seasoning, and imparting aroma or colour to foods and beverages. For the purposes of this discussion herbs, the aromatic leaves and flowers of certain plants, are included as spices. Condiments are spices and blends of spices which may be formulated with flavour accentuators and potentiators to enhance the flavour of foods. 'Spices and condiments' is the official nomenclature adopted by the International Organization for Standardization (ISO, 1972).

Gums include a wide range of plant and microbial polysaccharides, or their derivatives, which hydrate in either cold or hot water to form viscous solutions or dispersions. In addition to tree exudates, actual gums include seaweed extracts (e.g., agar, alginates, and carrageenan), gums from seed (e.g., locust bean, guar), and microbial gum (e.g., xanthan). As hydrocolloids, gums usually exhibit suspending, dispersing, and stabilizing properties, or they may function as emulsifiers, impart gelling properties, or be mucilaginous. Some gums serve as binders, flocculating agents, film formers, foam stabilizers, mould release agents, or lubricants (Peppier and Guarino, 1984).

A. INTERNATIONAL COMMERCE

Spice-bearing plants are chiefly indigenous to the Asian tropics but are grown commercially in many tropical and some temperate climates (e.g., in Hungary). Herbs are grown in more temperate climates of the Mediterranean, Middle East, North Africa, and North America and widely throughout Europe. India, one of the major spice producers and exporters, accounts for more than 50% of the international trade now estimated at 400 million dollars annually. The United States spice

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consumption, alone, is around 500 million pounds per year. The spice map of the world continues to change as some sources decline, others are revitalized, and new areas of spice production are developed (Peppier and Guarino, 1984).

The major sources of gums include India, Sudan, North Africa, Iran, Asia Minor, Pakistan, and Mediterranean countries (Peppier and Guarino, 1984).

B. COLLECTION OF SPICES AND GUMS

Spices are produced from botanically diverse plants grown in a wide variety of soils and climates. Depending on the spice, various parts of the plant are used, such as the seed (mustard and nutmeg), the berry (pepper), the fruit (celery seed and nutmeg), the leaves (oregano and bay), the root (horseradish), or the bark (cinnamon) (ICMSF, 1980, pp. 731-751).

As with grains, the primary consideration after harvesting is to dry the plant portion to a water activity level sufficiently low to prevent spoilage. Subsequently, further processing may involve, depending on the spice, such treatments as grinding, pulverizing, cleaning, and sorting. Some of these operations may be carried out at or near the point of harvest; others may be done at a remote site after shipment in international commerce. The microflora of spices reflects the diversity of raw materials from which they are derived, together with the varied and climatic conditions under which the plants are grown. These factors are further influenced by conditions of drying and storage, both of which may be extremely primitive (ICMSF, 1980, pp. 731-751).

Processing methods for many gums are proprietary, but patents and other literature provide some insight. Most tree gums are collected by hand and sorted, graded, packed, and shipped from the growing area. The processor further grades, cleans, mills, and blends the gum. As with spices, the water activity of the crude material is reduced to a level to preclude microbial growth. For example, gum ghatti is sundried and pulverized. Carrageenan is extracted from red algae with hot water, filtered and concentrated to about 3% gum content, precipitated with ethanol, dried, and milled (Peppier and Guarino, 1984).

C. MICROBIOLOGY OF SPICES, GUMS, AND HERBS

These products commonly carry large numbers of bacteria and moulds, in the case of herbs mainly of soil origin. Conditions of handling after

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harvest often permit extensive contamination and microbial growth although drying with heat somewhat reduces microbial numbers. The predominating flora is generally composed of aerobic spore-forming bacteria. Non-spore-forming bacteria, indicator organisms, and pathogens may be found.

The numbers of microbial contaminants can be reduced by treatment with ethylene oxide and to a lesser extent with propylene oxide. Irradiation is also effective in reducing microbial populations (ICMSF, 1980, p. 746).

Bacterial spoilage subsequent to harvesting and drying constitutes no problem, but spoilage by fungi may occur during storage and shipping. Of major concern is the role of contaminating microorganisms as spoilage agents in foods where spices and gums are used as ingredients. Spices contaminated with excessive numbers of spore-forming bacteria may be responsible for the spoilage of canned foods and processed meats (ICMSF, 1980, pp. 747-748). Though spore-forming organisms capable of causing gastroenteritis are found in small numbers in spices, they present no public health concern unless the foods into which the spices are incorporated as ingredients are mishandled to the extent that the small number of *Bacillus cereus* and *Clostridium perfringens*, for example, are permitted to multiply extensively. *Salmonella* is found infrequently, but in a wide variety of spices, and on rare occasions has been responsible for outbreaks of human salmonellosis (Laidley *et al*., 1974; Lystad *et al*., 1982; Gustavsen and Breen, 1984).

