

# codex alimentarius commission

FOOD AND AGRICULTURE  
ORGANIZATION  
OF THE UNITED NATIONS

WORLD HEALTH  
ORGANIZATION

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ALINORM 78/13

## CODEX ALIMENTARIUS COMMISSION Twelfth Session 1978

### REPORT OF THE THIRTEENTH SESSION OF THE CODEX COMMITTEE ON FOOD HYGIENE Rome, 10-13 May 1976

#### INTRODUCTION

1. The Thirteenth Session of the Codex Committee on Food Hygiene, hosted by the Government of the United States of America, was held at FAO headquarters, Rome, from 10-13 May 1976.
2. The session was opened by Mr. C.A. Norred, Permanent Representative of the United States of America to FAO, who welcomed the participants. Dr. J.C. Olson was Chairman of the session.
3. The session was attended by representatives and observers of 62 countries, and observers from 2 international organizations. The list of participants, including the representatives of FAO and WHO, is attached as Appendix I to this Report.

#### ADOPTION OF AGENDA

4. Following a brief discussion the Committee adopted the agenda with a change in the order of items to be discussed.

#### INFORMATION ON ACTIVITIES WITHIN WHO AND FAO OF INTEREST TO THE COMMITTEE

5. The representative of WHO reviewed recent WHO activities relating to the work of the Codex Committee on Food Hygiene. Further to information supplied at the recent Codex Alimentarius Commission session (ALINORM 76/44, paras 41-67), he referred to the Expert Committee on Public Health Aspects of Food Microbiology held in March 1976; the present status of the WHO Food Virology Programme; the Consultation on Post-Graduate Training in Food Microbiology held in Berlin (West) in November 1975 at the newly designated FAO/WHO Collaborating Centre for Research and Training in Food Hygiene; training courses in the field of food microbiology organized and/or coordinated by WHO; guides and manuals published or in preparation and WHO activities in the field of hygiene and sanitation in aviation.
6. With regard to the development of microbiological specifications for foods, he particularly stressed the importance of advice from the Codex Committee on Food Hygiene concerning the approach for future work in this field. The next FAO/WHO Expert Consultation on Microbiological Specifications will be convened in early 1977.
7. The representative of the Animal Production Division of FAO gave a resumé of activities with regard to meat hygiene which was mainly concentrated on meat inspection and quality control as well as on hygiene aspects related to meat production and distribution. Advice to Member Governments was given on:
  - animal and human health, and the economy of the meat industry;
  - ante-mortem and post-mortem inspection;
  - post-mortem judgement of meat;
  - sanitary handling of sound and condemned meat;
  - hygienic aspects of meat preservation, storage and meat technology;
  - control of sanitation and protection against microbiological, chemical and other contamination;
  - organization of meat inspection services at country and abattoir level, meat inspection legislation and international regulations.

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8. The Codex Committee on Meat Hygiene had collaborated with the Division in elaborating a Code on Post-Mortem Judgement of Slaughter Animals, which after consultation with WHO was published in the FAO Manual on Standards of Veterinary Services, Meat Hygiene and Meat Inspection, Post-Mortem Judgement of Slaughter Animals and Establishment of Specific Disease-Free Zones. This Code needs further elaboration to take account of chemical residues in meat. A consultation is planned in 1977 by FAO/WHO to assist in the amendment of the Code which will then serve as a first Draft Code to be considered by the Codex Committee on Meat Hygiene.
9. An organizational plan for a meat inspection service, mainly aimed at assisting developing countries, is being prepared, part of which will be published, probably as a supplement to the Animal Health Yearbook.
10. Training is provided for veterinary auxiliaries, meat hygiene and meat inspection staff, in various countries, including the establishment of standard curricula and training centres and the organization of inter-regional training courses.
11. The effect of sanitary barriers on meat export and import received particular consideration at the 15th Session of the FAO Conference. For implementation of the decisions made by the Conference, a Working Group on non-tariff trade barriers was set up in January 1971 with the main objective of studying possible ways of minimizing the adverse effects of those sanitary barriers affecting meat trade which are connected with animal health and meat hygiene regulations. This group was later incorporated into the Interdivisional Working Group on Policy for Meat Development.
12. The publication of the interdivisional study, Non-tariff barriers to International Meat Trade arising from Health requirements, which appeared in 1973, sets out a summary of provisions contained in bilateral veterinary conventions, proposals for a standard of veterinary services to be adopted in the framework of multilateral conventions, and a summary of the present policies of European countries to prevent the introduction of foot-and mouth disease.
13. The representative of the Fisheries Department of FAO referred to the successful collaboration with the Codex Committee on Fish and Fishery Products and the Codex Committee on Food Hygiene, which had resulted in the elaboration of the Codes for Fresh Fish and Canned Fish. In addition to the Draft Code of Practice for Frozen Fish which would be considered at the present session, he informed the Committee that further Codes of Practice for Fish Products were under elaboration; the most immediate were Codes for Smoked Fish and for Shrimps and Prawns, Lobsters, salted fish, and later, Minced Fish Products, Crabs and Frozen Battered and Breaded Fish Products.

REVIEW OF MATTERS RELEVANT TO THE CODEX COMMITTEE ON FOOD HYGIENE AS DISCUSSED BY THE CODEX ALIMENTARIUS COMMISSION AND VARIOUS CODEX COMMITTEES

Codex Alimentarius Commission

14. The Committee noted that questions regarding its area of competence had been considered by the Executive Committee at its 22nd Session (ALINORM 76/4).
15. The Committee had previously expressed the view (ALINORM 76/13A, paras 27-33) that all codes of practice containing hygiene provisions except those for which specific hygiene committees had been given complete responsibility should be referred to it for endorsement of such provisions. It was also of the opinion that it should provide the direct link between Codex Commodity Committees and meetings of experts on microbiological specifications. The Committee had in particular asked for the advice of the Executive Committee on:
- (i) whether all hygiene provisions included in codes of practice being elaborated by Codex Commodity Committees should be referred to it for endorsement; and
  - (ii) whether, in view of its increasing activity in the area of microbiological specifications, it should be the body to advise on and ultimately to endorse microbiological specifications for food and associated methodology.
16. The Commission had noted that the Executive Committee was of the opinion that it was clear, both from a previous decision of the Commission and the action of Codex Commodity Committees themselves, that hygiene matters in codes of practice should be referred to the Food Hygiene Committee. Furthermore, it was clear that it was the responsibility of the Codex Committee on Food Hygiene to approve all provisions on food hygiene in standards or codes of practice, including microbiological specifications and associated methodology.

17. The Commission agreed with the recommendation of the Executive Committee (ALINORM 76/4, para 25) that in order to remove any doubts concerning the rôle of the Codex Committee on Food Hygiene, the Terms of Reference of the Food Hygiene Committee be amended as follows (words underlined added):

- (a) to draft basic provisions on food hygiene applicable to all foods;
- (b) (i) to consider, amend if necessary, and endorse provisions on hygiene prepared by Codex Commodity Committees and contained in Codex Commodity Standards, and
  - (ii) to consider, amend if necessary, and endorse provisions on hygiene prepared by Codex Commodity Committees and contained in Codex codes of practice unless, in specific cases, the Commission has decided otherwise,  
or
  - (iii) to draft provisions on hygiene in respect of a particular food commodity within the terms of reference of a Codex Commodity Committee at the request of that Committee;
- (c) to draft, where necessary, provisions on hygiene in respect of any food not assigned to any Codex Commodity Committee;
- (d) to consider specific hygiene problems assigned to it by the Commission.

Note: The term "hygiene" includes where necessary microbiological specifications for foods and associated methodology.

Draft Code of Hygienic Practice for Poultry Processing (ALINORM 76/13, Appendix II)

18. The Committee noted that the Commission, after agreeing to some minor textual amendments, had adopted the Draft Code of Hygienic Practice for Poultry Processing at Step 8 of the Procedure as a Recommended Code.

Draft Code of Hygienic Practice for Egg Products (ALINORM 76/13, Appendix III)

19. The Committee noted that the Commission had adopted the Draft Code at Step 8 of the Procedure as a Recommended Code. It was pointed out that the Microbiological Specifications for Egg Products, which would later be considered by the Committee at Step 4, formed part of the End Product Specifications of the Recommended Code.

Proposed Draft Code of Practice for Fresh Fish (ALINORM 76/13A, Appendix II)

Proposed Draft Code of Practice for Canned Fish (ALINORM 76/13A, Appendix III)

20. The Committee noted that the Commission had concurred with the recommendation of the Codex Committee on Food Hygiene and the Codex Committee on Fish and Fishery Products and had adopted the two codes at Step 8 of the Procedure.

Draft Code of Hygienic Practice for Molluscan Shellfish (ALINORM 76/13A, Appendix VI)

21. The Committee noted that although the Commission recognized that the code was in an advanced state of preparation it nevertheless agreed with those delegations who held the view that omission of Steps 6 and 7 could not be recommended and that the code should await consideration by the Codex Committee on Fish and Fishery Products and subsequent re-examination by the Codex Committee on Food Hygiene. It therefore advanced the code to Step 6 of the Procedure.

Codex Committee on Foods for Special Dietary Uses (ALINORM 76/26A - 9th Session)

22. The Committee noted that the Commodity Committee had differentiated in different sections of various standards under elaboration between non-microbial contaminants resulting from the production of the raw materials or from processing, and toxic substances arising from microbiological contamination. The Committee agreed with this distinction.

23. The Committee endorsed the hygiene sections in the Standards for Infant Formula, Canned Baby Foods and Processed Cereal Based Foods for Infants and Children (ALINORM 76/26A, Appendices II, III and IV).

Codex Committee on Fish and Fishery Products (ALINORM 76/18A, 10th Session)

24. The Committee discussed briefly the hygiene provisions in the Draft Standards for Quick Frozen Fillets of Hake and for Quick Frozen Shrimps and Prawns. The provisions were endorsed with the deletion of the word "toxic" in sub-section 5.3(c) of the Hake Standard, in line with the relevant decision of the Committee at its 10th Session (ALINORM 74/13, para 10).

25. It was pointed out that the end product specifications of the Code of Practice for Frozen Fish differed slightly from the endorsed provisions. It was agreed to consider harmonization of texts when discussing the Code later during the session.

Codex Committee on Edible Ices (ALINORM 76/11, 2nd Session)

26. The Committee noted the request of the Commodity Committee to provide guidance with respect to selection of methods of microbiological examination of edible ices (ALINORM 76/11, para 57). It was agreed to return to this matter when discussing microbiological specifications for Egg Products, taking into account a summary of government observations as compiled in document CX/EI 76/4 and distributed during the session.

Codex Committee on Fats and Oils (ALINORM 76/19, 8th Session)

27. The Committee endorsed the Hygiene sections of the Standards for Low Fat Spreads and for Edible Low Erucic Acid Rapeseed Oil (ALINORM 76/19, Appendices III and XIII).

Codex Committee on Sugars (ALINORM 76/27)

28. The Committee endorsed the Hygiene provisions of the Standard for Fructose (ALINORM 76/27, Appendix II).

Coordinating Committee for Africa (ALINORM 76/28, 2nd Session)

29. The Committee noted the observations of the Coordinating Committee about the usefulness of microbiological specifications for certain commodities traded extensively in the Region. It concurred with these views and requested the Coordinating Committee to make specific proposals which the Committee could consider at its next session.

CONSIDERATION AT STEP 4 OF THE PROPOSED DRAFT CODE OF HYGIENIC PRACTICE FOR PEANUTS (GROUNDNUTS)

30. The Committee considered the above mentioned proposed draft code as contained in ALINORM 76/13A, Appendix VII, in the light of comments received from the Netherlands (CX/FH 76/8).

Section II - Definitions

Safe Moisture Level

31. It was pointed out that for a constant water activity the moisture content of ground nuts could vary according to the variety.

32. The Committee agreed to delete the reference to total moisture. To avoid any misinterpretation it was decided to state that the specified water activity was applicable to peanuts "in shell" or "shelled".

Section III - Raw Material Sanitation Requirements

33. In line with the decision taken to delete the reference to total moisture in the definition for safe moisture level the reference to upper limit for moisture (7%) was deleted in the provision on Curing (III.B(1)).

34. The provision for "purchasing of farmers' stock" was amended slightly by deleting an explanatory clause (III.D.(1)).

35. The Committee agreed to expand the provision regarding the cleanliness of warehouses and bins for storage in bulk of peanuts by specifying that not only static but also extraneous material should be removed (III.D.(2)).

Section IV - Plant Facilities and Operating Requirements

36. With respect to storage of peanuts in rooms with new concrete floors or walls it was agreed to amend the requirement to read "For the first year of new concrete it is safest to use an approved plastic cover spread over the entire new floor as a moisture barrier prior to filling with peanuts. Other means of storage such as stacking of containers on plastic pallets to protect the peanuts against moisture from 'sweating' of concrete can be used." (IV.D.(1)(b)).

37. The Committee discussed in detail the transport of peanuts in refrigerated vehicles. It was agreed that due to the varying climatic conditions under which peanuts could be grown a general provision would be preferable to the present text which was amended to read: "Refrigerated vehicles are recommended for transport when climatic conditions indicate such a need. Extreme care should be taken to prevent condensation when unloading peanuts from cold storage or from a refrigerated vehicle. In warm,

humid weather the peanuts should be allowed to reach ambient temperature before exposure to external conditions. This tempering may require 1-3 days." (IV.D.(6)).

38. The Committee thought it sufficient to indicate certain temperature and relative humidity ranges for optimum storage conditions. It agreed to delete a reference to storage in temperate areas (IV.D.(6)(b)(i)).

39. The question was raised whether in the Code methods for aflatoxin analysis would be included. The Committee agreed that this was not the intention but governments were specifically requested to give their views on this matter.

#### Status of the Code

40. Following some discussion on how best to proceed further with the elaboration of the Code the Committee decided to return the Code to Step 3 of the Procedure for a further round of government comments - observations from producing countries were specially requested. The Secretariat undertook to bring the matter to the attention of the Coordinating Committee for Africa. The Committee held the view that at its next session it might well recommend the omission of Steps 6 and 7 when advancing the Code to Step 5 of the Procedure. The revised Code is contained in Appendix III to this Report.

41. As a matter of general importance the Committee discussed the desirability of reviewing, after a certain number of years, those Codes which had been advanced to Step 8 of the Procedure. The Committee agreed to discuss the matter at its next session. In this connection the delegation of Senegal agreed to provide comments on the present Code.

#### CONSIDERATION OF THE PROPOSED DRAFT CODE OF HYGIENIC PRACTICE FOR PROCESSING OF FROG LEGS

42. The Committee considered the above mentioned proposed draft code as contained in ALINORM 76/13A, Appendix VIII, in the light of comments received from Canada, the Netherlands, Poland and the United Kingdom (CX/FH 76/9).

#### Section I - Scope

43. The Committee discussed in considerable detail the wording for the Scope section. It was finally agreed that harmonization of texts in Codes was desirable and the wording used in the Code for Foods for Infants and Children was considered most appropriate: "This Code of Hygienic Practice applies to frog legs. It contains the minimum requirements of hygiene in the production, processing, handling, packing, storage, transportation and distribution of frog legs to ensure a healthful and wholesome supply of this product."

44. The question was raised whether the species of frog from which frog legs were derived should be listed as was the case, for example, in the Code for Peanuts.

45. After some discussion it was agreed that listing all the species was not feasible nor, indeed, desirable.

#### General Discussion

46. At the request of the Chairman of the Committee a small working group consisting of representatives of the delegations of Canada, the Netherlands, UK and USA reviewed the Code taking into account the written comments received. The Committee discussed and concurred with the suggestions made by the working group which were in the main editorial. Several other revisions took into account the wording used in, for example, the Code of Hygienic Practice for Poultry Processing and for Molluscan Shellfish.

47. The Committee discussed at some length the necessity to include in the Code a provision for the method of slaughter and, in particular, whether a reference should be made to slaughtering "humanely".

48. Whilst agreeing that it was essential that the frogs should not suffer excessive stress during slaughtering, the Committee agreed that the method of slaughter was not relevant to a Code of Hygienic Practice. However, in order to take into account the views expressed by some delegations a provision was included in the Code stating that the frogs should be slaughtered under conditions of minimum stress. The Committee agreed to request producing countries to comment specifically on this new provision.

49. With regard to End Product Specifications it was agreed to replace the present text by the wording used in the Code for Frozen Fish (ALINORM 76/18A, Appendix VI), suitably amended.

50. The Committee discussed at length the need to include quantitative microbiological specifications in the Code. Some delegations held the view that these were not necessary, taking into account that the product would be cooked prior to consumption and that therefore microbiological specifications were of no direct value. Other delegations were of the opinion that specific microbiological requirements were necessary because of the danger of transfer of contamination before cooking.

51. The delegation of the United Kingdom pointed out that this risk could be avoided by proper attention to hygienic practices.

52. The Committee agreed to request the 2nd Joint FAO/WHO Expert Consultation for Microbiological Specifications for Foods to consider not only the establishing of micro-biological specifications but also the general question of whether such specifications would serve a practical purpose.

53. It was agreed to request governments to provide data on the microbiology of products which would assist in the development of microbiological specifications. Data should be sent to the Secretariat of the Consultation, Dr. Reinius.

#### Status of the Code

54. The Committee agreed to advance the Code of Hygienic Practice for Processing of Frog Legs to Step 5 of the Procedure. The revised Code is contained in Appendix II to this Report.