D. SAMPLING PLANS

In the first edition of this book (p.115) sampling plans and microbiological limits were suggested for spices and gums. In retrospect, it is felt that these recommendations were inappropriate. A considerable portion of these commodities in international commerce would not meet the suggested limits; furthermore, failure to meet the limits might or might not have borne a relationship to food quality or safety.

It is now our recommendation that spices and gums be treated as raw agriculture commodities. Accordingly, the ultimate use of such products will be the determinant. A spice or gum which is unsatisfactory for one purpose may be quite adequate for another. For example, black pepper containing large numbers of aerobic spore-forming bacteria may cause spoilage when used as an ingredient in canned foods or cooked sausage, but it could be used as a condiment on the table

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for seasoning cooked foods. A manufacturer using spices and gums must determine whether the microbiological quality of these products is satisfactory for the use intended (NCA, 1968). If it is not, his alternatives are to seek another source or to render the available product satisfactory. Treatment with ethylene oxide has been practised extensively for many years but there is some concern over its continued use. Gamma-irradiation may be an effective alternative provided flavours are not impaired.

In commercial practice the microbial and colour problems, and to some extent variability, are overcome by using extracts of the spices. For convenience in use these are often diluted on a carrier such as salt or a finely ground cereal (usually rusk, i.e., ground non-sweetened biscuit). Another approach is to use microencapsulated extracts using gelatin or starch as the encapsulant. Use of these forms is increasing at the expense of 'raw spices'.

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Sampling plans for fats and oils

Four categories of foods were discussed under fats and oils in ICMSF, 1980, pp. 752-777, namely mayonnaise and salad dressings, peanut butter, margarine, and butter. Product criteria would serve no useful purpose for any of these products except nut butters, including peanut butter.

The safety of mayonnaise and salad dressings is related directly to the pH and acetic acid content of the moisture (continuous) phase of the emulsions. The safety and stability of butter and margarine is primarily dependent on heat-treatment of the ingredients and the stability on emulsification to ensure uniformly small water droplets within the fat (continuous) phase. While microbiological criteria may be effectively applied at various stages in the production of these foods, product criteria, for application at ports of entry, would be of extremely limited value. Other spreads of different composition, however, may be less stable microbiologically.

With peanut and other nut butters microbiological stability is related to reduced water activity in the moisture phase of the product. The greatest hazard is the possibility of contamination with salmonellae, and if these organisms are present they will survive for long periods in a finished product although they would not grow in it. Peanut butter is generally eaten without either heating or a treatment that would destroy microorganisms. Therefore, for *Salmonella,* case 11 would apply (see Table 30), unless peanut butter is to be used as an ingredient in a high-moisture food, in which instance case 12 would apply. Thus 2–class plans are suggested with $n = 10$, $c = 0$ for case 11 and $n = 20$, $c = 0$ for case 12.

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TABLE 30
Sampling plans and recommended microbiological limits for peanut butter and other nut butters

Product use	Test	Method reference[a]	Case	Plan class	*n*	*c*	Limit per gram *m*	Limit per gram *M*
Consumed without heating or other treatment to destroy microbes	*Salmonella*	160[b]	11	2	10	0	0	–
Used as ingredient in high-moisture food	*Salmonella*	160[b]	12	2	20	0	0	–

a Refers to page numbers in ICMSF, 1978.
b See also ISO 6579 (1981).

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Sampling plans for sugar, cocoa, chocolate, and confectionery

1. SUGAR AND RELATED SWEETENERS

Sugar (sucrose) is obtained from sugar cane and sugar beets. It is sold for commercial use in both crystalline and liquid form. The liquid form may be sucrose or invert sugar obtained by hydrolysing sucrose. Corn sweeteners (glucose syrups) are obtained by acid and/or enzymatic hydrolysis of corn starch to dextrose, maltose, and higher polymers. They are sold both in solid form and as syrups. An increasingly important sweetener is high-fructose corn syrup (HFS, also known as isoglucose or isosyrup) made by enzymic conversion of glucose syrup to fructose.

The world production of sucrose and other sweeteners mentioned above in 1980 was 113 million tonnes (mt) of which 5 mt was HFS (Earley, 1980). It is predicted that by 1990 about 150 mt of sweeteners will be produced of which 8.2% will be from hydrolysed corn starch, and most of the corn-based sweeteners will be HFS. Sucrose obtained from various fruits and fructose by hydrolysis of inulin contributes only a minor portion of the world supply of sweeteners.

The concentrations of the liquid sweeteners most commonly used in commerce are listed in Table 31 (Marignetti and Mantovani, 1979/80; Minifie, 1980). Sugar syrups with concentrations of 67% w/w or higher, if properly prepared and stored, are usually not subject to spoilage by surface moulds or osmophilic yeasts.