#### CONSIDERATION OF THE HYGIENE PROVISIONS IN THE DRAFT CODE OF PRACTICE FOR FROZEN FISH

55. The Committee had before it the above Draft Code (ALINORM 76/18A, Appendix VI) and written comments received from the USA (CX/FH 76/5).

56. A small working group consisting of representatives of the delegations of the Netherlands and the USA, together with representatives of the FAO Fisheries Department, reviewed the suggested changes and presented to the Committee a number of proposals for amendment of the text of the Code.

57. The Committee noted that most changes were editorial in nature or were revisions to bring the text into conformity with the corresponding provisions in the Codes for Fresh Fish and for Canned Fish. The Committee concurred with the proposals of the working group.

#### Status of the Code

58. The Committee recommended that the Codex Committee on Fish and Fishery Products at its next session advance the Code to Step 8 of the Procedure. The various amendments are listed in Appendix IV to this Report.

#### CONSIDERATION AT STEP 4 OF MICROBIOLOGICAL SPECIFICATIONS FOR EGG PRODUCTS

59. The Committee considered the report of a working group consisting of the representatives of the delegations of the Netherlands (Chairman), United Kingdom and USA which had examined the Report of the Joint FAO/WHO Expert Consultation (EC/Microbiol/75/Report 1) and in particular the Microbiological Specifications contained in Annex V of the report, in conjunction with government comments.

60. The delegate of the Netherlands pointed out that the Joint Expert Consultation had recommended microbiological specifications for Salmonella, mesophilic aerobic bacteria and coliform bacteria for dried and frozen whole eggs and salmonella specifications only for other egg products.

61. More generally applicable methods for mesophilic aerobic plate count and coliforms were at present under consideration by ISO Technical Committee 34. Sub Committee 9 (TC 34/SC9) and it was expected that these would be published as ISO International Standards before the next session of the Committee.

62. The Committee agreed with the view of the working group that while suggested amendments should be incorporated into the Code where feasible, provision should be made for reference to the ISO Codes when these were available.

63. Standardization of the method for detecting Salmonella had not been completed by ISO TC 34/SC9. It was the opinion of the working group that the method for Salmonella as described in the Report on Microbiological Specifications for Egg Products and as amended by the Working Group was adequate.

64. The Committee agreed to the amendments to Annex V of the Report on Microbiological Specifications for Egg Products as proposed by the Working Group. The amended Salmonella method as proposed by the Working Group is attached as Appendix VI to this Report.

65. The delegation of the United Kingdom, while endorsing the methodology described in Annex V, was of the opinion that the  $\alpha$  amylase test as an indicator of effective pasteurization gave sufficient information on the safety of egg products.

#### Status of the Code

66. The Committee agreed to advance the Code to Step 5 of the Procedure, on the understanding that reference to the ISO specifications would be incorporated when available, and to request the Commission that Steps 6 and 7 be omitted. The UK entered a reservation that in the UK's opinion microbiological specifications were not necessary for egg products.

#### CONSIDERATION AT STEP 4 OF THE PROPOSED DRAFT CODE OF HYGIENIC PRACTICE FOR LOW ACID CANNED FOODS

67. The Committee had before it the above-mentioned proposed draft code as contained in ALINORM 76/13A, Appendix IV. Government comments had been received from Australia, Israel, Switzerland, UK and USA. At the outset of the session, the Committee had requested representatives from the delegations of Canada (Coordinator), the Netherlands, the UK and USA to review the code in the light of the comments received.

#### Section I - Scope

68. The delegation of Canada, acting as rapporteur of the Working Group, proposed that the scope be amended to apply to rigid hermetically-sealed containers as it was thought that for flexible pouches specific requirements with regard to hygienic practices were needed. The Committee concurred with this proposal and the scope section was amended accordingly. A footnote would point out that for flexible pouches a separate section within the code would be elaborated at a later date.

#### Section II - Definitions

69. The Working Group had made proposals for a large number of amendments to the definitions section. The Committee discussed these and some further changes were made. Several delegations pointed out that in a number of codes the same terms had been defined in slightly different ways and that harmonization seemed to be desirable. This matter was further taken up during the deliberations on the revised General Principles of Food Hygiene (see paras 75 and 76(i) of this Report). The Committee concurred with the various changes proposed and the definitions section was amended accordingly.

#### Low Acid Foods - pH value

70. There was some discussion on whether the equilibrium pH value of 4.6 should be reduced to 4.5 to provide a greater safety margin. Reference was made to a previous discussion at the 11th Session of the Committee (ALINORM 76/13, para 9) in which it was decided to change the pH of 4.5 to its present value. The Committee noted that at that time it was agreed that the pH defined in this manner allowed an adequate margin of safety and decided to maintain the equilibrium pH value for low acid foods at 4.6.

#### Section III - Raw Material Requirements

71. The Working Group had come to the conclusion that the raw material requirements as contained in Section III would, in the main, be equivalent to the relevant requirements in the General Principles of Food Hygiene and had therefore not made any proposals for amendments.

#### Section IV - Plant Facilities and Operating Requirements

72. Similar reasoning applied to part of this section with regard to the inclusion of provisions which were considered adequately covered in the General Principles of Food Hygiene and which related to all canned foods. An exception was made for the sub-sections on plant equipment and operating practices and production requirements (IV. C and D).

73. The Working Group proposed to make available by 1 September 1976 the revised Sections IV and V of the code to enable return of comments on the revised code from governments to arrive by 1 January 1977. This revision would consider changes suggested by those government comments received prior to and at the current meeting. The Group also proposed to meet prior to the next session of the Codex Committee on Food Hygiene to consider further government comments on this revised text.

#### Status of the Code

74. The Committee agreed to return the draft code to Step 3 of the Procedure for a further round of specific government comments (see para 73 above). The revised Scope (I) and Definitions (II) sections are attached as Appendix V to this Report.

#### CONSIDERATION OF THE REVISION OF THE CODE OF HYGIENIC PRACTICE - GENERAL PRINCIPLES OF FOOD HYGIENE

75. The Committee was informed that the Working Group consisting of representatives of delegations of the Netherlands, UK and USA and representatives of FAO and WHO which had been set up at the last session (Washington, May 1975) had had various deliberations on the revised text for the General Principles of Food Hygiene but that unfortunately an integrated proposal could not be presented at this session. Dr. K. Büchli (Netherlands) acted as rapporteur.

76. The Committee discussed various aspects of the principles involved in relation to the revision:

(i) Definitions. It was suggested that in an annex to the code a large number of terms could be defined including terms which might not appear in the text of the code proper but which had relevance to hygiene and were used in other codes. The Committee agreed with the principle of listing the definitions in an annex but reserved its position with regard to "extraneous" definitions (but see also para 92 of this Report). Several delegations pointed out that harmonization of definitions was highly desirable. In this connection it was pointed out that WHO had set up a Technical Terminology Service as a reference centre for definitions. It was agreed that this Service would be consulted when drawing up definitions.

(ii) Explanatory Notes. Several delegations suggested that, in revising the present code, a procedure be adopted similar to that followed in the various Fish and Fishery Product Codes with regard to explanatory notes to the Code proper. Other delegations held the view that this was a code of general principles and not a technological code and that explanatory notes might overburden the text and detract from its value. The Committee decided to leave the decision on the inclusion of explanatory notes to the judgment of the Working Group. It was pointed out however that there was a danger that such notes might lead to the General Principles taking the form of a Hygiene Text Book.

(iii) Guidelines for Disinfection. The delegation of the United Kingdom had prepared a document on Guidelines for Disinfection. It was agreed that these guidelines might also be appended to the code.

#### Status of the Code

77. The Committee agreed that the code would be revised by the Working Group which was to meet in October 1976 and that the revised document would be distributed for government comments at Step 3 towards the end of the year. If this was found desirable the Working Group would reconvene prior to the next session of the Committee to discuss the revised text in the light of government comments received. In view of the relevance of the General Principles of Food Hygiene to the Proposed Draft Code of Hygienic Practice for Foods for Infants and Children it was agreed to request the Federal Republic of Germany, one of the author countries of the latter code, to attend the meeting of the Working Group.



CONSIDERATION OF THE PROPOSED DRAFT CODE OF HYGIENIC PRACTICE FOR FOODS FOR INFANTS AND CHILDREN AT STEP 4

78. The Committee received an oral report from the delegation of the Federal Republic of Germany on the development of the above-mentioned Code as contained in ALINORM 76/13A, Appendix V.

79. The Rapporteur pointed out that comments had been received from Australia, Canada, the Federal Republic of Germany, Netherlands, Poland, Switzerland, United Kingdom and USA. The major part of the observations applied, however, to that portion of the text which was directly related to the General Principles of Food Hygiene which was being revised.

80. It had therefore not seemed appropriate to revise the Code until a more definite text for the General Principles had been agreed upon. The Committee concurred with the view of the Rapporteur but noted that the demand for the Code and the attached microbiological specifications required that action be taken as soon as possible. It was agreed that the Rapporteur would revise the Code following the session of the Working Group on the Code of Hygienic Practice - General Principles of Food Hygiene scheduled for October 1976 and which would also take into account to the extent appropriate the comments received on the present Code.

81. It was further agreed that the Rapporteur would convene, tentatively in November, at the FAO/WHO Collaborative Centre for Research and Training in Food Hygiene, Berlin, a meeting of experts to discuss the microbiological specifications for Foods for Infants and Children. An initial proposal for such specifications was attached as Annex A to the Code.

82. The report of the Berlin meeting would be sent to the Chairman of the Food Hygiene Committee for general distribution early 1977 to provide the opportunity for another round of government comments prior to the next session. The report and government comments would also be made available to the 2nd Joint FAO/WHO Expert Consultation on Microbiological Specifications to be convened in the first quarter of 1977 with a request that it review the findings of the Berlin Group.

83. The Committee would at its next session then be able to discuss at Step 4 the Revised Code together with the Revised Microbiological Specifications (Annex A).

OTHER BUSINESS

84. The Committee noted the recommendation of the ISO Sub Committee 9 of TC 34 (May 1976) which expressed concern that in many instances microbiological specifications were not based on sound principles when set for certain foods. SC 9 recommended that FAO and WHO convene a Joint FAO/WHO Expert Committee to consider the matter.

85. After some discussion the Committee agreed to request the 2nd Joint FAO/WHO Consultation on Microbiological Specifications for Foods to establish Guidelines for the development and application of microbiological specifications for foods, taking account of the purpose, need, relevance and administrative feasibility of the application of such specifications.

86. It was brought to the attention of the Committee that another body within FAO was elaborating standards for gelatine, including microbiological specifications and that examination of such specifications might well come within the terms of reference of the Committee.

87. It was agreed that the Secretariat should inform the Committee at its next session on the status of this and other similar standards so that the Committee could then express its views on the matter.

FUTURE WORK

88. The Committee noted a suggestion that there was a need for the elaboration of a code for acidified low acid canned foods which would cover such products as pimentoes, peppers and certain canned tomatoes in which the growth of C. botulinum had presented problems.

89. The Committee also noted the observations of the delegations of Senegal and France with regard to the control of aflatoxin, particularly in groundnuts. It was pointed out that at its 11th Session (ALINORM 76/44) the Commission had agreed that data on levels of contaminants should be submitted to the appropriate Codex Committees who should make proposals on the limits of contaminants in various foods for further consideration and endorsement by General Subject Committees.

90. The Committee noted (see para 13) that it could expect further work on Codes of Practice for Fish and Fishery Products at its next session and to examine the conclusions of the 2nd Joint FAO/WHO Consultation on Microbiological Specifications for Foods which would also cover microbiological specifications for Edible Ices.

91. In addition further revised drafts of the Codes of General Principles of Food Hygiene, Hygienic Practice for Foods for Infants and Children, and Low-acid Canned Foods would be ready for examination.

92. Following further discussions on the harmonization of definitions, including mycotoxins, it was agreed that the Committee would receive background information on the work and potentialities of the WHO Terminology Reference Centre. The delegation of the Netherlands undertook to provide a paper with regard to harmonization of definitions of some different descriptions of terms used in various Codes which could be discussed at the next session of the Committee.

DATE AND PLACE OF NEXT SESSION

93. The Committee noted that its 14th Session was planned to be held in Washington in May 1977 but that the exact date had not yet been fixed.

NOTE: Summary Status of Work is on page 71.

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APPENDIX III

DRAFT CODE OF HYGIENIC PRACTICE FOR PROCESSING OF FROGEGGS

(Advanced to Step 5)

This code of hygienic practice applies to froglegs. It contains the minimum requirements of hygiene in the production, processing, handling, packing, storage, transportation and distribution of froglegs to ensure a healthful and wholesome supply of the product. The transport vehicle should be inspected on arrival. (2) RECEIVING AND INSPECTION OF FROGEGGS. Suppliers of stock should be inspected at the shellings plant.

SECTION III - RAW MATERIAL REQUIREMENTS

A. Environmental Sanitation in Production Areas

(1) Sanitary disposal of all human and animal wastes. Adequate precautions should be taken to ensure that human and animal wastes are disposed of in such a manner as not to constitute a public health or hygienic hazard and extreme care should be taken to protect products from contamination with these wastes.

(2) Animal, plant pest and disease control. Control measures that are undertaken by treatment with chemical or biological or physical agents should be done only in accordance with the recommendations of the appropriate official agency, by or under the direct supervision of personnel with a thorough understanding of the hazards involved, including the possibilities of toxic residues being retained by the product.

(3) Harvest areas. The environment from where frogs are caught or collected should not constitute a public health hazard to the consumer through the product.

B. Sanitary Production of Raw Materials

(1) Equipment and product containers. Equipment and product containers should not constitute a hazard to the product. Containers which are reused should be of such material and construction as to facilitate thorough cleaning, and should be so cleaned and maintained as not to constitute a source of contamination to the product.

(2) Sanitary techniques. (a) Frogs obtained from habitats which may be polluted should be subjected to cleansing and sanitizing in running potable water for at least 24 hours. For this purpose a cleaned and sanitized cemented tank or preferably stainless steel or non-corrosive metal tank with an outlet at the bottom may be employed.

(b) To prevent deterioration in the quality of froglegs, it is essential that steps should be taken to prevent the following:

- (i) injury or bruising of the fresh froglegs during catching, handling, and from use of unsuitable gear.
- (ii) contamination of the froglegs with dirt or any other extraneous matter;
- (iii) exposures to unfavourable temperatures;
- (iv) rough handling, such as improper stacking of full containers.



(3) Removal of obviously unfit materials. Unfit frogs, for example, those less active that are injured or have blood clots or parasites in the flesh, should be segregated during collection to the fullest extent practicable and should be disposed of in a manner that will prevent contamination of other frogs or water supplies.

(4) Protection of product from contamination. Suitable precautions should be taken to protect the raw product from being contaminated by animals, insects, vermin, birds, chemicals or microbiological contaminants or other objectionable substances during handling and storage.

C. Transportation

(1) Facilities. Conveyances for transporting the raw product from the production area or storage should be adequate for the purpose intended and should be of such material and construction as will permit thorough cleaning and sanitizing (disinfection) and should be so cleaned and maintained as not to constitute a source of contamination to the product.

(2) Handling procedures. All handling procedures should be such as will prevent the product from being contaminated. Extreme care should be taken in transporting the frogs, for example vans should be covered.

SECTION IV - PLANT, FACILITIES AND OPERATING REQUIREMENTS

A. Plant Construction and Lay-out

(1) Location, size and sanitary design. The building and surrounding areas should be such as can be kept reasonably free from objectionable odours, smoke, dust or other contaminations; should be of sufficient size for the purpose intended without crowding of equipment or personnel; should be of sound construction and kept in good repair; should be of such construction as to prevent the entrance and harbouring of insects or birds or vermin; and should be so designed as to permit easy and adequate cleaning. The working premises, walls, floors and ceiling should be constructed of such a material and be so finished as to allow them to be effectively cleaned and drained and should be kept in good repair and prevent as far as practicable any risk of infestation. Areas used for waste disposal should be paved.

(2) Sanitary facilities and controls

(a) Separation of processes. Areas where raw materials are received or stored should be so separated from areas in which final product preparation or packaging is conducted as to preclude contamination of the finished product. Areas and compartments used for storage, processing and handling of the product should be separated and distinct from those used for inedible materials. The processing area should be completely separated from any part of the premises used as living quarters.

(b) Water supply. An ample supply of potable water should be available. Standards of potability should not be less than those contained in the latest published edition of "International Standards for Drinking-Water", World Health Organization, Geneva.

(c) Ice. Ice should be made from water of potable quality and should be manufactured, handled, stored and used so as to protect it from contamination.

(d) Auxiliary water supply. Where non-potable water is used - for such purposes as fire control - it must be carried in completely separate lines, identified preferably by colour and with no cross-connection or back-siphonage with the lines carrying potable water.

(e) Plumbing and waste disposal. All plumbing and waste disposal lines (including sewer systems) must be large enough to carry peak loads. All lines must be water-tight and have adequate traps and vents. Disposal of

waste should be effected in such a manner as not to permit contamination of potable water supplies. The plumbing and the manner of waste disposal should be approved by the official agency having jurisdiction.

(f) Lighting and ventilation. Premises should be well lit and ventilated. Special attention should be given to the venting of areas and equipment producing excessive heat, steam, obnoxious fumes or vapours or contaminating aerosols. Good ventilation is important to prevent both condensation (which may drip into the product) and mould growth in overhead structures, which growth may fall into the product. Light bulbs and fixtures suspended over the product in any step of preparation should be of the safety type or otherwise protected to prevent product contamination in the case of breakage. The illumination in any part of a working room should not be less than 325 lux units (30 foot candles) and at points requiring close examination of the product they should be illuminated at an intensity of not less than 540 lux units (50 foot candles). Reflector filaments should be designed to allow easy dismantling, cleaning and reassembling. Ventilation should be planned to allow for adequate circulation or changes of air and to ensure that the direction of air flow is never from a dirty area to a clean one.