A. REASONS FOR SAMPLING

Dry refined sugar and dried glucose syrups are remarkably stable, have not been vehicles of food-borne pathogens, and for many industrial uses (e.g., jam, confectionery, bakery products) do not contain microbes

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TABLE 31
Concentrations of commercial liquid sweeteners

Liquid sweetener (syrup)	Percentage (w/w)
Sucrose	67–68
Invert sugar	76–77
Glucose (26–65 DE)*	75–82
High fructose (42%, 55%, and less frequently up to 90% of sugar is fructose)	76–77

* DE = dextrose equivalent = the percentage of reducing sugars, on a dry basis, calculated as dextrose

that will spoil products in which they are used. Some liquid sweeteners may become mouldy or ferment unless stored to preclude condensation on the surface and formation of microenvironments permitting growth of surface moulds or osmophilic yeasts. Similarly, contamination with osmophilic yeasts must be avoided to prevent their contaminating sensitive products (Coleman and Bender, 1957; ICMSF, 1980, pp. 778-821). Dry and liquid sugars used in certain canned foods, and for soft drinks, may introduce sufficient numbers of spoilage microorganisms for some to survive the process and spoil the product. For this reason, sugars used in the canning and soft drinks industries are usually subjected to microbiological testing. Both the microbial limits and the analytical methods have been defined (NCA, 1968; 1972; NSDA, 1975; AOAC, 1972).

B. SAMPLING PLANS

It has been suggested (ICMSF, 1980, pp. 802-803) that case 2 be used for dry sugar that is for purposes other than canning or bottling, and case 3 for both dry and liquid sugar to be used for canning or bottling. The basis for this suggestion was the potential for such sweeteners to introduce spoilage organisms into these classes of products. Additionally, liquid sugars (and other liquid sweeteners) may be spoiled by osmophilic yeasts. On further consideration, however, sampling plans and microbiological criteria are not recommended for the following reasons: (a) sugars and other sweeteners have not been involved in microbial food-borne disease; (b) the microbial content of sugars and other sweeteners is mainly the concern of manufacturers using them as ingredients in other products (see NCA, 1972 for limits suitable for purchase specifications).

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II. COCOA AND CHOCOLATE

Cocoa beans are the source of material for a wide variety of cocoa and chocolate products. The annual crop is relatively stable at about 1.5 million tonnes, but the price is sometimes subject to enormous changes within a few years (Minifie, 1980). The combined annual per capita consumption of cocoa and chocolate among 19 countries that consume the bulk of the product varies from 0.6 kg in Portugal to 8.9 kg in Switzerland; the median is 4.3 kg (Minifie, 1980).

A. REASONS FOR SAMPLING

Cocoa beans are harvested, fermented, and dried mostly in technically underdeveloped or in developing countries, frequently under primitive jungle conditions. Most of the dried beans have in the past been processed to cocoa, cocoa butter, and chocolate in more technically advanced countries, where most of the product is consumed. However, there is a trend towards processing an increasing percentage of the crop to intermediate products such as chocolate liquor, cocoa press cake, and cocoa butter in the country of origin to increase local economic return. This practice may cause some concern because one step in processing, roasting, is the main germicidal barrier between a raw agricultural product and the consumer. Unless roasting is adequate and handling of roasted beans well controlled, the intermediate products may be contaminated with *Salmonella.*

Salmonellae are not readily destroyed in chocolate liquor or in confectionery made with cocoa or chocolate liquor because of their increased resistance to heat at the low a_w of such products. In cocoa and in chocolate confectionery, salmonellae may persist for years. Chocolate even appears to protect these bacteria against destruction by acid in the stomach. Small numbers (<1 per gram) of salmonellae in chocolate have caused at least one large outbreak of salmonellosis (Craven *et al.*, 1975; D'Aoust *et al.*, 1975) and larger numbers (about 25 per gram) have caused another outbreak (Gill *et al.*, 1983). *Salmonella*-contaminated cocoa has been associated with a similar large outbreak (Gastrin *et al.*, 1972). Even when no illness has been reported from *Salmonella*-contaminated products, the risk has on occasions prompted their recall (Silliker, 1967; Lennington, 1967; Anonymous, 1978).

Moulds are frequently found in cocoa beans, and it is not uncommon to find mycotoxin-producing moulds and occasionally low levels of mycotoxins in cocoa. However, raw cocoa beans do not readily

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TABLE 32

Sampling plans and recommended microbiological limits for cocoa, chocolate, and confectionery

Product	Test	Method reference[a]	Case	Plan class	*n*	*c*	Limit *m*
Cocoa	*Salmonella*	163	11	2	10[b]	0	0
Chocolate and other confectionery	*Salmonella*	163	11	2	10[b]	0	0

a Refers to page number in ICMSF, 1978. Methods as described (pp. 163-172) using non-selective enrichment medium made with skim milk and brilliant green (ICMSF, 1978, p. 324).

b The 25 g analytical units may be composited.

support the production of mycotoxins although they do support abundant growth of mycotoxin-producing fungi (Llewellyn *et al.*, 1978). Therefore, there is always a threat of mycotoxins in cocoa products, but toxin has been found only infrequently and only at low levels.

B. SAMPLING PLANS

The ICMSF (1980, p. 818) has suggested that case 2 be used for aerobic plate counts (APC) and mould counts for cocoa, chocolate, and compound coatings containing cocoa, and that case 5 be applied for coliforms and *Escherichia coli*, and case 11 with $n = 10$ and $c = 0$ for *Salmonella*. However, on further consideration, only *Salmonella*, as the main food-borne hazard in cocoa and cocoa products, need be tested for at ports of entry. The total microbial population and the presence of indicator oganisms are generally controlled by suppliers as part of purchase specifications. Based on the inability of these products to support microbial growth, and their non-involvement in food-borne disease other than salmonellosis, there appears little point in testing them for microbes other than *Salmonella* (see Table 32).

III. CONFECTIONERY

Sugar confectionery is eaten in most countries. Minifie (1980) reported that the annual per capita consumption in 17 countries varied from 1.2 kg in Portugal to 6.6 kg in Ireland, with a median of 3.6 kg.

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A. REASONS FOR SAMPLING

Confectionery, because of its low a_w is generally free from microbial hazards and microbial spoilage problems. The main hazards, when they exist, may come from such ingredients as cocoa, coconut, milk powder, and egg albumen, all of which sometimes contain *Salmonella*, and nuts and cocoa, which may contain mycotoxins. Cocoa has been discussed above and the other ingredients in other chapters. The main microbial spoilage problems are (1) explosion of enrobed preparations of fruit and fondant as a result of growth and gas production by osmophilic yeasts introduced with the fruit, (2) explosion of marzipan from gas produced by osmophilic yeasts introduced with nuts or from the environment, and (3) 'Wasserflecken' (globular areas with watery appearance) in marzipan resulting from growth of moulds (Windisch and Neumann, 1965; Windisch *et al*., 1978). Moulds may also cause spoilage as a result of condensation of moisture in water-impermeable packages (Jarvis, 1982).

B. SAMPLING PLANS

The ICMSF (1980, p. 821) suggested that case 3 was appropriate for spoilage organisms and case 11 for *Salmonella* in confectionery. Spoilage organisms are mainly the concern of the manufacturer. At port-of-entry sampling for *Salmonella* only, case 11 is recommended (see Table 32).

IV. SAMPLING AND TESTING PROCEDURES FOR COCOA, CHOCOLATE LIQUOR, COATINGS CONTAINING COCOA, CHOCOLATE, AND OTHER CONFECTIONERY

For general directions on collecting and handling samples, see Chapter 9 and ISO, 1981.

Among the many factors to be considered when examining food for *Salmonella*, the anti-*Salmonella* activity in some cocoa which is negated by casein is especially pertinent when isolating this organism from cocoa and combinations of cocoa with milk (e.g., milk chocolate) (Park *et al.*, 1976; Zapatka *et al.*, 1977). In the interest of uniformity, we recommend that reconstituted skim milk with brilliant green be used as the non-selective enrichment medium for cocoa-containing products whether or not they already contain milk. This method appears

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to be most useful for the products of concern, although it differs in some details from several other recommendations (IOCC, 1977; ICMSF, 1978; FDA, 1978; Wilson *et al*., 1980).

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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 microfiora 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

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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

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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,

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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 endproduct specifications truly represents some fault in GMP. Products are present on the market, made according to GMP, which contain higher

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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 Commerical 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 drymix 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 dryblending (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

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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.

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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*	Limit per gram *M*
Coated or filled, dried shelf-stable biscuits	Coliforms	126-131 (Method 3)[b]	5	3	5	2	10	10^2
	Salmonella[ef]	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
	Salmonella[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
	Salmonella[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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Sampling plans for natural mineral waters, other bottled waters, process waters, and ice

A. DEFINITIONS AND ECONOMIC IMPORTANCE OF WATERS IN INTERNATIONAL TRADE

Natural mineral water comes from underground water-bearing strata and is characterized by its mineral salt and trace element constituents. It is subjected to only minimal treatment such as carbonation, aeration, sedimentation, and filtration. A more complete definition is cited by Codex, 1978; a portion of this definition is given below (extracted from the Draft European Regional Standard for Natural Mineral Waters).

2.1 *Definition of Natural Mineral Water.* Natural mineral water is a water clearly distinguishable from ordinary drinking because: (a) it is characterized by its content of certain mineral salts and their relative proportions and the presence of trace elements or of other constituents; (b) it is obtained directly from natural or drilled sources from underground water bearing strata; (c) of the constancy of its composition and the stability of its discharge and its temperature, due account being taken of the cycles of natural fluctuations; (d) it is collected under conditions which guarantee the original bacteriological purity; (e) it is bottled close to the point of emergence of the source with particular hygienic precautions; (f) it is not subjected to any treatment other than those permitted by this standard; (g) it is in conformity with all the provisions laid down in this standard.