(g) Toilet rooms and facilities. Adequate and convenient toilets should be provided and toilet areas should be equipped with self-closing doors. Toilet rooms should be well lit and ventilated and should not open directly into the product handling area. They should be kept in a sanitary condition at all times. There should be associated hand-washing facilities within the toilet area and notices should be posted requiring personnel to wash their hands after using the toilet.

(h) Hand-washing facilities. Adequate and convenient facilities for employees to wash and dry their hands should be provided wherever the process demands. They should be in full view of the processing floor. Single-use towels are recommended, where practicable, but otherwise the method of drying should be approved by the official agency having jurisdiction. The facilities should be kept in a sanitary condition at all times.

(i) Cleaning and sanitizing (disinfection). Premises, equipment and utensils should be cleaned at frequent intervals during the day. They should be cleaned and sanitized (disinfected) immediately and thoroughly, whenever circumstances make it necessary. Additionally, they should be cleaned and sanitized (disinfected) at the end of each working day.

#### B. Equipment and Utensils

(1) Materials. All product-contact surfaces should be smooth; free from pits, crevices and loose scale; non-toxic; unaffected by the product; capable of withstanding repeated exposure to normal cleaning; and non-absorbent.

(2) Sanitary design, construction and installation. Equipment and utensils should be so designed and constructed as will prevent hygienic hazards and permit easy and thorough cleaning. Stationary equipment should be installed in such a manner as will permit easy and thorough cleaning. All contact surfaces should be of stainless steel or any other non-corroding, non-absorbing materials. Approved plastic materials used should be free from cracks and scratches and should be capable of withstanding the regular cleaning and disinfection process.

(3) Equipment and utensils. Equipment and utensils used for inedible or contaminating materials should be so identified and should not be used for handling edible products.

C. Hygienic Operating Requirements

(1) Sanitary maintenance of plant, facilities and premises

(a) The building, equipment, utensils and all other physical facilities of the plant should be kept in good repair and should be kept clean and maintained in an orderly, sanitary condition at all times. Waste materials should be frequently removed from the working area during plant operation and adequate waste receptacles should be provided. Detergents and disinfectants employed should be appropriate to the purpose and should be so used as to present no hazard to public health.

(b) Cleaning and sanitizing (disinfection). Premises, equipment and utensils should be cleaned at frequent intervals during the day. They should be cleaned and sanitized (disinfected) immediately and thoroughly, whenever circumstances make it necessary. Additionally, they should be cleaned and sanitized (disinfected) at the end of each working day.

(c) Waste materials should be stored in such a manner as not to cause nuisance from offensive odours or flies or vermin. They should be removed from the premises at least once daily. Immediately after emptying, the receptacles should be thoroughly washed out with hot water and detergent. The area used for storage of waste receptacles should be thoroughly cleaned and sanitized (disinfected).

(2) Vermin control. Effective measures should be taken to protect against the entrance into the premises and the harborage on the premises of insects, rodents, birds or other vermin. The processing halls should be adequately fly-proofed and provided with self-closing doors.

(3) Exclusion of domestic animals. Dogs, cats and other domestic animals should be excluded from areas from where the product is processed or stored.

(4) Personnel Health. Plant management should advise personnel that any person afflicted with infected wounds, sores, or any illness, notably diarrhoea, should immediately report to management. The plant management should take care to ensure that no person, while known to be affected with a disease capable of being transmitted through food, or known to be a carrier of such disease, or while afflicted with infected wounds, sores, or any illness, is permitted to work in any area of a food plant in a capacity in which there is a likelihood of such a person contaminating the product or surfaces with which the product may come into contact.

(5) Toxic substances. All rodenticides, fumigants, insecticides or other toxic substances should be stored in separate locked rooms or cabinets and handled only by or under the direct supervision of personnel with a thorough understanding of the hazards involved, including the possibility of contamination of the products.

(6) Personnel hygienic and product handling practices

(a) All persons working in the plant should maintain a high degree of personal cleanliness while on duty. Clothing, including suitable head-dress should be appropriate to the duties being performed and should be kept clean.

(b) Hands should be washed as often as necessary to conform to hygienic operating practices.

(c) Spitting, eating and the use of tobacco or chewing gum should be prohibited in the product handling areas.

(d) All necessary precautions should be taken to prevent the contamination of the product with any foreign substances.

(e) Minor cuts and abrasions on the hands should be appropriately treated and covered with a suitable water-proof dressing. Adequate first aid facilities should be provided to meet these contingencies so that there is no contamination of the product.

(f) Gloves used in product handling should be maintained in a sound, clean and sanitary condition. Gloves should be made of an impermeable material.

(7) Drainage. There should be adequate drainage facilities for carrying away water used in the plant premises and to discharge it into a channel at least 3 metres from the plant. The drainage system inside the premises should be properly covered. The sewage from the toilet should be disposed of in such manner that it cannot contaminate the water supply to the plant. Water, including waste or rain water, should not be allowed to accumulate inside the premises.

(8) Floor. The floor of the plant should be smooth, impervious and should be sloping so that the water always runs into the drain.

#### D. Operating Practice and Production Requirements

##### (1) Raw-material handling

(a) Acceptance criteria. It is recommended that unfit frogs should be segregated prior to delivery to the processing plant. Similarly, on arrival, unfit frogs should be removed as soon as possible and segregated for disposal in an appropriate manner. Arrangements for removal and segregation should be approved by the official agency having jurisdiction.

(b) Storage. Raw materials stored in the plant premises should be maintained under conditions that will protect them against contamination and infestation and minimize deterioration.

(2) Inspection and sorting. Prior to introduction into the processing line or at a convenient point within it, the raw materials should be inspected, sorted or culled as required to remove unfit materials. Such operation should be carried out in a clean and sanitary manner. Only clean sound materials should be used in further processing.

(3) Washing or other preparation. Raw materials should be washed as needed to remove any contamination. Water used for washing and rinsing should be of potable quality. Water used for such purpose should not be re-circulated unless suitably treated to maintain in a condition as will not constitute a public health hazard.

##### (4) Preparation and processing

(a) Preparation. Only healthy frogs should be slaughtered. Slaughter should be carried out under conditions of minimal stress to the animal. After slaughter the hind legs should be cut at the abdomen not more than 2.5 cm above the waist. Immediately after cutting, the legs should be de-skinned and put into 5% chilled brine for proper bleeding and to prevent clotting of blood inside. The de-skinned legs should be washed and further cleaned by trimming of the claws. Hanging pieces of flesh should also be removed. The dressed material should be washed (3-4 times) to remove bacteria coming from broken viscera or from contamination during cutting and handling. The water used for washing should be freshly running potable water and should not be re-circulated unless it is restored to a level of potable quality. The water may be chlorinated in concentrations approved by the official agency having jurisdiction. The product should then be preserved under chilled conditions.

(b) Grading. The material should be given a final wash in clean water and graded in different sizes on the basis of count per kg.

(c) Freezing. The legs should be frozen in the minimum possible time. Bruised, squeezed or broken legs should not be used for freezing.

##### (5) Packing of finished products

(a) Materials. Packaging materials should be stored in a clean and sanitary manner and should not transmit to the product objectionable substances beyond limits acceptable to the official agency having jurisdiction and should provide appropriate protection from contamination.

(b) Techniques. Packaging should be done under conditions that preclude the introduction of contamination into the product. The legs may be wrapped individually in either polyethylene film or other suitable covering.

(c) Lot Identification. Each container should be embossed or otherwise permanently marked in code or in clear so that information regarding the processor and date of processing can be retrieved and products identified in consumer markets when associated cases of food-borne illness have occurred.

(6) Storage of finished products. The following provisions should apply where the product is placed in chilling room and cold storage:

(a) The product should be stored under such conditions as will preclude the contamination with, or growth of pathogenic or toxigenic microorganisms or infestation and protect against deterioration of the product or of the containers. Special care should be taken to ensure that the air circulation in between the stacked product is proper and adequate;

(b) Entry should be restricted to personnel necessary to carry out operations efficiently;

(c) Doors should not be left open for extended periods and should be closed immediately after use;

(d) No chilling room and cold storage should be loaded beyond its designed capacity;

(e) Where recording thermometers are not used temperatures should be read at regular intervals and the readings recorded in a log book.

(7) Transport of finished products. The finished products should be transported under such conditions as will preclude the contamination with, or growth of pathogenic microorganisms or infestation and protect against deterioration of the product or of the containers.

#### E. Sanitation Control Programme

It is desirable that each plant in its own interest designate a single individual, whose duties are preferably divorced from production, to be held responsible for the cleanliness of the plant. His staff should be a permanent part of the organization and should be well trained in the use of special cleaning tools, methods of disassembling equipment for cleaning, and in the significance of contamination and the hazards involved. Critical areas, equipment and materials should be designated for specific attention as part of a permanent sanitation schedule.

#### F. Laboratory Control Procedures

In addition to any control by the official agency having jurisdiction, it is desirable that each plant in its own interest should have access to laboratory control of the sanitary quality of the product processed. Such control should reject all products that are unfit for human consumption. Analytical procedures used should follow recognized or standard methods in order that the results may be readily interpreted.

### SECTION V - END PRODUCT SPECIFICATION

Appropriate methods should be used for sampling and examination to determine the compliance with the following specifications:

A. Frog legs should, to the extent possible in good manufacturing practice, be free from objectionable matter and parasites.

B. Frog legs should be free from microorganisms in amounts harmful to man, free from parasites harmful to man and should not contain any substances originating from microorganisms in amounts which may represent a hazard to health.

C. Froglegs should be free from chemical pollutants in amounts which may represent a hazard to health.

D. Froglegs should comply with any requirements set forth by the Codex Alimentarius Commission on pesticide residues and food additives as contained in permitted lists of Codex commodity standards, or should comply with the requirements on pesticide residues and food additives of the country in which the froglegs will be sold.

PROPOSED DRAFT CODE OF HYGIENIC PRACTICE FOR PEANUTS (GROUND NUTS)  
(Returned to Step 3)

To be read in conjunction with the Recommended International Code of Practice - General Principles of Food Hygiene. Sidelined portions indicate material which is particular to this Code of Hygienic Practice and therefore does not appear in the General Principles of Food Hygiene.

SECTION I - SCOPE

This Code of Hygienic Practice applies to peanuts, also known as ground nuts (Arachis hypogaea). It contains the minimum requirements of hygiene for farm handling, transportation, storage, in-shell operations and commercial shelling. It covers all types and forms of raw, dried, in-shell and shelled peanuts.

SECTION II - DEFINITIONS

"Blows", (pops) means in-shell nuts which are unusually light-weight due to extensive damage from physiological, fungous, insect, or other causes and which can be removed mechanically, for example, by air flow.

"Curing" means drying of in-shell peanuts to a safe moisture level whether by natural or mechanical means, or a combination of both.

"Farmer's stock peanuts" means in-shell peanuts as they come from the field, after separation from the vines by hand and/or mechanical means.

"Safe moisture level" means one that will prevent growth of microorganisms normal to the nut harvesting, processing and storage environment. The maximum safe moisture level for peanuts is established by its water activity ( $a_w$ ). Water activity is defined as the quotient of the water vapour pressure of the substance (peanut - in-shell or shelled) divided by the vapour pressure of pure water at the same temperature. An  $a_w$  exceeding 0.70 at 25°C (77°F) is unsafe.

SECTION III - RAW MATERIAL SANITATION REQUIREMENTS

A. Environmental Sanitation in Growing, Harvesting and Food Production Areas

(1) Sanitary disposal of human, animal and plant wastes. Adequate precaution should be taken to ensure that human and animal wastes are disposed of in such a manner as not to constitute a public health or hygienic hazard, and extreme care should be taken to protect the products from contamination with these wastes. Vine and peanut waste should not be permitted to accumulate in such a manner as to attract rodents or insects.

(2) and (3) - As in the General Principles of Food Hygiene.

B. Sanitary Harvesting and Production

(1) Curing. After digging pods should be exposed for maximum rate of drying. This may be accomplished by turning the vines to leave the pods uppermost where they are away from the ground and exposed to sun and wind. Curing, whether by natural or mechanical means or a combination of both, should be completed as rapidly as possible to a safe moisture level, so as to prevent growth of microorganisms, particularly molds that produce aflatoxins. When using mechanical drying, excessive heat should be avoided since this causes some kernels to split after shelling. Close checks of moisture content or water activity of lots of farmers' stock peanuts should be maintained.

(2) Equipment and product containers. As in the General Principles of Food Hygiene.

(3) Sanitary techniques. Harvesting and production operations, methods and procedures should be clean and sanitary. Drying equipment should be so constructed as to be easily cleaned and maintained and should contain no pockets in which debris may become lodged.

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(4) Removal of obviously unfit materials. Damaged or imperfect peanuts and lots that contain any obvious contamination with human or animal wastes, insect infestation, decomposition, broken shells, embedded dirt, blows, or other defects to an extent which would render them unfit for human consumption, should be segregated during harvesting and production to the fullest extent practicable. Such segregated unfit peanuts should be disposed of in such place and manner to prevent contamination of sound nuts, water supplies, or other crops.

(5) Protection of peanuts from contamination. Suitable precautions should be taken to protect the nuts from contamination by domestic animals, rodents, birds, insects, mites and other arthropods, or other biological agents, or with chemical or other objectionable substances during handling and storage. The nuts should be moved to suitable storage, or to the processing area for immediate processing, as soon as possible after harvesting or drying. Where nuts are likely to become infested with insects, mites (and other arthropods) during or after harvesting, suitable treatment such as fumigation or application of an insecticide spray should be carried out as a preventive measure. Nuts held for processing should be stored in covered containers, buildings, or under covering. Fumigation or spray methods and chemicals used should be approved by the official agency having jurisdiction. High humidities which are conducive to proliferation of mould and elaboration of mycotoxins should be avoided in storage areas in order to maintain peanuts at a safe moisture level. Recommended storage conditions are specified in Section IV D.(1)(b).

C. Transportation

(1) Facilities. Conveyances for transporting the harvested crop from the place of harvest or storage should be adequate for the purpose intended and should be of such material and construction as will permit thorough cleaning and should be so cleaned and maintained as not to constitute a source of contamination to the product. In addition, bulk transport such as ship or rail car should be well ventilated with dry air to remove moisture resulting from respiration of the peanuts and to prevent moisture condensation as the vehicle moves from warm to cool regions or from day to night.

(2) Handling procedures. All handling procedures should be such as will prevent the product from being contaminated. Extreme care should be taken in transporting peanuts with an unsafe moisture level to prevent spoilage or deterioration. Special equipment - such as refrigerated transport - should be used if the nature of the product or distances involved so indicate.

D. Shelling Plant

(1) Purchasing of farmers stock. Most of the damage may have already been done to the peanuts during growing, harvesting, drying, handling, and storage. A buyer for a shelling plant, whether located at the plant or at an outlying commission buying point, should monitor the quality of peanut lots offered to him, and with the cooperative extension service assist suppliers in eliminating improper practices. Buyers should encourage suppliers of farmers stock peanuts to follow food production practices as described herein.

(2) Receiving and inspection. Farmers stock peanuts received at the shelling plant should be inspected on arrival. It is advisable to know the origin and history of each lot of peanuts. The transport vehicle should be examined for cleanliness, insect infestation, dampness or unusual odours. If the vehicle is not an enclosed van-type, it should have available a covering such as a tarpaulin to keep out the rain or moisture.

The general appearance of the peanuts should be observed during the process of unloading. If the peanuts are wet to the touch, insect infested, or contain an unusual amount of dirt, debris or other foreign material, they should not be co-mingled with known good peanuts in a bulk warehouse. The vehicle should be set aside until a decision is made for its disposition. If possible, remove a sample from each lot and shell it for peanut grade observation before an acceptance decision is made. Split all kernels and observe for possible presence of mould. A magnifying lens or microscope should be used to determine whether any mould observed resembles Aspergillus flavus. Excessive mould or presence of mould resembling A. flavus warrants a chemical test for aflatoxin.



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If the peanuts are to be stored in a bulk warehouse or storage bin, the warehouse or bin should be thoroughly cleaned of all static and extraneous material and fumigated before use. Peanuts should not be stored in a warehouse containing any openings which may permit entrance of rodents or birds or which may have leaks in the roof or walls that can allow the rain to enter. The warehouse should be checked frequently for leaks or infestation, both before and after filling. To prevent condensation drippage, warehouses should be ventilated as, for example, by screening around tops or eaves.

(3) Unloading equipment and area. Unloading equipment such as dumping pit, conveyor belt, bucket elevator, and dirt removing equipment should be so designed as to prevent accumulation of debris. A programme of periodic cleaning, together with preventive pest control measures, should be carried out. Peanuts should be handled so as to avoid cracking or tearing of hulls which may permit damage to the kernels.

(4) Precleaning. As much dust and dirt as possible should be removed from the farmers stock peanuts before they enter the shelling plant. Sand screens and aspirators will take out much of the dust and dirt and improve the overall sanitation of the shelling plant.

As much foreign material, loose shell, loose kernels, and pops as possible should be removed. Foreign material not removed by the cleaner can cause mechanical problems by clogging the sheller, as well as by requiring more picking and sorting of the shelled peanuts. Removal of loose kernels and blows before shelling will improve the quality of the peanuts as well as the sheller and plant performance.