2.2 *Supplementary Definitions*

2.2.1 *Naturally carbonated natural mineral water.* A 'naturally carbonated natural mineral water' is a natural mineral water which, after possible treatment in accordance with sub-section 3.1.1 and replacement of gas and after packaging, has the same content of gas from the source as at emergence of the natural mineral water taking into account the usual technical tolerance.

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2.2.2 *Non-carbonated natural mineral water.* A 'non-carbonated natural mineral water' is a natural mineral water which, by nature and after possible treatment in accordance with sub-section 3.1.1 and after packaging, does not contain free carbon dioxide in excess of the amount necessary to keep the hydrogen carbonate salts present in the water dissolved.

2.2.3 *Decarbonated natural mineral water and natural mineral water fortified with carbon dioxide from the source.* A 'decarbonated natural mineral water' or a 'natural mineral water fortified with carbon dioxide from the source' is a natural mineral water which, after possible treatment in accordance with subsection 3.1.1 and after packaging, does not have the same carbon dioxide content as at emergence.

2.2.4 *Carbonated natural mineral water. A* 'carbonated natural mineral water' is a natural water which, after possible treatment in accordance with subsection 3.1.1 and after packaging, has been made effervescent by the addition of carbon dioxide from another origin.

3.1.1 Treatments permitted include separation from unstable constituents by decantation and/or filtration, if necessary accelerated by previous aeration.

3.1.2 The treatments provided for in sections 2.2.1, 2.2.2, 2.2.3, 2.2.4 and 3.1.1 above may only be carried out on condition that the mineral content of the water is not modified in its essential constituents, which give the water its properties.

Natural mineral water may be either non-carbonated or carbonated. The latter may be either naturally carbonated at its origin or by adding carbon dioxide ($C0_2$) from its own source or from other sources. Hence, the $C0_2$ concentration may vary considerably. The pH of carbonated mineral waters is often rather low, between 3 and 4. Natural mineral waters without, or with very little, carbon dioxide are known in Europe as 'flat waters' (*eaux plates*).

Although specific figures are not available, bottled mineral waters are shipped internationally in considerable quantities. Water from similar sources, but not having any particular specified amount of mineral content, and potable* water from other sources are sometimes also bottled. These bottled waters may be sold in large quantities in certain

* Potable means that water must be free from microorganisms capable of causing disease and free from either minerals or organic substances that can produce adverse physiological effects. It must also be aesthetically acceptable and thus be free from apparent turbidity, colour, odour, and objectionable taste and have a reasonable temperature for its intended purpose. Hence, it can be ingested or used in food-processing in any amount without concern for adverse effects on health (Borchardt and Walton, 1971).

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countries or regions of a country, but are at present of limited importance in international trade.

Water is used widely in the food industry. It is used to move products, to wash vegetables, fruits, fish, and poultry, to freshen raw vegetables after harvesting and during distribution, and as an ingredient in some foods. Virtually all frozen foods carry a glaze of ice which is often derived from process water, and for certain frozen foods (such as fish and shellfish) a glaze is added as a protective measure. This process water is thus an integral part of some processed foods that are shipped internationally. For example, fish is typically shipped in ice; poultry may be cooled in water and slush ice and transported in ice.

B. REASONS FOR SAMPLING

Even the most protected and best drawn waters are never sterile and possess a certain natural microbial (autochthonous) flora which is rather constant for a particular supply. Subsequent contamination during catching, storing, transporting, or bottling accounts for the introduction of microbial species (allochthonous flora) normally present (ICMSF, 1980b, pp. 834-837).

(a) *Bottled waters*

Contaminated surface or ground (well or spring) waters have frequently been identified as vehicles in outbreaks of water-borne diseases (Holden, 1970; Mossel *et al.*, 1973, 1977; WHO, 1979, 1980). Particular care, therefore, is needed to obtain water from sources that are potable and to protect the sources and water in distribution from contamination. This is particularly important for natural mineral waters because, by definition, they cannot be disinfected before or after bottling and can be subjected only to certain treatments.

Water ingested between meals passes rapidly through the stomach because the pyloric area is relaxed at that time, and thereby avoids the bactericidal effect of intragastric retention (Levine and Nalin, 1976; Mossel and Oei, 1975). Thus, water containing even small numbers of pathogens can cause disease.

Bottled waters have been implicated in outbreaks of waterborne disease, but there is little published information. Bottled, noncarbonated mineral water was a vehicle of *Vibrio cholerae* in a cholera outbreak in Portugal (Blake *et al.*, 1977). Buttiaux (1960) attributed outbreaks of gastroenteritis in Hungary and of typhoid fever among

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Polish children to ingestion of bottled water. Persons from countries with a high standard of hygiene who travel to countries where standards are lower often drink bottled water in the hope of avoiding illness. However, they frequently develop travellers' diarrhoea, and sometimes the bottled water comes under suspicion (Gangarosa *et al*., 1980).

Bottled natural mineral water is sometimes used for domestic preparation of either infant formulas or dietetic foods on the assumption that it is safer than other waters. Consumers of these particular products are often highly susceptible to disease. *Pseudomonas aeruginosa*, for example, may be a hazard for infants (Panero *et al.*, 1970; Mossel, 1977). An epidemic of *P. aeruginosa* infection occurred among infants in a nursery when well water was contaminated by seepage of sewage and by infiltration of stream water (Weber *et al.*, 1977).

Thus, it is essential that the initial bacteriological quality of the water be high and maintained by strict control at its source and by monitoring of the bottling operation. In the absence of information on the efficacy of control at source, importing countries should seek assurance that bottled water is safe by appropriate microbiological monitoring of incoming lots.

(b) *Process waters*

Processing water and ice can be important sources of spoilage organisms and, if from non-potable sources or subsequently contaminated, potential sources of pathogens. They can introduce these organisms to foods during processing, storage, or preparation. If illness occurs as a result of such contamination, the contaminated food is considered as the vehicle, although the initial source of pathogens was process water. For example, raw untreated river water was used for washing fish that were later identified as a vehicle in an outbreak of salmonellosis (Gangarosa *et al.*, 1968). Contaminated cooling water was implicated as the source of *Salmonella typhi* in canned corned beef responsible for several outbreaks of typhoid fever (Howie, 1968). Water used to keep tofu (oriental soyabean curd) moist in packages was the source of *Yersinia enterocolitica* that was responsible for an outbreak of gastroenteritis (Nolan *et al.*, 1982).

C. SAMPLING PLANS

Unfortunately, no single organism, or group of organisms, serves as an ideal indicator of pollution of water. Tests for the following are sometimes used to evaluate the safety of water: APC (42°C, 37°C, and

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20°C), coliforms, faecal coliforms, *Escherichia coli*, Enterobacteriaceae, faecal streptococci, sulphite-reducing clostridia, *E. coli* phages, and specific pathogens. Many of these tests are used to examine bottled water in several countries (Morisetti, 1979). The decision as to which organisms should be sought to evaluate the potability of bottled waters is complicated because unique advantages and disadvantages are associated with each indicator organism or group. These have been reviewed by Cominazzini (1978), Geldreich (1978), Hoadley and Dutka (1976), and Mossel (1976).

(a) *Non-carbonated waters*

Although water-testing laboratories in some countries use the *E. coli* test for routine examination of water for the protection of public health, others, as recommended by WHO (1971) and an FAO/WHO Working Group (1981), use the coliform test. The coliform test, even with its disadvantages, is the method chosen by the Codex working group for examining natural mineral water (Codex, 1978). Microbiological data available to the Commission indicate that bottled natural mineral water can be produced without contamination by coliforms. Examination for coliforms provides a more stringent microbiological rejection criterion than testing for *E. coli*. Routine testing of bottled waters for both coliforms and *E. coli* offers little advantage and increases the cost of analysis.

Cases 4 to 6 apply when indicator organisms are sought. Although certain microorganisms multiply in bottle-stored water, the organisms usually employed as indicators decrease in number with time of storage (Schmidt-Lorenz, 1976). Because time of bottling is often unknown, case 5 seems the most appropriate. This case calls for a sampling plan of $n = 5$ and $c = 2$ (Table 10, p. 74) but such a plan is too lenient for a commodity of special public health concern. These concerns are: (1) the sampling plan employed should give at least the same protection of public health as those required for community water supplies; (2) bottled water is usually stored longer than water in community water supplies, and during the storage period certain indicators of faecal pollution (such as coliforms) may die off more rapidly than some pathogens, while autochthonous or other allochthonus organisms multiply; and (3) home-prepared formulas for infants are sometimes reconstituted with bottled mineral water. These formulas are sometimes subject to temperature abuse between preparation and feeding (e.g., when a child falls asleep during feeding and later wakes and feeds

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again). A 2–class sampling plan for coliforms with $n = 5$ and $c = 0$ and a limit of $m = 0$ is recommended for examination of bottled non-carbonated waters including natural mineral water (Table 34). Analytical units of 250 ml should be examined for coliforms by the membrane filter method. Decreasing the *c* value from 2 to 0 and increasing the size of the sample unit to 250 ml make a stringent plan to serve the above-mentioned considerations.

Pseudomonas aeruginosa, a frequent contaminant of the environment, may cause water-borne disease (Hoadley, 1976, Young, 1977, Weber *et al*., 1977). After adaptation, it can multiply in a mineral water of extremely low nutrient level (Schmidt-Lorenz, 1976). Indicator organisms are of little value in detecting the presence of this opportunistic pathogen. The finding of *P. aeruginosa* in natural mineral water should be cause for concern, particularly if the water is to be consumed by highly susceptible persons such as infants. Therefore, it is recommended that natural mineral water for such a purpose be tested for *P. aeruginosa*. Case 8 applies, and a 2–class plan with $m = 0$ is suggested (Table 34).