(5) Shelling and sizing. All foreign material should be removed from the shelled peanuts (using stoners, magnets, sorters, etc.). The shelled peanuts should be continuously inspected to determine whether the plant equipment is performing properly and the peanuts are free of foreign material, damage, and contamination. Any equipment adjustments indicated by the inspection should be made promptly.

Once the shelled peanuts are size graded, additional stoning should be done in order to remove small light stones, dirt balls and other foreign material which could not be removed in the farm stock stoners. Special care should be taken to avoid overloading size grading equipment.

(6) Sorting. Sorting is the final step for removing debris and defective kernels. It can be done by hand picking or photoelectric sorting machines or a combination of both. Sorting belts should be well lighted, loaded no more than one layer deep, and operated at a speed and with the number of sorters to assure removal of foreign material and defective kernels. Photoelectric sorting machines should be adjusted against standards selected to assure removal of foreign material and defective kernels. Adjustment should be checked on a frequent periodic basis. One contaminated kernel may contain sufficient aflatoxin to endanger as many as 10,000 comingled kernels. Foreign material and defective kernels (mouldy, discoloured, rancid, decayed, shriveled, damaged) should be separately bagged and red tagged as unsuitable for human or animal consumption. Bags of sorted out peanuts should be removed as soon as practicable from the processing room.

(7) Cleaning of special areas

- (a) Boots of elevators accumulate peanuts and peanut material. They should be cleaned out and sprayed regularly to prevent insect and rodent infestation. Fumigation or spray methods and chemicals used should be approved by the official agency having jurisdiction.
- (b) Canvas conveyor belts will accumulate product between belt and conveyor pan. Pulleys can accumulate crushed material. Undersides of moulding on conveyors can accumulate particles of peanuts. These areas should be cleaned and sprayed on regular schedule to prevent insect and rodent infestation.
- (c) Storage and surge hoppers should be cleaned and sprayed between runs.
- (d) Areas which can accumulate peanuts and debris and are difficult to inspect and clean regularly should not be used.
- (e) Every piece of machinery whether open or enclosed should be cleaned of lodged material on a regular schedule.
- (f) The area immediately surrounding the plant should be kept clean of all debris that might attract rodents or birds.

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- (g) Dry clean-up procedures should be utilized to avoid wet spots in which micro-organisms can propagate and contaminate contacted peanut kernels. Even though water may not be used directly on equipment, spray and elevated humidity from continuous use can increase moisture in organic matter trapped in crevices in equipment, such as conveyors, to the point where microorganisms can proliferate.

SECTION IV - PLANT FACILITIES AND OPERATING REQUIREMENTS

A. Plant Construction and Layout

- (1) Location, size, and sanitary design. As in the General Principles of Food Hygiene.  
(2) Sanitary facilities and controls. (a), (b), (d), (e), (f), (g) and (h) as in the General Principles of Food Hygiene.

B. Equipment and Utensils

- (1), (2) and (3) as in the General Principles of Food Hygiene.

C. Hygienic Operating Requirements

- (1), (2), (3), (4), (5) and (6) as in the General Principles of Food Hygiene (with the deletion of the introductory paragraph).

D. Operating Practices and Production Requirements

(1) Raw material handling

(a) Acceptance criteria. Peanuts should not be accepted by the plant if known to contain decomposed, toxic, or extraneous substances which will not be reduced to acceptable levels by normal plant procedures, sorting or preparation. Particular care should be taken to avoid contaminating in-shell peanuts or nut meats with animal or human faecal material; nuts suspected of being contaminated, should be rejected for human consumption. Special precautions must be taken to reject nuts showing signs of mould growth because of the danger of their containing mycotoxins such as aflatoxins. Aflatoxin test results should be known before allowing lots of raw peanuts to be processed. A lot of raw peanuts with an unacceptable level of aflatoxins, which cannot be reduced to permitted levels by the available sorting equipment should not be accepted.

(b) Storage. Raw materials stored on the plant premises should be maintained under conditions that will protect against contamination and infestation and minimize deterioration. Peanuts not scheduled for immediate use should be stored under conditions that prevent mould growth and infestation. See Section D, (7)(b).

The warehouse should be of sound construction, in good repair and built and equipped so that it will provide suitable storage and adequate protection for peanuts. All breaks or openings in the walls, floors, or roof shall have been repaired. Any breaks or openings around doors, windows and eaves shall have been repaired or screened. The use of screens should be restricted to areas of the building not subject to moisture entry. The building should have sufficient ventilation so as to prevent the build-up of condensation.

New concrete floors or walls should not be used for storage until it is absolutely certain that the new concrete is well-cured and free of excess water. For the first year of new concrete it is safest to use an approved plastic cover spread over the entire new floor as a moisture barrier prior to filling with peanuts. Other means of storage such as stacking of containers on plastic pallets to protect the peanuts against moisture from "sweating" of concrete can be used. The plastic can then be discarded when the warehouse is emptied. This system will ensure against sweating of the new concrete and possible moulding of the peanuts.

Products which affect the storage life, quality or flavour of peanuts should not be stored in the same room or compartment with peanuts. For example, such items as fertilizer, gasoline or lubricating oils should not be stored with peanuts, and some fruits or vegetables contribute objectionable odours or flavours.

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(2) Inspection and sorting. Prior to introduction into the processing line, or at a convenient point within it, raw materials should be inspected, sorted or culled as required to remove unfit materials. See Section III, D, (2) and (6).

Experience has shown that aflatoxin is most frequently associated with mouldy, discoloured, shriveled, or otherwise damaged peanuts. Mould contaminated peanuts may exhibit some of the following characteristics:

1. Darker skin colouring before and/or after roasting.
2. Darker flesh (after blanching) before and/or after roasting.
3. Resistance to splitting and/or blanching.

To remove effectively mould contaminated nuts, sorting should be performed before and after blanching and roasting. Where splitting is part of the processing operation, nuts that resist splitting should be removed. The effectiveness of sorting techniques should be checked by regular aflatoxin analyses of the sorted peanut stream or of the finished product, or both. This should be done frequently enough to give assurance that the product is completely acceptable.

Rejected peanuts from the sorting procedure (pickouts) should be destroyed or segregated from edible products. If they are to be used for crushing, they should be separately bagged and red tagged as unsuitable for human or animal consumption.

(3) and (4) as (4) and (5) in the General Principles of Food Hygiene.

(5) Preservation of product. In-shell nuts or nut meats should be dried to a moisture level low enough so that the product can be held under normal storage conditions without development of mould or significant deterioration by oxidative or enzymatic changes. Finished roasted products may be (a) treated with antioxidants at levels approved by the Codex Committee on Food Additives as referenced in the Commodity Standard; and (b) heat processed and/or packed in gas tight containers under nitrogen or vacuum, so that the product will not spoil under normal storage conditions.

(6) Storage and transport of product. Peanuts should be stored and transported under such conditions as will maintain the integrity of the container and the product within it. Carriers should be clean, dry weatherproof, free from infestation and sealed to prevent water, rodents or insects from reaching the peanuts. Peanuts should be loaded and unloaded in a manner that protects from damage or water. Refrigerated vehicles are recommended for transport when climatic conditions indicate such a need. Extreme care should be taken to prevent condensation when unloading peanuts from cold storage or from a refrigerated vehicle. In warm, humid weather, the peanuts should be allowed to reach ambient temperature before exposure to external conditions. This tempering may require 1-3 days. Peanuts that have been spilled are vulnerable to contamination and should not be used for edible products.

(a) All products should be stored in clean, dry buildings, protected from insects, mites and other arthropods, rodents, birds, or other vermin, chemical or micro-biological contaminants, debris and dust.

(b) Optimum storage conditions:

(i) Optimum storage conditions are 0-6°C (32-42°F) with a relative humidity between 55% to 65%. A dry environment should be maintained to protect quality and prevent mould growth. No peanuts should be stored closer than 0.5 metres (1 ½ feet) from any outside wall. An active programme should be maintained to detect and control hazards from damp pallets, damp floors and walls, overhead moisture, condensation, wet unloading and loading out conditions - all conducive to moisture pick-up and mould. Growth of toxigenic molds may be prevented by packing nut products that have been dried to a "safe moisture level" or by storing at a temperature sufficiently low to reduce both water activity and mold viability to a point that mold growth is prevented. Exposed nut products in storage may be maintained at or dried to a "safe moisture level" by control of the relative humidity of the circulating air. Those who use refrigerated storage should be aware that the water activity of nut meats increases with increased temperature; this fact should be taken into account when changing storage temperatures.

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(ii) Where peanuts are stored under conditions in which they may become infested by insects and/or mites, appropriate fumigation methods should be used regularly. Peanuts should be stored in such a manner that they can be fumigated in situ or alternatively they can be removed for fumigation in special facilities (e.g. fumigation chambers, steel barges). In the latter situation, the storage area should be separately sanitized. Cold storage can be used, either to prevent infestation in localities where insects are likely to be present in ordinary storage or to prevent insects already present from damaging the peanuts.

E. Sanitary Control Procedures

As in the General Principles of Food Hygiene.

F. Laboratory Control Procedures

In addition to any control by the official agency having jurisdiction, it is desirable that each plant should have its own or contracted laboratory control of the sanitary quality of the nut products processed. The amount and type of such control will vary with the different nut products as well as the needs of management. Such control should provide for rejection of all nuts that are unfit for human consumption and monitoring of the quality of the finished products. Analytical procedures used should follow recognized or standard methods so that the results may be readily interpreted.

SECTION V - END-PRODUCT SPECIFICATIONS

Standard methods should be used for sampling, analysis and other determinations to meet the following specifications:

- A. To the extent possible in good manufacturing practice, the products should be free from objectionable matter.
- B. When tested by appropriate methods of sampling and examination, the products:
  - (a) should be free from pathogenic microorganisms; and
  - (b) should not contain any substances originating from microorganisms in amounts which may represent a hazard to health in accordance with the standards of the official agency having jurisdiction, particularly mycotoxins, such as aflatoxins, formed by moulds.
- C. The products should comply with the provisions for food additives and contaminants laid down in Codex Commodity Standards and with maximum levels for pesticide residues recommended by the Codex Alimentarius Commission.

AMENDMENTS TO DRAFT CODE OF PRACTICE FOR FROZEN FISH, ALINORM 76/18A, APPENDIX VI  
(Advanced to Step 6 at the 11th Session of the Codex Alimentarius Commission in 1976)

The Committee appointed a working group to review the Proposed Draft Code of Practice for Frozen Fish, ALINORM 76/18A, Appendix VI, in the light of government comments received (CX/FH 76/5) (USA), April 1976.

The group consisted of members of the delegation of the USA and the Netherlands, and a representative of the Department of Fisheries, FAO (Chairman) and met on 10 and 11 May 1976 to review the hygiene provisions of the above document.

The group found that the government comments received were mainly of an editorial nature and included these in its proposal for revision of the text of the code.

The Committee agreed to the proposals of the working group which are listed below:

3.1.1 FISH INTENDED FOR FREEZING SHOULD BE OF THE HIGHEST POSSIBLE QUALITY.

FF  
(3.1.2  
Adapted)

Although there are many aspects that might be taken into account when defining what is meant by the "highest possible quality" fish, there are two major ones that should concern the fisherman as a primary producer:

1. quality of fish when caught, and
2. quality of fish on delivery to the buyer or the processor.

The first one is determined by the physical condition of the fish, and includes appearance, size, percentage of fat, amount of feed, damage to skin, presence of disease and of toxic substances. The second one will result from the methods and techniques employed in fishing, practices in handling and freezing, and conditions of storage in the freezer store.

The fisherman should discard any fish that is diseased or is known to contain toxic substances or has undergone deterioration or any process of decomposition or which has been contaminated with foreign matter to an extent which has made it unfit for human consumption.

Freezing and frozen storage cannot improve the quality of fish. At best, the process maintains the fish in much the same condition as it was immediately before freezing. It is therefore essential that the raw material be as fresh as possible.

4.1.1.1 THE FISHING VESSEL SHOULD BE DESIGNED FOR RAPID AND EFFICIENT HANDLING AND FREEZING OF FISH, EASE OF CLEANING AND DISINFECTION, AND SHOULD BE OF SUCH MATERIAL AND CONSTRUCTION AS NOT TO CAUSE ANY DAMAGE OR CONTAMINATION OF THE CATCH.

FF  
(4.1.1  
Adapted)

In designing a fishing vessel many factors, apart from the vessel's performance as a harvesting unit, should be considered. The fisherman's earnings are determined not only by the quantity of the fish caught but, to a great extent, by the quality of the catch delivered to the processing plant.

Fishing vessels should be designed and constructed so as not to cause contamination of the fish with bilge, water, sewage, smoke, fuel, oil, grease or other objectionable substances. Fish, if not frozen soon after capture, should be protected against physical damage, exposure to high temperatures and drying effects of sun and wind.

All surfaces with which the fish might come in contact should be of suitable corrosion-resistant material which is smooth and easily cleanable.

A vessel that is to be designed for freezing fish at sea should be large enough to allow for installation of proper processing and freezing equipment and for an adequate freezer store.

Such a vessel, to justify its cost, should be able to fish in more distant areas and remain on the fishing grounds till fully loaded. Fish which is frozen and stored on the vessel should be of the same quality as if it were processed and stored in a shore establishment.

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4.1.3 Sanitary Facilities

- 4.1.3.1 AREAS OF THE DECK WHERE FISH ARE UNLOADED AND HANDLED, OR THE FISH HOLD WHERE FISH ARE STOWED, SHOULD BE USED EXCLUSIVELY FOR THESE PURPOSES. FF (4.3.1)

All such areas should be well defined and should be kept clean or be readily capable of being maintained in a clean condition.

Storage of fuel and other petroleum products, or of different cleaning and sanitizing agents, should be so arranged that there is no possibility of contamination of surfaces with which fish come in contact.

Any exposure, even for a short time, of fish to petroleum products, very often results in rejection and eventual destruction of the whole load. The odour and the taste of fish contaminated with fuel or other similar compounds are very persistent and difficult to remove during the subsequent processing and should therefore be discarded.

- 4.1.3.7 ON LARGE FISHING VESSELS, ENGAGED IN FISHING AS WELL AS FISH PROCESSING AND FREEZING, SUITABLE WASHING FACILITIES SHOULD BE PROVIDED. FF (4.3.9)

Such facilities should be located in toilets and close to the fish handling or processing areas. They should be supplied with clean water, soap and towels (preferably disposable).

- 4.1.3.8 THE FISHING VESSELS SHOULD BE EQUIPPED WITH BRUSHES, SCRAPERS, WATER HOSES, SPRAY NOZZLES AND OTHER SUITABLE WASHING AND SANITIZING EQUIPMENT. FF (4.3.10)

Although there is a variety of cleaning and sanitizing equipment available on the market, good quality hand brushes of several sizes and shapes are still the most inexpensive and versatile tools for cleaning operations. Brushes should be kept in a clean and sound condition and, when not used, should be stored in a dry state. Brushes could spread dirt and micro-organisms. Micro-organisms will proliferate in a dirty brush when stored in a wet condition. The use of steel-wool for scouring should be avoided as there is a constant danger of introducing small, sometimes hardly visible, bits of wire into the final product. If for some reason cleaning cannot be done effectively with a good brush, then plastic, brightly coloured scouring pads might be used.

The high pressure and high frequency oscillating water or detergent spraying equipment has been found to be quite effective in cleaning, but it usually requires an experienced operator to prevent damage to painted surfaces.

/\*,disinfected after each use (rinsing in 50 ppm chlorine solution is recommended) and,..

4.2 Equipment and Utensils

- 4.2.1 ALL FISH STORAGE, HANDLING, CONVEYING, PROCESSING AND FREEZING EQUIPMENT USED ON BOARD FISHING VESSELS SHOULD BE DESIGNED FOR THE RAPID AND EFFICIENT HANDLING OF FISH, BE SUITABLE FOR EASY AND THOROUGH CLEANING AND SHOULD BE CONSTRUCTED SO AS NOT TO CAUSE CONTAMINATION OF THE CATCH. FF (4.4.1 Adapted)

Some of the equipment currently used in the fishing industries is quite unsuitable for the purpose in which it is employed. More thought should be given to the design and layout of fixtures and plant when obtaining equipment, only equipment which can be readily disassembled for thorough cleaning should be considered.

- 4.3.5 WHERE GUTTING BENCHES ARE INSTALLED THESE SHOULD BE PROVIDED WITH CHANNELS OR CHUTES WHICH HAVE A CONTINUOUS SUPPLY OF CLEAN SEA WATER TO CARRY THE GUTS OVER THE SHIPSIDE OR TO A SUITABLE COLLECTING CONTAINER.

Where fish are contaminated by offal and filth from the gutting operations, the spoilage rate will be increased and all surfaces with which the guts come in contact will also become contaminated. The installation of gutting benches makes the task easier, but care should be taken to ensure that the benches are kept in a hygienic condition.

4.4.1 Handling the Catch before Freezing

4.4.1.1 HANDLING THE CATCH SHOULD BEGIN AS SOON AS IT COMES ON BOARD. ANY FISH UNSUITABLE FOR HUMAN CONSUMPTION SHOULD BE REMOVED FROM THE CATCH AND KEPT SEPARATE

FF  
(4.6.2)

Sorting the catch should be done as soon as the fish are taken on board, to remove as quickly as possible fish unsuitable for human consumption. Mixed species catches should also be sorted rapidly not only for the reason stated above but also to avoid possible damage due to abrasion, particularly where the catch contains spiny and rough skin species and to prevent transferring undesirable odours and tastes which may affect the organoleptic quality of the differing species.