Aerobic plate counts (at 35–37°C for 24 hours and at 20–22°C for 72 hours) at source and within 12 hours after bottling may be considered, according to the general principles of FAO/WHO, as guidelines useful in interpreting water quality at its source and its place of bottling. To be acceptable, counts should not exceed those set for natural mineral water by EEC (1980a). APC at 20°C may be useful for quality control within certain food-processing plants.

(b) *Carbonated waters*

Pathogenic organisms (other than spores) should succumb rapidly (usually within hours) after exposure to a pH value of 3.5 or lower (ICMSF 1980a, pp. 92-111). Therefore, it is not necessary to test carbonated natural mineral waters, or other carbonated bottled waters having a pH value of 3.5 or lower, for indicator organisms. To determine whether testing is necessary, a sampling plan that includes testing the pH of five bottles (*n*) from a lot is recommended. If the pH of any sample unit exceeds 3.5, the sampling plans for testing non-carbonated bottled waters should be followed (see Table 34). If it is not possible to test the pH of the water, the sampling plan for non-carbonated waters should be followed.

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TABLE 34

Sampling plans and recommended microbiological limits for bottled water

Product	Test	Method reference	Case	Plan	*n*	*c*	Limit per 250 ml *m*	Limit per 250 ml *M*
Non-carbonated natural mineral waters (*eaux plates*) and bottled non-carbonated waters, not classified as mineral waters	Coliforms	MF[a]	5	2	5	0	0	–
	Pseudomonas aeruginosa[b]	See text	8	2	5	0	0	–
Carbonated waters (natural mineral or other bottled waters	pH	See text		2	5	0	3.5[c]	–

a MF = membrane filter method (see Greenberg *et al*., 1981). N. B. Resuscitate prior to recovery on selective media (Morisetti, 1979).
b This test is used only if it is anticipated that bottled waters are to be used in infant formulae or for other highly susceptible persons.
c If any sample unit is greater than pH 3.5, proceed with the above sampling plans for non-carbonated waters.

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(c) Process waters and ice

Guidelines for microbiological criteria of process water and ice must be applied at the processing or manufacturing plant, at their sources, and/or at appropriate distribution points. Process water and ice should be made from potable water, and should meet the microbiological criteria cited for potable water by EEC (1980b), Greenberg *et al*. (1981), or WHO (1971). Ice glaze is considered as part of the frozen food itself and will be tested during analysis of the food.

D. SAMPLING PROCEDURES

Sample units of bottled water should be collected randomly from the lot as described in Chapter 9 and brought to the laboratory within 1 hour of collection. If this is not practicable, they should be cooled to 10°C or below, iced, and transported to the laboratory in insulated containers. Water should be collected at its source or during processing, distribution, or storage as described by Bryan *et al*. (1979). When sampling waters at their source, samples should be stored chilled and APCs made within 12 hours. Ice should be brought frozen to the laboratory, either as unopened bags of ice or as ice sampled in bulk with a sterile pick, scoop, or tongs and put into sterile bags or jars.

E. TEST PROCEDURES

The coliform test by the membrane filter technique is recommended because it facilitates sampling large volumes of water. Laboratories not able to use this technique should test for coliforms using the multiple tube dilution (MPN) method. Test procedures for water and ice are not described in ICMSF, 1978, but those given in *Standard Methods for the Examination of Water and Wastewater* (Greenberg *et al*., 1981) are applicable. Carbonated waters should be degassed before microbiological testing. The pH should be measured by the method specified in Greenberg *et al*. (1981). There is no standard method for detection *of P. aeruginosa* in water, but methods are given by Greenberg *et al*. (1981), Mossel *et al*. (1976), and WHO (1980).

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Shelf-stable canned foods

Shelf stable canned foods are packed in hermetically sealed containers and are commercially sterile. Commercial sterility of thermally processed food means the condition achieved by the application of heat, alone or in combination with other treatments, to render the food free from microorganisms capable of growing in the food under normal conditions of distribution and storage. Such processes include: (a) the 'botulinum cook' used for low-acid foods (i.e., those having an equilibrium pH above 4.6); (b) the lesser heat-treatment applied to products containing curing ingredients such as sodium chloride or sodium nitrite; (c) the relatively mild heat-treatment given to products of pH 4.6 and below ('acid' foods); and (d) the mild heat-treatment given to foods in which the water activity, or combination of water activity with other preservation factors such as pH, inhibits growth from bacterial spores.

Many canned foods have the potential to cause botulism. However, *Clostridium botulinum* is present in such low numbers, and so infrequently, in such products that no realistic sampling plan would be adequate as a direct measure of its presence.