5.1.2.1 FISH PROCESSING AND FREEZING PLANT SHOULD BE SPECIALLY DESIGNED FOR THE PURPOSE

FF  
5.1.1

Raw fish spoils considerably faster than raw meat of warm blooded animals.

The keeping time of the fish delivered to the processing plant has been already reduced by time and conditions of handling and storage on the fishing vessel. There is little that could be done by the processing and freezing to improve the quality of fish delivered. Even with the best of treatment fresh fish, depending on geographical area, species and physical condition of the animal when caught, will, in most cases after ten to twelve days in ice, be considered as unfit for human consumption.

Because of this highly perishable nature of fish, the processing plant demands special facilities and materials which, as compared to other food processing establishments, are in some cases rather unique.

The technological and hygienic operating and production requirements also differ in being often more demanding and critical.

The processing and freezing plant therefore should meet the same requirements for construction and sanitary facilities as the fresh fish processing establishment detailed in the "Code of Practice for Fresh Fish" and repeated in this Code under sub-sections 5.1.2 and 5.1.3 respectively.

5.1.3.4 AN AMPLE SUPPLY OF COLD AND HOT WATER OF POTABLE QUALITY UNDER ADEQUATE PRESSURE SHOULD BE AVAILABLE AT NUMEROUS POINTS THROUGHOUT THE PREMISES AT ALL TIMES DURING THE WORKING HOURS.

FF  
(5.1.3.4)

All water available for use in those parts of establishments where fish is received, held and processed should be of potable quality. If sea water is used, it must be clean sea water.

An adequate supply of hot water of potable quality at a minimum temperature of 82°C (180°F) should be available at all times during the plant operation.

The cold water supply used for cleaning purposes should be fitted with an inline chlorination system allowing the residual chlorine content of the water to be varied at will in order to reduce multiplication of micro-organisms and prevent the build-up of fish odours.

Water used for washing or conveying raw materials should not be re-circulated unless it is restored to a level of potable quality.

5.3.8 EFFECTIVE MEASURES SHOULD BE TAKEN TO PROTECT AGAINST THE ENTRANCE INTO THE PREMISES AND THE HARBOURAGE ON THE PREMISES OF INSECTS, RODENTS, BIRDS OR OTHER VERMIN.

FF  
(5.3.11)

An effective and continuous programme for the control of insects, rodents, birds or other vermin within the establishment should be maintained. The plant and surrounding area should be regularly examined for evidence of infestation. Where control measures are necessary, treatment with chemical, biological or physical agents should meet the requirements of the official agency having jurisdiction and should be under the direct supervision of personnel with a thorough understanding of the hazards involved, including the possibility of toxic residues being retained by the fish, or their products.

The use of insecticides, during the plant operation, without any provision for collection of dead insects, should be discouraged. Instead, the use of adhesive insect

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traps or very efficient "black light insecticutor" lamps with the attached collecting trays, is recommended. Insect traps should not be located directly over the processing areas and should be away from windows and doors.

All rodenticides, fumigants, insecticides or other toxic substances should be of an approved type and should be stored in separate locked rooms or cabinets and handled only by properly trained personnel.

5.4.1.5 FISH WHICH CANNOT BE PROCESSED IMMEDIATELY ON ARRIVAL AT THE PLANT SHOULD BE WELL ICED IN CLEAN CONTAINERS AND STORED IN SPECIALLY DESIGNATED AREAS WITHIN THE PLANT WHERE THEY WILL BE PROTECTED FROM HEAT AND WEATHER CONDITIONS AND WILL NOT BE CONTAMINATED BY DUST, INSECTS OR VERMIN. WHERE POSSIBLE, THE ICED FISH SHOULD BE STORED IN A CHILL ROOM, THE TEMPERATURE OF WHICH IS JUST FF ABOVE THAT OF MELTING ICE, 0°C (32°F). (5.4.3.1)

In order to produce good quality frozen products, the quality of the raw fish must be maintained by protecting it from heat, contamination from other sources and physical damage.

It must be stressed again that placing quantities of fish in a chill room does not remove the need for adequate icing. Chill rooms are designed to maintain a chill temperature and to keep already cool fish from warming up. The refrigeration machinery used in chill room operations is not adequate to lower the temperature of a mass of fish in a short time. The initial cooling must be done by the addition of ice.

It is poor practice, therefore, to load the chill room with large quantities of fresh fish that were not prechilled effectively to the temperature of melting ice.

The chill room should be equipped with a recording thermometer and an automatic temperature control and should be so designed that it can be kept in a clean sanitary condition at all times. The chill room should also be equipped with an automatic alarm system to alert the proper personnel when the temperature drops below 0°C (32°F).

6. SECTION V - END PRODUCT SPECIFICATIONS

6.1 Appropriate methods should be used for sampling and examination to determine the compliance with the following specifications:

- A. Fishery products should be, to the extent possible in good manufacturing practice, free from objectionable matter and parasites.
- B. Fishery products should be free from micro-organisms in amounts harmful to man, free from parasites harmful to man and should not contain any substances originating from microorganisms in amounts which may represent a hazard to health. FF (6.1)
- C. Fishery products should be free from chemical pollutants in amounts which may represent a hazard to health.
- D. Fishery products should comply with any requirements set forth by the Codex Alimentarius Commission on pesticide residues and food additives as contained in permitted lists of Codex commodity standards, or should comply with the requirements on pesticide residues and food additives of the country in which the fish will be sold.
- E. Specifications A, B, C and D should, to the extent possible, also apply to frozen fish.

In addition to the requirements 4.1.1.1, 4.4.1.1 and 5.1.2.1 (as shown), also 5.1.2.9 and 5.1.2.10 will be amended to bring the document into compliance with the Code of Practice for Fresh Fish (ALINORM 76/13A and Corrigendum), and sections 5.1.2.11 and 5.3.3 will also be altered to the form as approved by the 10th Session of the Codex Committee on Fish and Fishery Products.



DRAFT CODE OF HYGIENIC PRACTICE FOR LOW-ACID CANNED FOODS

SECTION I - SCOPE

This Code of Practice applies to the canning and safe heat processing of low-acid canned foods packed in rigid hermetically sealed containers, and which depend for the preservation of the product on the heat applied by the process.\*

SECTION II - DEFINITIONS

1. "Aseptic processing" means the filling of a commercially sterile product into pre-sterilised containers followed by hermetically sealing with a presterilised closure in an atmosphere free of microorganisms.
2. "Bleeders" means small orifices through which steam escapes throughout the entire heat process.
3. "Broken heating curve" means heat penetration data plotted against time on semi-log graph paper which shows that the product changes its rate of heating during sterilization.
4. "Canned" means product packed in rigid containers which have been hermetically sealed and sufficiently heated to destroy or inactivate all microorganisms that are able to grow in the product at temperatures at which the canned product is normally likely to be held during manufacture, distribution and storage.
5. "Cleaning" means the removal of residues from equipment and of objectionable matter from production surfaces, raw material or product.
6. "Coming-up-time" means the time which elapses between the introduction of heating medium into the closed retort and the time when the temperature in the retort including venting time reaches the required processing temperature.
7. "Commercial sterility of food" means the condition achieved by application of heat which renders such food free from viable microorganisms capable of reproducing in the food under expected conditions of storage and distribution and which will include microorganisms of known public health significance.
8. "Commercial sterility of equipment and containers used for aseptic processing and packaging of food" means the condition achieved by application of heat, chemical sterilant(s), or other appropriate treatment which renders such equipment and containers free from viable microorganisms capable of reproducing in the food under expected conditions of storage and distribution and which will include microorganisms of known public health significance.
9. "Cooling time" means the time necessary to cool the contents of a container from the sterilisation temperature to approximately 40°C (104°F).
10. "Disinfection" means the application of effective chemical or physical agents or processes to clean surfaces with the intention of eliminating microorganisms and preventing infection of food products.
11. "Flame sterilizer" means an apparatus in which hermetically sealed containers are agitated at atmospheric pressure, by either continuous, discontinuous, or reciprocating movement, over gas flame to achieve commercial sterility of food. A holding period in a heated section may follow the initial heating period.
12. "Headspace" means the volume in a closed container not occupied by the product.

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\* This Code of Practice does not apply to low-acid foods packed in flexible or semi-rigid containers, and which also depend for the preservation of the product on the heat applied by the process, nor does it apply to those foods which have been pre-cooked or pasteurized and therefore should be stored under refrigeration.

APPENDIX V

13. "Heat process" means the treatment of product with sufficient heat to achieve commercial sterility. The heat process is defined in terms of time of treatment of product at a specified temperature.
14. "Hermetically sealed container" means a container which is designed and intended to be secure against the entry of microorganisms during and after processing.
15. "Holding time", see sterilisation time.
16. "Incubation tests" means tests in which the heat processed product is kept at a specific temperature for a specified period of time in order to determine if outgrowth of microorganisms occurs under these conditions.
17. "Initial temperature" means the temperature of the contents of the coldest container to be processed at the time the sterilising cycle begins, as determined after thorough stirring or shaking of the contents.
18. "Lot" means the product produced under one code mark.
19. "Low acid foods" means any foods, other than alcoholic beverages, with an equilibrium pH value greater than 4.6.
20. "Potable water" means fresh water fit for human consumption. Standards of potability should not be lower than those contained in the latest edition of the "International Standards for Drinking Water", World Health Organization.
21. "Retort" means a pressure vessel designed for heat processing food packed in hermetically sealed containers by appropriate heating medium and where necessary with superimposed air pressure.
22. "Scheduled process" means the process selected by the processor as adequate under the conditions of manufacture for a given product and container size to achieve commercial sterility.
23. "Simple heating product" means a product that heats in a continuous patten and can be represented by a straight line when the heating data is plotted against time on semi-log graph paper.
24. "Sterilisation temperature" means the operating temperature maintained in the retort as given in the scheduled process.
25. "Sterilisation time" is the time between the moment that the required sterilisation temperature is achieved and the moment that the cooling is started.
26. "Venting" means the process of flushing the air out of steam retorts at the beginning of a heat process by the means of openings controlled by adequate valves.

SECTION III - RAW MATERIAL REQUIREMENTS

- A. Environmental Sanitation in Growing and Raw Food Material Production Areas
  - (1) Sanitary disposal of human and animal wastes as in the General Principles of Food Hygiene.
  - (2) Sanitary quality of irrigation water as in the General Principles of Food Hygiene.
  - (3) Animal, plant pest and disease control as in the General Principles of Food Hygiene.
- B. Sanitary Harvesting and Production of Raw Food Materials
  - (1) Equipment and product containers as in the General Principles of Food Hygiene.
  - (2) Sanitary techniques as in the General Principles of Food Hygiene.
  - (3) Removal of obviously unfit materials as in the General Principles of Food Hygiene.
  - (4) Protection of product from contamination as in the General Principles of Food Hygiene.
- C. Transportation
  - (1) Facilities as in the General Principles of Food Hygiene.
  - (2) Handling procedures as in the General Principles of Food Hygiene.

Note: The remaining sections of this Code are to be revised and will be issued in due course (CX/FH 78/4).

DRAFT PROPOSAL FOR MICROBIOLOGICAL SPECIFICATIONS FOR  
DA SCHNITTZTEN EGG PRODUCTS

This draft proposal for microbiological specifications for egg products contains:-

- (1) Number of field samples from a lot 1/
- (2) Sampling methods
- (3) Reference methods for the detection of salmonellae, and for the enumeration of mesophilic aerobic bacteria and coliform bacteria
- (4) Microbiological sampling plans and limits

1. Number of field samples from a lot

1.1 Dried whole eggs

Take 10 field samples, all of which are used for the detection of salmonellae, and select at random 5 of these field samples to be examined also for mesophilic aerobic bacteria and coliform bacteria.

1.2 Frozen whole eggs

Take 10 field samples, all of which are used for the detection of salmonellae, and select at random 5 of these field samples to be examined also for mesophilic aerobic bacteria and coliform bacteria.

1.3 Other egg products

Take 10 field samples, all of which are used for the detection of salmonellae.

2. Sampling methods

For all egg products take field samples of at least 200 grammes. 2/

2.1 Dried whole eggs 3/

Equipment. Sterile grain trier long enough to reach to bottom of containers to be sampled. Sterile sample containers with tight closures, sterile spoon, alcohol lamp or other burner, alcohol, cotton, clean cloth or towel and water pail.

Methods. For small packages, randomly take one unopened package for each of the required number of field samples required. For larger containers, such as boxes, bags, etc., remove top layer with sterile spoon or other sterile instrument, and with a sterile trier, remove at least 3 cores from the centre, midway between the centre and periphery and from the periphery respectively. Aseptically transfer the cores to a sterile sample container. Samples should be stored in a refrigerated or a cool place until analysis takes place.

- 1/ A lot is a quantity of food produced under identical conditions, all packages of which should bear a lot number that identifies the production during a particular time interval, and usually from a particular "line" or other critical processing unit.
- 2/ For further information see International Commission on Microbiological Specifications for Foods (1974) Microorganisms in Foods II. Sampling for Microbiological analysis: principles and specific applications. Toronto, University of Toronto Press.
- 3/ For further information see the latest edition of "Official Methods of the Association of Official Analytical Chemists" section 41.003 and 41.004.

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2.2 Frozen whole eggs <sup>1/</sup>

Equipment. Electric or hand drill with a sterile 40 x 2.5 cm auger, hammer and steel strip 30 x 5 x 0.5cm or other suitable tool for opening cans, sterile spoon, precooled sterile containers (screw-cap jars or friction-top cans), alcohol lamp or other burner, alcohol, cotton, clean cloth or towel, and water pail.

It is advisable when using an electrically powered drill when sampling to fit a baffle on the drill to prevent aerial contamination of the product.

Methods. Drill 3 cores from top to bottom of container: first core in centre, second core midway between centre and periphery, and third core near edge of container. Transfer drillings from container with a sterile spoon to a prechilled sample container. Keep field samples refrigerated with solid CO<sub>2</sub> or other suitable refrigerant, if analysis is to be delayed or sampling point is at some distance from laboratory.

2.3 Other egg products

Proceed as for dried or frozen egg products, whichever is appropriate.

3. Reference Methods

3.1 EGG PRODUCTS - DETECTION OF SALMONELLAE (REFERENCE METHOD)

1. SCOPE

A Reference Method for the detection of salmonellae (including Arizona but excluding Salmonella typhi) in egg products.

2. FIELD OF APPLICATION

The method can be applied to egg products covered by the Code of Hygienic Practice for Egg Products.

3. REFERENCE

Modification of ISO/DIS 3565.

4. DEFINITIONS

4.1 salmonellae: Micro-organisms which form typical colonies on solid selective media and which possess the biochemical and serological characteristics described when the test is carried out according to this method.

4.2 detection of salmonellae: Determination of the presence or absence of these micro-organisms, in a particular mass, when the test is carried out according to the method described.

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<sup>1/</sup> For further information see the latest edition of "Official Methods of the Association of Official Analytical Chemists" section 41.003 and 41.004.

5. PRINCIPLE

The detection of salmonellae necessitates four successive stages, because they are usually present in low numbers and often in the presence of considerably larger numbers of other members of Enterobacteriaceae.

5.1 Pre-enrichment: incubating the samples in a non-selective liquid medium at 37°C.

5.2 Enrichment: incubated pre-enrichment media of samples from a single lot are incubated in groups of ten into single flasks of each of two liquid selective media.

5.3 Plating out: inoculation of the two enrichment media onto solid, selective diagnostic media which, after incubation at 37°C, are examined for the presence of colonies which by their characteristics are considered presumptive salmonellae.

5.4 Confirmation: subculturing of colonies of presumptive salmonellae and determining their appropriate biochemical and serological characteristics.

6. CULTURE MEDIA, DILUENTS AND REAGENTS

6.1 Basic materials

For uniformity of results, it is recommended that either dehydrated culture medium components of uniform quality and analytical grade chemicals, or a dehydrated complete medium, be used. The water used shall be distilled water or water of at least equivalent purity.

The manufacturers' instructions should be rigorously followed when dehydrated complete media are used.

NOTE - With regard to brilliant-green, note the specification given in the annex.

6.2 Culture media

6.2.1 BUFFERED PEPTONE WATER

Composition

peptone	10.0 g
sodium chloride	5.0 g
disodium hydrogen phosphate ( $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ )	9.0 g
potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ )	1.5 g
water	1 000 ml

Preparation

Dissolve the components in the water by boiling.

Adjust the pH so that after sterilization it is  $7.0 \pm 0.1$  at 20°C.

Transfer the medium in quantities of 225 ml into bottles of 500 ml capacity.

Sterilize the medium for 20 min at  $121 \pm 1^\circ\text{C}$ .

## 6.2.2 TETRATHIONATE MEDIUM (MULLER KAUFFMANN)

## 6.2.2.1 Base

Composition

meat extract	5.0 g
peptone	10.0 g
sodium chloride	3.0 g
calcium carbonate	45 g
water	1 000 ml

Preparation

Add the dehydrated base components or the dehydrated complete base to the water and boil until complete dissolution of the soluble components.

Adjust the pH so that after sterilization it is  $7.0 \pm 0.1$ , at  $20^{\circ}\text{C}$ .

Sterilize the base for 20 min at  $121 \pm 1^{\circ}\text{C}$ .

## 6.2.2.2 Sodium Thiosulphate Solution

Composition

sodium thiosulphate ( $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ )	50.0 g
water to a final volume of	100 ml

Preparation

Dissolve the sodium thiosulphate in a part of the water.