In practice the controls applied consist of in-plant control to ensure that (i) the containers are sound and correctly seamed or sealed, (ii) the correct thermal process has been applied, (iii) the cooling water is of suitable microbiological quality, either by chlorination and chemical monitoring or by microbiological monitoring, and (iv) hygienic practices during and after cooling are satisfactory.

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A. RECOMMENDED HYGIENIC PRACTICES FOR CANNED FOODS

Within recent years a number of codes of practice for commercial processing of foods in hermetically sealed containers have been published. For example, the United States Food and Drug Administration has issued a code of good manufacturing practice for low-acid canned foods (FDA, 1979) as has the UK Department of Health and Social Security (DHSS, 1981). The Codex Alimentarius Commission has drafted a *Recommended International Code of Hygienic Practice for Low-Acid and Acidified Low-Acid Canned Foods* (Codex, 1983).

B. INTEGRITY OF CONTAINERS

Even when an adequate heat-process has been applied, the integrity of hermetically sealed containers used for canned foods is critical to safe processing and requires constant surveillance by the can manufacturer and canner. A defective or unsound container is one that is of faulty manufacture, is improperly closed, or has been damaged in such a manner as to permit recontamination of the can contents following the heat-process (post-processing contamination). If the contaminant is a pathogen and the food is capable of supporting its growth, a health hazard exists, as demonstrated by type E botulism from canned tuna (Johnston *et al.*, 1963), typhoid from canned corned beef (Howie, 1968); and staphylococcal food-poisoning from canned peas (Bashford *et al.*, 1960). Even a sound container, if mishandled, may leak, and if hygiene is poor, contamination of the contents may occur (Put *et al.*, 1972; Stersky *et al.*, 1980). To minimize the chances of contamination caused by defective containers, the container manufacturers, as well as the canners, should maintain a strict quality assurance program including pressure-testing and can seam measurements to ensure that predetermined tolerances are met. Minimum inspection programs for seam and seal checks are detailed in FDA, 1978, and in guidelines issued by the NFPA (1979). More detailed guidance on can inspection is available in HPB, 1983; OSU 1982; and Thorpe and Barker, 1984, which also includes information on containers with welded side seams.

C. COOLING WATER

In addition to container-integrity checks and process controls, the canner should use water of suitable microbiological quality for cooling

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the retorted cans. If appropriate, the water should be chlorinated or otherwise sanitized. Examination of the cooling water is the responsibility of the canner and should involve regular microbiological examination and, if a sanitizer is used, frequent analysis for the concentration of the sanitizer present.

D. INCUBATION TESTS

Opinions differ concerning the value of incubating and testing a small number of cans immediately after processing, but there is no doubt that such incubation tests are of no value in ensuring the safety of shelf-stable canned foods. It must also be borne in mind that such limited incubation tests will reveal only gross underprocessing and/or extensive post-processing contamination. However, limited incubation tests are still required by some agencies for certain low-acid canned foods which do not receive a full botulinum cook, including some cured meats which are shelf-stable as a result of the presence of curing agents. Incubation tests may also be of use to the manufacturer to monitor spoilage trends over long periods of time. It is accepted practice to examine all cans subjected to incubation tests for swelling, but it must be realized that a variable proportion of contaminated cans produce swells. Hence, the contents of as many of the incubated cans as practicable should be examined for pH change and other evidence of spoilage. The practice of deliberate storage (or incubation) of all cans after thermal processing, and removal of swollen cans prior to labelling or distribution, is to be discouraged. It can lead to the marketing of an unsatisfactorily processed product, which may be a health hazard as some pathogens (e.g., staphylococci and *Salmonella typhi)* do not produce gas in many canned products. It should be emphasized that no sampling procedure ensures adequate safety with respect to the botulism hazard. Industrial experience suggests that greater reliance should be placed on the following: thermal-processing records, constancy of product formula (solid-to-liquid ratios), volume of fill, the use of automated instrumentation such as time-temperature recording devices, and alarm warning systems to signal any interruption of the specified thermal process.

E. PROCESS VALIDATION AND RECORDS

In addition to data on seam inspection (e.g., tear-down and visual inspection), thermal processing, and cooling-water quality, it is

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recommended that information on appropriate chemical composition (e.g., salt and nitrite content), pH, or any other critical factors be recorded and made available to the controlling agency. The data on chemical composition should give, at least, mean values and the standard deviation or range. If this information gives cause for concern, or is lacking or inadequate, further can examinations appear advisable (see Chapter 5, Section N: Investigational Sampling).

F. SAMPLING

For reasons stated above, a sampling plan for the routine surveillance of shelf-stable canned foods to determine microbiological safety is not recommended.

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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.

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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 inICMSF, *Microorganisms in Foods, I. 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

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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,*

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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 (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).

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