Dilute to the final volume.

Sterilize the solution for 20 min at  $121 \pm 1^{\circ}\text{C}$ .

## 6.2.2.3 Iodine Solution

Composition

iodine	20.0 g
potassium iodide	25.0 g
water to a final volume of	100 ml

Preparation

Dissolve the potassium iodide in a minimal volume of water and add the iodine.

Shake till complete solution.

Dilute to the final volume.

Store the solution in a tightly closed opaque container.

## 6.2.2.4 Brilliant-Green Solution

Composition

brilliant-green	0.5 g
water	100 ml

Preparation

Add the brilliant-green to the water.

Store the solution at least for one day in the dark to allow auto-sterilization to occur.

6.2.2.5 Ox Bile Solution

Composition

ox bile, desiccated	10.0 g
water	100 ml

Preparation

Dissolve the desiccated ox bile in the water by boiling.

Sterilize the solution for 20 min at  $121 \pm 1^\circ\text{C}$ .

6.2.2.6 Complete Medium

Composition

base (6.2.2.1)	900 ml
sodium thiosulphate solution (6.2.2.2)	100 ml
iodine solution (6.2.2.3)	20 ml
brilliant-green solution (6.2.2.4)	2 ml
ox bile solution (6.2.2.5)	50 ml

Preparation

Add to the base, under aseptic conditions, the other ingredients in the above-mentioned order.

Mix the liquids well after each addition.

Transfer the complete medium in quantities of 1000ml aseptically into sterile flasks.

Store it at  $4^\circ\text{C}$  in the dark until needed but use it within one week after preparation.

6.2.3 SELENITE CYSTINE BROTH

6.2.3.1 Base

Composition

tryptone	5.0 g
lactose	4.0 g
disodium hydrogen phosphate ( $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ )	10.0 g
sodium acid selenite	4.0 g
water	1 000 ml

Preparation

Dissolve the ingredients with the exception of sodium acid selenite in the water by boiling for 5 min. After cooling add the sodium acid selenite. Adjust the pH to  $7.0 \pm 0.1$  at  $20^\circ\text{C}$  store at  $4^\circ\text{C}$ .

6.2.3.2 L-Cystine Solution

Composition

L-cystine	0.1 g
N sodium hydroxide (NaOH)	15 ml

Preparation

Dilute to 100 ml with distilled water, do not autoclave.

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6.2.3.3 Complete Medium

Cool base and add l-cystine solution at the rate of 0.1 ml per 10 ml of base.

Adjust pH to 7.0±0.1 at 20°C.

Transfer the complete medium in quantities of 1000ml to sterile flasks.

Use the medium on the day of preparation.

6.2.4 BRILLIANT-GREEN/PHENOL RED AGAR (EDEL AND KAMPELMACHER)

6.2.4.1 Base

Composition

meat extract	4.0 g
peptone	10.0 g
sodium chloride	3.0 g
disodium hydrogen phosphate ( $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ )	0.8 g
sodium dihydrogen phosphate ( $\text{NaH}_2\text{PO}_4$ )	0.6 g
agar, readily soluble <sup>1</sup>	12.0 g
water	900 ml

Preparation

Dissolve the dehydrated base components or the dehydrated complete base in the water by boiling.

Adjust the pH so that after sterilization it is 7.0±0.1 at 20°C.

Transfer the base to tubes or bottles of not more than 500 ml capacity.

Sterilize the base for 15 min at 121±1°C.

6.2.4.2 Sugar/Phenol Red Solution

Composition

lactose	10.0 g
sucrose	10.0 g
phenol red	0.09 g
water to a final volume of	100 ml

Preparation

Dissolve the ingredients in the water.

Heat in a water bath for 20 min at 70°C.

Cool to 55°C and use immediately.

<sup>1</sup>

The material known by the brand name of Oxoid No. 1 Agar is suitable



### 6.2.4.3 Brilliant-Green Solution

For composition and preparation of this solution, see 6.2.2.4.

### 6.2.4.4 Complete Medium

#### Composition

base (6.2.4.1)	900 ml
sugar/phenol red solution (6.2.4.2)	100 ml
brilliant-green solution (6.2.4.3)	1 ml

#### Preparation

Under aseptic conditions, add the brilliant-green solution to the sugar/phenol red solution cooled to approximately 55°C.

Add to the base at 50 to 55°C and mix.

### 6.2.4.5 Preparation of Agar Plates

Add to sterile large-size Petri dishes (7.2.5.1) about 40 ml of the freshly prepared complete medium (6.2.4.4) having a temperature of approximately 45°C, and allow to solidify. (When large Petri dishes are not available, transfer about 15 ml of the melted medium (6.2.4.4) to sterile small Petri dishes (7.2.5.2) and allow to solidify.)

Immediately before use, dry the plates carefully, preferably with the lids off and the agar surface downwards, in an oven or incubator at 50±5°C for 30 min.

If prepared in advance, the undried plates shall not be kept longer than 4 h at room temperature or one day in a refrigerator.

### 6.2.5 BISMUTH SULFITE AGAR (WILSON AND BLAIR, MODIFIED)

#### Composition

beef extract	5.0 g
peptone or polypeptone	10.0 g
glucose	5.0 g
disodium hydrogen phosphate ( $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ )	4.0 g
ferrous sulfate ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ )	0.3 g
bismuth sulfite	8.0 g
brilliant-green	0.025 g
agar	20.0 g
water	1 000 ml

#### Preparation

Dissolve the dehydrated base components or the dehydrated complete base in water by boiling with frequent agitation to dissolve soluble materials. Cool to 40-45°C, do not autoclave. Final pH should be approximately 7.7.

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6.2.5.1 Preparation of Agar Plates

Add to sterile large-size Petri dishes (7.2.5.1) about 40 ml of the freshly prepared complete medium (6.2.5) and allow to solidify. (When large Petri dishes are not available, transfer about 15 ml of the melted medium (6.2.5) to small sterile Petri dishes (7.2.5.2) and allow to solidify.) Store in a refrigerator and do not use before 24 h storage or after 5 days storage.

6.2.6 NUTRIENT AGAR

Composition

meat extract	3.0 g
peptone	5.0 g
agar	12.0 g
water	1 000 ml

Preparation

Dissolve the dehydrated medium components or the dehydrated complete medium in the water by boiling.

Adjust the pH so that after boiling it is  $7.0 \pm 0.1$  at  $20^{\circ}\text{C}$ .

Transfer the culture medium to tubes or bottles of not more than 500 ml capacity.

Sterilize the medium for 20 min at  $121 \pm 1^{\circ}\text{C}$ .

6.2.6.1 Preparation of Agar Plates

Transfer about 15 ml of the melted medium (6.2.6) to sterile small Petri dishes (7.2.5.2), and proceed as in 6.2.4.5.

6.2.7 TRIPLE SUGAR/IRON AGAR (TSI AGAR)

Composition

meat extract	3.0 g
yeast extract	3.0 g
peptone	20.0 g
sodium chloride	5.0 g
lactose	10.0 g
sucrose	10.0 g
glucose	1.0 g
iron (III) citrate	0.3 g
sodium thiosulphate	0.3 g
phenol red	0.024 g
agar	12.0 g
water	1 000 ml

Preparation

Dissolve the dehydrated medium components or the dehydrated complete medium in the water by boiling.

Adjust the pH so that after sterilization it is  $7.4 \pm 0.1$  at  $20^\circ\text{C}$ .

Transfer the medium in quantities of 10 ml to tubes of diameter 17 to 18 mm.

Sterilize the medium for 10 min at  $121 \pm 1^\circ\text{C}$ .

Allow to set in a sloping position to give a butt of depth 2.5 cm.

6.2.8 UREA AGAR (CHRISTENSEN)

6.2.8.1 Base

Composition

peptone	1.0 g
glucose	1.0 g
sodium chloride	5.0 g
potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ )	2.0 g
phenol red	0.012 g
agar	15.0 g
water	1 000 ml

Preparation

Dissolve the dehydrated base components or the dehydrated complete base in the water by boiling.

Sterilize the base for 20 min at  $121 \pm 1^\circ\text{C}$ .

6.2.8.2 Urea Solution

Composition

urea	400 g
water to a final volume of	1 000 ml

Preparation

Dissolve the urea in the water.

Sterilize by filtration and check sterility.

(For details of the technique of sterilization by filtration, reference should be made to any appropriate textbook on microbiology.)

6.2.8.3 Complete Medium

Composition

base (6.2.8.1)	950 ml
urea solution (6.2.8.2)	50 ml

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Preparation

Under aseptic conditions, add the urea solution to the base.

Adjust the pH so that it is  $6.8 \pm 0.1$  at  $20^{\circ}\text{C}$ .

Transfer the complete medium in quantities of 10 ml to sterile tubes.

Allow to set in a sloping position.

6.2.9 SEMI-SOLID NUTRIENT AGAR

Composition

meat extract	3.0 g
peptone	5.0 g
agar	4.0-8.0 g (depending on the "gel strength")
water	1 000 ml

Preparation

Dissolve the dehydrated base components in the water by boiling.

Adjust the pH so that after sterilization it is  $7.0 \pm 0.1$  at  $20^{\circ}\text{C}$ .

Transfer the medium to bottles of not more than 500 ml capacity.

Sterilize the medium for 20 min at  $121 \pm 1^{\circ}\text{C}$ .

Preparation of agar plates

Add to sterile small Petri dishes (7.2.5.2) about 15 ml of the freshly prepared complete medium (6.2.9). The plates shall not be dried.

6.2.10 SALINE SOLUTION

Composition

sodium chloride	8.5 g
water	1 000 ml

Preparation

Dissolve the sodium chloride in the water by boiling.

Adjust the pH so that after sterilization it is  $7.0 \pm 0.1$  at  $20^{\circ}\text{C}$ .

Transfer such quantities of the solution to bottles or tubes that they will contain 90 to 100 ml after sterilization.

Sterilize the solution for 20 min at  $121 \pm 1^{\circ}\text{C}$ .

6.2.11 LYSINE DECARBOXYLATION MEDIUM

Composition

l-lysine monohydrochloride	5.0 g
yeast extract	3.0 g
glucose	1.0 g
bromocresol purple	0.015 g
water	1 000 ml

Preparation

Dissolve the components in the water by boiling.

Adjust the pH so that after sterilization it is  $6.8 \pm 0.1$  at  $20^{\circ}\text{C}$ .

Transfer the medium in quantities of 5 ml to narrow culture tubes approximately 8 mm in diameter and 160 mm in length for anaerobic conditions.

Sterilize the medium for 10 min at  $121 \pm 1^{\circ}\text{C}$ .

6.2.12  $\beta$ -GALACTOSIDASE REAGENT (ONPG test)

6.2.12.1 Buffer Solution

Composition

sodium dihydrogen phosphate ( $\text{NaH}_2\text{PO}_4$ )	6.9 g
sodium hydroxide, approximately $0.1^{\text{N}}$ (4 g/l) solution	3 ml
water to a final volume of	50 ml

Preparation

Dissolve the sodium dihydrogen orthophosphate in approximately 45 ml of water.

Adjust the pH to  $7.0 \pm 0.1$  with approximately 3 ml of the sodium hydroxide solution.

Add water to a final volume of 50 ml.

Store under refrigeration.

6.2.12.2 ONPG Solution

Composition

o-nitrophenyl $\beta$ -D-galactopyranoside (ONPG)	80 mg
water	15 ml

Preparation

Dissolve the ONPG in the water at  $50^{\circ}\text{C}$ .

Cool the solution.

6.2.12.3 Complete Reagent

Composition

buffer solution (6.2.12.1)	5 ml
ONPG solution (6.2.12.2)	15 ml

Preparation

Add the buffer solution to the ONPG solution.

Store the complete reagent at  $4^{\circ}\text{C}$  but not for longer than one month.

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6.2.13 VOGES-PROSKAUER REACTION (RAPID METHOD BY BARRY AND FEENEY)

6.2.13.1 VP Medium

Composition

peptone	7.0 g
glucose	5.0 g
dipotassium hydrogen phosphate ( $K_2HPO_4$ )	5.0 g
water	1 000 ml

Preparation

Dissolve the components in the water.

Adjust the pH to 6.9 and filter.

Sterilize the medium for 20 min at 115°C.

6.2.13.2 Creatine Solution

Composition

creatine monohydrate	0.5 g
water	100 ml

Preparation

Dissolve the creatine monohydrate in the water.

6.2.13.3  $\alpha$ -Naphthol Reagent

Composition

$\alpha$ -naphthol	6 g
ethanol, 96% (V/V)	100 ml

Preparation

Dissolve the  $\alpha$ -naphthol in the ethanol.

6.2.13.4 KOH Reagent

Composition

potassium hydroxide	40 g
water	100 ml

Preparation

Dissolve the potassium hydroxide in the water.

6.2.14 INDOL REACTION

6.2.14.1 Tryptone Medium

Composition

tryptone	10 g
sodium chloride	5 g
water	1 000 ml

Preparation

Dissolve the components in the water.  
Sterilize for 20 min. at  $121 \pm 1^{\circ}\text{C}$ .

6.2.14.2 Reagent (Kovacs)

Composition

p-dimethylaminobenzaldehyde	5 g
hydrochloric acid, p 1.19 g/ml	25 ml
tert amyl alcohol	75 ml

Preparation

Mix the components.

6.3 Sera

Several anti-Salmonella sera may be obtained commercially, i.e. anti-sera containing one or more "O" groups (so called mono- or polyvalent anti O-sera), and anti-sera containing one or more "H" groups (so called mono- or polyvalent anti H-sera). The precise description may vary and careful reading of the labels is advised. The sera should be certified for potency and specificity by an appropriate controlling authority.

7. APPARATUS AND GLASSWARE

7.1 Apparatus

7.1.1 Mechanical blender, operating at not less than 8 000 rev/min and not more than 45 000 rev/min, with glass or metal blending jars of an appropriate capacity, fitted with lids and resistant to the conditions of sterilization.

7.1.2 Apparatus for sterilization of glassware, blender jars, culture media, etc. and equipment for filter sterilization, for example asbestos pad, membrane filter, or filter candle of a suitable porosity.

7.1.3 Drying cabinet, oven or incubator for drying the surface of agar plates preferably at  $50 \pm 5^{\circ}\text{C}$ .

7.1.4 Incubator for maintaining the inoculated liquid media, plates and tubes at  $37^{\circ}\text{C}$ .

7.1.5 Incubator or water bath for maintaining inoculated liquid media at 42 to  $43^{\circ}\text{C}$ .

APPENDIX VI

7.1.6 Water baths for heating and cooling solutions and culture media to the appropriate temperatures.

7.2 Glassware

7.2.1 The glassware shall be resistant to repeated sterilization.

7.2.2 Culture tubes and bottles for sterilization and storage of culture media, and culture tubes 8 mm in diameter and 160 mm in length for lysine decarboxylation medium (6.2.11).

7.2.3 Measuring cylinder of 100 ml capacity, subdivided in 10 ml, for preparation of the complete media.

7.2.4 Graduated pipettes with a nominal capacity of 10 ml and 1 ml, subdivided respectively in 1.0 and 0.1 ml.

7.2.5 PETRI DISHES

7.2.5.1 Large-Size Dish

Dish

external diameter	140 $\pm$ 2 mm
external height	30 $\pm$ 2 mm
glass thickness	1.5 $\pm$ 0.5 mm

The rim shall be ground in a plane parallel to the base.

The bottom of the dish shall be flat and parallel to the base.

Lid

external diameter	150 $\pm$ 2 mm
external height	15 $\pm$ 2 mm
glass thickness	1.5 $\pm$ 0.5 mm

7.2.5.2 Small-Size Dish

Dish

internal diameter	90 $\pm$ 2 mm
external height, minimum	18 mm

The rim shall be ground in a plane parallel to the base.

The bottom of the dish shall be flat and parallel to the base.

Lid

external diameter, maximum	102 mm
----------------------------	--------



7.2.5.3 Alternatively, plastic Petri dishes may be used, even if of slightly different dimensions from the glass dishes described in 7.2.5.1 and 7.2.5.2.

### 7.3 Sterilization of glassware, etc.

Sterilize the glassware, etc. by one of the following methods:

- wet sterilization at not less than 121°C for not less than 20 min;
- dry sterilization at not less than 170°C for not less than 1 h.

## 8. SAMPLING

Proceed from the 200 g field samples. (see pages 47 and 48)

The frozen field samples must be kept frozen until analysis.

## 9. PROCEDURE

### 9.1 Pre-treatment of the sample

Field samples of dried eggs must be well mixed by shaking before the sample units are withdrawn. Field samples of frozen eggs should be thawed by placing them in cold running water only long enough to completely thaw them. The thawed field sample must be well mixed by shaking, before removal of the sample units.

### 9.2 Sample unit

Weigh 25 g of the mixed field sample (9.1) into a sterile blender jar (7.1.1).

### 9.3 Blending

Add 225 ml of the buffered peptone water (6.2.1) to the jar.

Operate the blender according to its speed, for sufficient time to give a total number of 15 000 to 20 000 revolutions. Thus, even with the slowest blender, this time will not exceed 2.5 min.

### 9.4 Pre-enrichment

9.4.1 Transfer the contents of the blender jar aseptically to a sterile 500 ml bottle.

9.4.2 Incubate the bottle at 37±1°C for not less than 16 h and not more than 20 h.

### 9.5 Enrichment

9.5.1 After the incubation period, transfer 10 ml from each of 10 bottles. (9.4.2) to 1000 ml of tetrathionate medium (6.2.2), and 10 ml from each of the same 10 bottles to 1000 ml of selenite medium (6.2.3). Both enrichment broths should be warmed to 42-43°C prior to inoculation.

9.5.2 Incubate the inoculated tetrathionate and selenite media up to 2 days at 42 to 43°C. The temperature must not exceed 43°C.

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9.6 Plating out

9.6.1 After an incubation period of 18 to 24 h, streak from each flask (9.5.2), using a loop with a diameter of 2.5 to 3 mm, onto the surface of brilliant-green/phenol red agar plates (6.2.4) and to bismuth sulphite agar (6.2.5), so that well-isolated colonies are obtained. (When large Petri dishes are not available, two small Petri dishes may be streaked one after the other, using the same loop.)

9.6.2 Incubate the plates with the bottom of the Petri dishes uppermost in an incubator at  $37 \pm 1^{\circ}\text{C}$ .

9.6.3 After an incubation period of 2 days (see 9.5.2), repeat the plating out of the two enrichment media and place the plates in an incubator at  $37 \pm 1^{\circ}\text{C}$ .

9.6.4 Examine the plates after an incubation of 20 to 24 h for the presence of typical colonies of salmonellae.

9.6.5 If growth is slight and no typical colonies of salmonellae are present, reincubate at  $37 \pm 1^{\circ}\text{C}$  for a further 20 to 24 h.

Re-examine the plates for the presence of typical colonies of salmonellae.

NOTE - Subject any typical or suspect colony to a confirmation (9.7) because the recognition of colonies of salmonellae is to a large extent a matter of experience and their appearance may vary somewhat, not only from species to species of salmonellae, but also from batch to batch of medium. In this respect agglutination of colonies with an omnivalent Salmonella antiserum may help to recognize suspected colonies.

9.7 Confirmation of presumptive salmonellae colonies

9.7.1 SELECTION OF COLONIES FOR CONFIRMATION

9.7.1.1 From each plate of each selective medium (see 9.6.1) select five typical or suspect colonies for confirmation.

9.7.1.2 If on one plate there are less than five typical or suspect colonies, take for confirmation all the typical or suspect colonies.

9.7.1.3 Streak the selected colonies onto the surface of nutrient agar plates (6.2.6), in a manner which will allow well-isolated colonies to develop.

9.7.1.4 Incubate the inoculated plates at  $37 \pm 1^{\circ}\text{C}$  for 20 to 24 h.

9.7.1.5 Select isolated colonies for biochemical and serological confirmation.

9.7.2 BIOCHEMICAL CONFIRMATION

9.7.2.1 Inoculation and Incubation of Media

Inoculate the following media with the selected colonies (9.7.1.5) by means of an inoculating wire:

9.7.2.1.1 TSI agar (6.2.7)

Streak the agar slope surface and stab the butt.

Incubate for 1 or 2 days at  $37 \pm 1^\circ\text{C}$ .

Interpret the changes in the medium as follows:

Butt

yellow	.....	glucose converted
red or unchanged	.....	no conversion of glucose
black	.....	formation of hydrogen sulphide
bubbles or cracks	.....	gas formation from glucose

Slant surface

yellow	.....	lactose, and/or sucrose converted
red or unchanged	.....	neither lactose nor sucrose converted

9.7.2.1.2 Urea agar (6.2.8)

Streak the agar slope surface.

Incubate for 1 or 2 days at  $37 \pm 1^\circ\text{C}$ .

Splitting of urea liberates ammonia, which changes the colour of phenol red to rose-pink and later on to deep cerise.

9.7.2.1.3 Lysine decarboxylation medium (6.2.11)

Inoculate just below the surface of the liquid medium.

Incubate for 1 day at  $37 \pm 1^\circ\text{C}$ .

A purple colour after growth has occurred indicates a positive reaction.

A yellow colour indicates a negative reaction.

9.7.2.1.4  $\beta$ -galactosidase reagent (6.2.12)

Suspend a loopful of the suspected colony in 0.25 ml of the saline solution (6.2.10) in a tube.

Add 1 drop of toluene.

Put the tube in a water bath at  $37 \pm 1^\circ\text{C}$  for several minutes.

Then add 0.25 ml of the  $\beta$ -galactosidase reagent and mix.

Replace the tube in the water bath at  $37 \pm 1^\circ\text{C}$  for 24 h (see note).

A yellow colour indicates a positive reaction.

NOTE - The reaction is often apparent after 20 min.

## 9.7.2.1.5 Voges-Proskauer reaction (6.2.13)

Inoculate two tubes by suspending a loopful of the suspected colony in 0.2 ml of the medium (6.2.13.1) in each tube.

Incubate one tube at room temperature and the other at  $37 \pm 1^\circ\text{C}$  for 48 hrs.

After suspension, add to each tube 2 drops of the creatine solution (6.2.13.2), 3 drops of the ethanolic naphthol solution (6.2.13.3) and then 2 drops of the KOH reagent (6.2.13.4); shake after the addition of each reagent.

A pink to bright red colour within 15 min indicates a positive reaction.

## 9.7.2.1.6 Indol reaction (6.2.14)

Inoculate a tube containing 5 ml of the medium (6.2.14.1) with the suspected colony.  
Incubate for 24 h at  $37 \pm 1^\circ\text{C}$ .

After incubation, add 1 ml of the indol reagent (6.2.14.2).

The forming of a red ring indicates a positive reaction.

A yellow-brown ring indicates a negative reaction.

## 9.7.2.2 Interpretation of the Results

Salmonellae show the following reactions<sup>1)</sup>:

		Salmonella serotypes confirming <sup>2)</sup>	
TSI glucose (acid formation) (9.7.2.1.1)	+	100 %	} figures will be updated
TSI glucose (gas formation) (9.7.2.1.1)	+	91.9 %	
TSI lactose (9.7.2.1.1)	- <sup>3)</sup>	99.2 %	
TSI sucrose (9.7.2.1.1)	-	99.5 %	
TSI hydrogen sulphide (9.7.2.1.1)	+	91.6 %	
Urea splitting (9.7.2.1.2)	-	100 %	
Lysine decarboxylation (9.7.2.1.3)	+	94.6 %	
$\beta$ -galactosidase reaction (9.7.2.1.4)	- <sup>3)</sup>	98.5 %	
Voges-Proskauer reaction (9.7.2.1.5)	-	100 %	
Indol reaction (9.7.2.1.6)	-	98.9 %	

## 9.7.3 SEROLOGICAL CONFIRMATION

Examine pure (9.7.1.5) non-auto-agglutinable (9.7.3.1) colonies for the presence of Salmonella O or H antigens by slide agglutination with sera according to the following procedure.

## 9.7.3.1 Elimination of Auto-Agglutinable Strains

Put on a carefully cleaned slide 1 drop of saline solution (6.2.10).

Disperse in this drop an amount of the culture under test to obtain a homogeneous and turbid suspension.

1/ Edwards and Ewing, 1972.

2/ These percentages only indicate that not all strains of salmonellae show the reactions as marked by + or -. These percentages may vary from country to country and from food product to food product.

3/ The Salmonella subgenus III (tricona) may give a positive lactose and  $\beta$  galactosidase reaction; the salmonella subgenus II may give a negative lactose, but a positive  $\beta$  galactosidase reaction.

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10. EXPRESSION OF RESULTS

If salmonellae after plating out (9.6) are detected in neither of the enrichment media, report: "No salmonellae isolated from the 10 (or 30) sample units of product examined".

If salmonellae after plating out (9.6) are detected in one or both of the enrichment media, report: "Salmonellae isolated from the 10 (or 30) sample units of product examined", and whether serotyping has been used. "The identified salmonellae belong to the following types: ..."

11. TEST REPORT

Indicate the method of test by quoting this Reference Method.

Give the exact name of the Centre which helped to identify the strains.

Rock the slide gently for 30 to 60 s.

Observe the reactions against a dark background, preferably with the aid of a magnifying glass.

The strains are considered auto-agglutinable if the bacteria have clotted to more or less distinct units.

The serological confirmation of these auto-agglutinable strains by the procedures 9.7.3.2 and 9.7.3.3 is impossible.

#### 9.7.3.2 Examination of the O-Antigens

Use pure (9.7.1.5) non-auto-agglutinable (9.7.3.1) colonies.

Proceed according to 9.7.3.1, using anti-O serum (6.3) instead of saline solution.

The mono- or polyvalent sera shall be used one after another.

#### 9.7.3.3 Examination of the H-Antigens

Inoculate the semi-solid nutrient agar (6.2.9) with a pure non-auto-agglutinable (9.7.3.1) colony.

Incubate the medium for 18 to 24 h at  $37 \pm 1^\circ \text{C}$ .

Use this culture for the examination of the H-antigens according to the procedure in 9.7.3.1 but using a drop of anti-H serum (6.3) instead of saline solution.

#### 9.7.4 INTERPRETATION

9.7.4.1 Strains which show typical biochemical reactions (9.7.2) and give positive serological reactions according to 9.7.3.2 or 9.7.3.3, are considered to be salmonellae.

9.7.4.2 Strains which show typical biochemical reactions (9.7.2) but do not give positive serological reactions according to 9.7.3.2 or 9.7.3.3, strains which do not show typical biochemical reactions (9.7.2), but give positive serological reactions according to 9.7.3.2 or 9.7.3.3, and auto-agglutinable (9.7.3.1) strains which show typical biochemical reactions (9.7.2), could be salmonellae.

9.7.4.3 Strains which do not show typical biochemical reactions (9.7.2) and which do not give positive serological reactions according to 9.7.3.2 or 9.7.3.3 are not considered to be salmonellae.

#### 9.7.5 DEFINITIVE CONFIRMATION

Strains which are considered to be salmonellae (9.7.4.1) or which may be salmonellae according to 9.7.4.2, shall be sent to a recognized Salmonella Reference Centre for definitive typing.

This dispatch shall be accompanied by all possible information concerning the strain(s).

**SPECIFICATION FOR BRILLIANT-GREEN**

**A.1 BACTERIOLOGICAL PERFORMANCE**

Suppression of spreading of proteus on brilliant-green/phenol red agar (6.2.4), while the growth of salmonellae is not inhibited.

**A.2 METHOD OF TEST**

**A.2.1 Medium**

Prepare brilliant-green/phenol red agar according to 6.2.4 with various concentrations of brilliant-green, viz. 4.5 mg/l to 6 mg/l.

**A.2.2 Procedure**

Inoculate a set of plates with different brilliant-green concentrations with a pure culture of a swarming proteus and another set with a pure culture of salmonellae, and incubate these plates at 37°C for no longer than 24 h.

A satisfactory concentration of the stain should allow growth of salmonellae with typical pink colonies, 1 to 2 mm in diameter, and limited growth of proteus, i.e. no spreading.

The concentration of brilliant-green which shows this pattern should be used for the preparation of the brilliant-green solution (6.2.2.4).

APPENDIX VI

3.2 EGG PRODUCTS - ENUMERATION OF MESOPHILIC AEROBIC BACTERIA (REFERENCE METHOD)

1. SCOPE

A Reference Method for enumeration of mesophilic aerobic bacteria in egg products.

2. FIELD OF APPLICATION

The method can be applied to dried or frozen whole egg products covered by the Code of Hygienic Practice for Egg Products.

3. REFERENCE

Modification of ISO/TC 34/SC 9 method for enumeration of mesophilic aerobic bacteria.

4. DEFINITION

By "mesophilic aerobic bacteria" are meant micro-organisms growing aerobically at 30°C under the conditions described in the present method.

5. PRINCIPLE

Inoculation in Petri dishes of melted defined culture medium, with the food homogenate (1 in 10) and decimal dilutions.

Incubation of this medium aerobically at 30°C for 72 hrs.

Calculation of the number of mesophilic aerobic bacteria per gramme of sample unit from the number of colonies obtained in selected Petri dishes at levels of dilution giving a significant result.

6. CULTURE MEDIA, DILUENTS AND REAGENTS

6.1 Basic materials

For uniformity of results, it is recommended that either dehydrated culture medium components of uniform quality and analytical grade chemicals, or a dehydrated complete medium, be used. The water shall be distilled water or water of at least equivalent purity.

The manufacturers' instructions should be rigorously followed when dehydrated complete media are used.

If the media are not used on the day of preparation, keep them in darkness at +5°C for not more than one month, taking precautions to prevent evaporation.

6.2 Culture media

6.2.1 BUFFERED PEPTONE WATER

Composition

peptone	10.0 g
sodium chloride	5.0 g
disodium hydrogen phosphate ( $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ )	9.0 g
potassium dihydrogen phosphate ( $\text{K}_2\text{HPO}_4$ )	1.5 g
water	1 000 ml



Preparation

Dissolve the components in water by boiling.  
Adjust the pH so that after autoclaving it is  $7.0 \pm 0.1$  at  $20^{\circ}\text{C}$ .  
Transfer to tubes or dilution bottles in quantities of 9 ml.  
Sterilize for 20 min at  $121 \pm 1^{\circ}\text{C}$ .

6.2.2 PLATE COUNT AGAR

Composition

Dehydrated yeast extract	2.5 g
Pancreatic digest of casein	5.0 g
Glucose	1.0 g
Powdered or flaked agar-agar	12 to 18 g depending on gelatinizing properties of the product
water	1 000 ml

Preparation

Dissolve, in boiling water, the components or the dehydrated complete medium.  
If necessary, adjust the pH so that after sterilization, it is  $7.0 \pm 0.2$  at  $20^{\circ}\text{C}$  (Measurement performed at  $45^{\circ}\text{C}$  with temperature correction).  
Distribute the medium in tubes (e.g. 18 mm x 180 mm), with 15 ml per tube, or in bottles not exceeding 500 ml, filling only about half of the volume of the bottle.  
Sterilize in an autoclave at  $121^{\circ}\text{C} \pm 1^{\circ}\text{C}$  for 20 min.  
Before beginning the analysis, to avoid delay in pouring the agar, melt the medium completely in a bath of boiling water and cool to  $45-48^{\circ}\text{C}$ , preferably in a water bath.

6.2.3 NON-NUTRITIVE AGAR, CALLED "WHITE AGAR"

Composition

Powdered or flaked agar-agar	12 to 18 g depending on the gelatinizing properties of the product.
water	1 000 ml

Preparation

Dissolve the agar-agar in boiling water.  
If necessary, adjust the pH so that after sterilization it is  $7.0 \pm 0.2$  at  $20^{\circ}\text{C}$  (Measurement performed at  $45^{\circ}\text{C}$  with temperature correction).  
Distribute the agar in tubes (e.g. 18 mm x 180 mm), 4 ml per tube, or in 150 ml bottles, 100 ml per bottle.

Sterilize in an autoclave at  $121^{\circ}\text{C} \pm 1^{\circ}\text{C}$  for 20 min.

Before beginning the analysis, to avoid delay in pouring the agar, melt the medium completely in a bath of boiling water, and cool to  $45-48^{\circ}\text{C}$ , preferably in a water bath.

## APPENDIX VI

### 7. APPARATUS AND GLASSWARE

Standard laboratory equipment, and especially:

- 7.1 Apparatus for sterilizing glassware, culture media, etc.
- 7.2 Incubator regulated to  $30^{\circ}\text{C} \pm 1^{\circ}\text{C}$ .
- 7.3 Glass Petri dishes or plastic dishes, diameter 90 to 100 mm.
- 7.4 Culture tubes or bottles for sterilization and storage of culture media.
- 7.5 Total-flow pipettes, nominal capacity 1 ml and graduated in 0.1 ml.

#### Sterilization of the glassware

Sterilize the glassware by one of the following methods:

dry sterilization at not less than  $170^{\circ}\text{C}$  for not less than 1 h.

wet sterilization at not less than  $120^{\circ}\text{C}$  for not less than 20 min.

### 8. SAMPLING

Proceed from the 200 g field samples (see paras 1. and 2).

The frozen field samples must be kept frozen until analysis.

### 9. PROCEDURE

#### 9.1 Preparation of the sample unit, of the Food Homogenate (1 in 10) and of the decimal dilutions

9.1.1 For the pre-treatment of the field sample, the sample unit and blending to obtain the food homogenate (1 in 10), refer to Salmonella reference method 9.1, 9.2 and 9.3 of section 3.1.

##### 9.1.2 DILUTION

9.1.2.1 Mix the contents of the jar by shaking, and pipette (with 7.5) 1 ml into a tube containing 9 ml of dilution fluid (6.2.1).

9.1.2.2 Mix the liquids carefully by aspirating 10 times with a pipette.

9.1.2.3 Transfer with the same pipette 1.0 ml to another dilution tube containing 9 ml of dilution fluid, and mix with a fresh pipette.

9.1.2.4 Repeat steps 9.1.2.2 and 9.1.2.3 until the required number of dilutions are made. Each successive dilution will decrease the concentration 10-fold.

#### 9.2 Pour Plating

9.2.1 Take two sterile Petri dishes (7.3).  
Transfer into each of these dishes, with a sterile pipette (7.5), 1 ml of the food homogenate (1 in 10).

9.2.2 Take two other sterile Petri dishes.  
With a new sterile pipette, transfer to each of these dishes 1 ml of

APPENDIX VI

10.1.2 OTHER PRODUCTS (TABLE II)

10.1.2.1 General case: At least one dish exists which contains between 30 and 300 colonies (examples 3, 4 and 5)

Retain all dishes corresponding to the dilution or to the two successive dilutions in which this dish or these dishes are located.

For each dilution, calculate the average number of colonies. Retain only two significant digits. Thus, for a three-digit number, round off to the nearest zero. If the third digit is 5, round off to the lower zero.

Multiply the value obtained by the inverse of the corresponding dilution to obtain the number of bacteria per gramme of product.

In a case in which there are two values for the number of bacteria per gramme of product (as when two dilutions have been retained) average these two values if the ratio of the higher value to the lower value is less than 2. If not, retain the lower value.

10.1.2.2 Special cases: There are no dishes containing between 30 and 300 colonies

If the numbers of colonies differ slightly from these limits at the level of two successive dilutions (example 6), proceed as for 10.1.2.1 (case for two retained dilutions).

If the dishes corresponding to 1 dilution contain spreading colonies, and if the number of colonies of the next dilution is lower than 30 (example 7), proceed with this dilution as for 10.1.2.1.

11. TEST REPORT

Indicate the method of test by quoting this Reference Method.

The test report must give the information needed for complete identification of the sample.

Pour into each Petri dish 15 ml of medium (6.2.2). The time elapsing between commencing to prepare the dilutions and pouring the agar into the dishes must not exceed 15 min.

Carefully mix the inoculum with the medium and allow the latter to solidify by placing the Petri dishes on a cool horizontal surface.

Where it is suspected that the product to be analyzed contains bacteria whose colonies are likely to spread over the surface of the media, pour onto the surface of the inoculated agar, after the latter has solidified, about 4 ml of medium (6.2.3) to provide a layer of approximately 2 mm in thickness. Allow the medium to solidify.

### 9.3 Incubation of the dishes

Invert the prepared dishes and place them in the incubator at  $30^{\circ}\text{C} \pm 1^{\circ}\text{C}$  (7.2) for  $72 \pm 3$  h.

### 9.4 Counting the Colonies

Examine the dishes after the prescribed incubation period. If this is not possible, they may be held at  $4^{\circ}\text{C}$  for a maximum of 24 h.

Count the colonies in each dish suitable for use in the calculation of the number of bacteria per gramme of product, in principle those containing between 30 and 300 colonies (unless exception, see section 9).

## 10. EXPRESSION OF RESULTS

### 10.1 Method of calculation

Give the result as the number of mesophilic aerobic bacteria per gramme of dried or frozen whole egg product. Express it by a number in the range 1.0 to 9.9 multiplied by  $10^x$ , x being the appropriate power of 10.

When counting, several situations may be encountered:

#### 10.1.1 PRODUCTS CONTAINING RELATIVELY FEW MICROORGANISMS (TABLE 1)

##### 10.1.1.1 The dishes examined contain no colonies:

Give the result in the form:

less than  $1 \times 10^1$  bacteria per gramme of product,  $10^1$  being the inverse of the dilution of the food homogenate (ex.1).

##### 10.1.1.2 The dishes corresponding to the food homogenate (1 in 10) contain less than 30 colonies:

Give the result in the form:

less than  $3 \times 10^2$  bacteria per gramme of product (example 2).

\* New text from ISO document when available.

TABLE I (will be revised)

Examples	No. of colonies from 1 gramme of food homogenate (1 in 10)	Results (in no. of bacteria per g of product)	Explanation of Calculations
No. 1	0	fewer than 1 x 10 bacteria	$1 \times 10^1 = 1 \times 10$
No. 2	18 17	fewer than $3 \times 10^2$ bacteria	$30 \times 10^1 = 3 \times 10^2$

APPENDIX VI

TABLE II (will be revised)

Examples	Number of Colonies		Ratio	Results (in number of bacteria per gramme of product)	Explanation of Calculations
	dilution at $\frac{1}{100}$	dilution at $\frac{1}{1000}$			
No. 3	175 208	16 17	-	$1.9 \times 10^4$	175 + 208 $\frac{383}{2} = 191 \rightarrow 190 \rightarrow 190 \times 10^2 = 1.9 \times 10^4$
No. 4	322 278	23 29	-	$3 \times 10^4$	322 + 278 $\frac{600}{2} = 300 \rightarrow 300 \times 10^2 = 3 \times 10^4$
No. 5	296 378	40 24	< 2	$3.3 \times 10^4$	296 + 378 $\frac{674}{2} = 337 \rightarrow 340 \rightarrow 340 \times 10^2 = 3.4 \times 10^4$  40 +24 $\frac{64}{2} = 32 \rightarrow 32 \times 10^3 = 3.2 \times 10^4$  $\frac{3.4 \times 10^4}{3.2 \times 10^4} < 2$  $\rightarrow 10^4 \frac{(3.4 + 3.2)}{2} = 3.3 \times 10^4$
No. 6	327 330	18 25	< 2	$2.7 \times 10^4$	327 + 330 $\frac{657}{2} = 328 \rightarrow 330 \rightarrow 330 \times 10^2 = 3.3 \times 10^4$  18 + 25 $\frac{43}{2} = 21.5 \rightarrow 21 \times 10^3 = 2.1 \times 10^4$  $\frac{3.3 \times 10^4}{2.1 \times 10^4} < 2$  $\rightarrow 10^4 \frac{(3.3 + 2.1)}{2} = 2.7 \times 10^4$
No. 7	spreaders spreaders	18 24			18 + 24 $\frac{42}{2} = 21 \rightarrow 21 \times 10^3 = 2.1 \times 10^4$

3.3 EGG PRODUCTS - ENUMERATION OF COLIFORM BACTERIA; DETERMINATION OF THE MOST PROBABLE NUMBER (MPN) (REFERENCE METHOD)

1. SCOPE

A Reference Method for the detection of coliform bacteria in egg products.

2. FIELD OF APPLICATION

The method can be applied to dried or frozen whole egg products covered by the Code of Hygienic Practice for Egg Products.

3. REFERENCE

Thatcher, F.S. and Clark, D.S., ed. (1968): Microorganisms in foods. Their significance and methods of enumeration. Toronto, University of Toronto Press.

4. DEFINITION

Coliform bacteria: Microorganisms that form gas in the two media described below when the test is carried out according to the method.

5. PRINCIPLE

5.1 Enrichment

Inoculation in tubes of an enrichment medium with the food homogenate (1 in 10) and decimal dilutions.

Incubation of this medium at 37°C for 48 hours.

5.2 Confirmation

From tubes with gas formation, inoculation in a confirmatory medium in tubes.

Incubation of these confirmatory tubes at 37°C for 48 hours and calculation on basis of a table the most probable number of coliform bacteria per gramme of the egg product.

6. CULTURE MEDIA, DILUENTS AND REAGENTS

6.1 Basic materials

For uniformity of results, it is recommended that either dehydrated culture medium components of uniform quality and analytical grade chemicals, or a dehydrated complete medium, be used. The water used shall be distilled water or water of at least equivalent purity.

The manufacturers' instructions should be rigorously followed when dehydrated complete media are used.

If the media are not used on the day of preparation, keep them in darkness at +5°C for not more than one month, taking precautions to prevent evaporation.

APPENDIX VI

6.2 Culture media

6.2.1 BUFFERED PEPTONE WATER

Composition

peptone	10.0 g
sodium chloride	5.0 g
disodium hydrogen phosphate ( $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ )	9.0 g
potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ )	1.5 g
water	1 000 ml

Preparation

Dissolve the components in water by boiling.  
Adjust the pH so that after autoclaving it is 7.0±0.1 at 20°C.  
Transfer to tubes or dilution bottles in quantities of 9 ml.  
Sterilize for 20 min at 121±1°C.

6.2.2 LAURYL SULPHATE TRYPTOSE BROTH

Composition

Tryptose, tryptone, or trypticase	20 g
Lactose	5 g
Potassium monohydrogen phosphate ( $\text{K}_2\text{HPO}_4$ )	2.75 g
Potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ )	2.75 g
Sodium chloride	5 g
Sodium lauryl sulphate	0.1 g
water	1 000 ml

Preparation

Dissolve ingredients in water and dispense in 10 ml volumes in tubes (e.g. 18 mm x 180 mm) (7.3) containing inverted Durham fermentation vials (10 mm x 75 mm) (7.4).  
Sterilize in an autoclave at 121°C for 10 minutes. Final pH should be approximately 6.8.

6.2.3 BRILLIANT-GREEN LACTOSE BILE BROTH 2%

Note: For preparation of ox bile solution and Brilliant-green solution, see Salmonella Reference Method.

Composition

Peptone	10 g
Lactose	10 g
Ox bile	20 g
Brilliant-Green	0.0133 g
water	1 000 ml

Preparation

Dissolve the peptone and lactose in 500 ml of water and add the ox bile dissolved in 200 ml of water. Bring the volume to approximately 975 ml with water and adjust the pH to 7.4.



Add 13.3 ml of a 1% aqueous solution of brilliant-green, bring the total volume to 1 litre, stir, and filter through cotton if necessary.

Disperse in 10 ml volumes into tubes (e.g., 18 mm x 180 mm) (7.3) containing inverted Durham fermentation vials (10 mm x 75 mm) (7.4).

### 7. APPARATUS AND GLASSWARE

Standard laboratory equipment, and especially:

- 7.1 Apparatus for sterilizing glassware, culture media, etc.
- 7.2 Incubator regulated to  $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ .
- 7.3 Tubes for sterilization and storage of culture media.
- 7.4 Durham tubes to be inserted in 7.3.
- 7.5 Total-flow pipettes, nominal capacity 1 ml and graduated in 0.1 ml.

#### Sterilization of the glassware

Sterilize the glassware by one of the following methods:

dry sterilization at not less than  $170^{\circ}\text{C}$  for not less than 1 hour;

wet sterilization at not less than  $121^{\circ}\text{C}$  for not less than 20 min.

### 8. SAMPLING

Proceed from the 200 g field samples (see pages 47 and 48).

The frozen field samples must be kept frozen until analysis.

### 9. PROCEDURE

#### 9.1 Preparation of the sample unit, of the food homogenate (1 in 10), and of the decimal dilutions

9.1.1 For the pre-treatment of the sample, the sample unit and blending to obtain food homogenate (1 in 10), refer to Salmonella method 9.1, 9.2 and 9.3.

#### 9.1.2 DILUTION

9.1.2.1 Mix the contents of the jar by shaking, and pipette (with 7.5) 1 ml into a tube containing 9 ml of dilution fluid (6.2.1).

9.1.2.2 Mix the liquids carefully by aspirating 10 times with a pipette.

9.1.2.3 Transfer with the same pipette 1.0 ml to another dilution tube containing 9 ml of dilution fluid, and mix with a fresh pipette.

APPENDIX VI

9.2 Inoculation of enrichment medium

9.2.1 Take three tubes of lauryl sulphate tryptose broth (6.2.2). Transfer into each of these tubes with a sterile pipette (7.5) 1 ml of the food homogenate (1 in 10).

9.2.2 Take three other tubes of lauryl sulphate tryptose broth (6.2.2). With a new sterile pipette, transfer to each of these tubes 1 ml of the contents of the first dilution tube.

9.2.3 Carry out the same operation from the last dilution tube.

9.3 Incubation of the tubes

Incubate tubes at  $37 \pm 1^\circ\text{C}$  for 24 and 48 hours.

9.4 Reading of enrichment tubes

After 24 hours, record tubes showing gas production, and proceed to step 9.5 for these tubes. Reincubate negative tubes and read these after 48 hours. Record tubes showing gas production, and proceed to step 9.5.

9.5 Confirmation of coliforms

Confirm that the tubes of lauryl sulphate tryptose broth selected in step 9.4 are positive for coliform bacteria by transferring a loopful of each to separate tubes of brilliant-green lactose bile broth 2% (6.2.3).

9.6 Incubation of confirmatory tubes

Incubate confirmatory tubes 48 hours at  $37 \pm 1^\circ\text{C}$  and note gas production.

9.7 Reading of confirmatory tubes

The formation of gas confirms the presence of coliform bacteria.

9.8 Recording the number of positive confirmatory tubes

Record the number of enrichment tubes (9.4) in each dilution that were confirmed as positive for coliform bacteria.

If, for example, the number of positive tubes in the three dilutions were 3, 1, and 0, respectively, the results are recorded as 1:10 dilution = 3, 1:100 dilution = 1, and 1:1000 dilution = 0.

10. EXPRESSION OF RESULTS

10.1 Method of calculation

To obtain the most probable number (MPN) of coliform bacteria, proceed as follows

10.1.1 Refer to the MPN table (Table 1) and note the MPN appropriate to the number of positive tubes. For example, in the illustration given in step 9.8 above, the values for each dilution are 3, 1 and 0 respectively. The table shows that these results indicate an MPN of 40 per gram of the egg product.

Aromatizantes artificiales

Maltol de etilo

Referencia 1/

(1)

Agente activo de superficie

Sodio sulfosuccinato dioctílico

(3)

Referencia

- (1) Normas de identidad y pureza para algunos disolventes de extracción y otros, FAO: Reuniones sobre nutrición Informe No. 48B (o OMS/Food Add./70.40)
- (2) Normas de identidad y pureza para algunas enzimas y otros, FAO: Reuniones sobre nutrición, No. 50B (o OMS Serie de Aditivos Alimentarios, 1972, No. 2)
- (3) Normas de identidad y pureza de algunos aditivos alimentarios, FAO: Reuniones sobre nutrición, Informe No. 54B (o OMS/Food Add./7).

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1/ Los documentos pueden solicitarse a los Servicios de Distribución y Ventas de la FAO y la OMS, que los distribuyen a los puntos de contacto del Codex.

Aditivo

6.1 Hexametilentetramina

6.2 El Grupo Especial de Trabajo no examinó las especificaciones para color de caramelo, por no haber recibido información de la 21ª reunión del Comité Mixto de Expertos.

7. Se recomienda que por el momento las siguientes especificaciones no se presenten a la Comisión para su aprobación:

Aditivo

Objeciones/observaciones

7.1 Todas las enzimas

- (a) Hay que revisar los criterios microbiológicos
- (b) Hay que revisar el límite para aflatoxinas
- (c) La nomenclatura de las enzimas debe hacer referencia a los números IUB.

7.2 Estearoil-lactilato de sodio

- (a) Las especificaciones para índice de ácido, índice de ester, contenido de sodio y contenido de ácido láctico deben revisarse para describir los productos comerciales actuales.
- (b) Hay que corregir el nombre químico.

7.3 Estearoil-lactilato de calcio

... véase 7.2(a)

APENDICE XI

ESPECIFICACIONES DE IDENTIDAD Y PUREZA DE ADITIVOS ALIMENTARIOS  
(presentadas a la Comisión en el Trámite 5 del Procedimiento del Codex para la elaboración de Especificaciones) 1/

Acentuadores del sabor

Referencia 2/

Acido L(+)glutámico  
L(+)glutamato, amonio  
L(+)glutamato, calcio  
L(+)glutamato, potasio  
5'-guanilato, calcio  
5'-guanilato, sodio  
5'-inosinato, calcio  
5'-inosinato, sodio  
5'-nucleotido, calcio  
5'-nucleotido, sodio

(3)  
(3)  
(3)  
(3)  
(3)  
(3)  
(3)  
(3)  
(3)  
(3)

Sales de ácidos orgánicos

Gluconato cálcico  
Lactato cálcico  
Gluconato ferroso  
Acetato potásico  
Lactato potásico (solución)  
Acetato sódico  
Lactato sódico (solución)

(3)  
(3)  
(3)  
(3)  
(3)  
(3)  
(3)

Sales de ácidos inorgánicos

Sulfato cúprico  
Cloruro estannoso

(1)  
(1)

Sustancias conservadoras

Hexametilentetramina

(2)

1/ Véanse párrafos 125-129 de este informe

2/ Los documentos pueden solicitarse a los Servicios de Distribución y Ventas de la FAO y la OMS, que los distribuyen a los puntos de contacto del Codex.

11. TEST REPORT

Indicate the method of test by quoting this Reference Method.

The test report must give the information needed for complete identification of the sample.

APPENDIX VI

TABLE 1<sup>1</sup>

MOST PROBABLE NUMBER (MPN) OF COLIFORM BACTERIA IN EGG PRODUCTS PER GRAMME

3 x 0.1 g; 3 x 0.01 g; 3 x 0.001 g

Result	MPN	Confidence limits			
		99%		95%	
0 1 0	3	<1	23	<1	17
1 0 0	4	<1	28	1	21
1 0 1	7	1	35	2	27
1 1 0	7	1	36	2	28
1 2 0	11	2	44	4	35
2 0 0	9	1	50	2	38
2 0 1	14	3	62	5	48
2 1 0	15	3	65	5	50
2 1 1	20	5	77	8	61
2 2 0	21	5	80	8	63
3 0 0	23	4	177	7	129
3 0 1	40	10	230	10	180
3 1 0	40	10	290	20	210
3 1 1	70	20	370	20	280
3 2 0	90	20	520	30	390
3 2 1	150	30	660	50	510
3 2 2	210	50	820	80	640
3 3 0	200	<100	1900	100	1400
3 3 1	500	100	3200	200	2400
3 3 2	1100	200	6400	300	4800

The table contains only the most likely results which would be obtained in 95% of the cases with series of 5 tests. If one of the results does not figure in the table, it is too unlikely to be acceptable and the series of 5 tests must be repeated.

<sup>1</sup> The MPN table reproduced here is calculated according to : J. C. de Man (1975) The Probability of Most Probable Numbers. European J. Appl. Microbiol., 1, 67-78.

4. Sampling Plans and Microbiological Limits

4.1 Dried and Frozen Whole Egg

Salmonellae: Salmonella organisms should not be recovered from any of ten sample units examined when the test is carried out according to the method described. (n = 10, c = 0, m = 0).

In products intended for special dietary purposes, salmonella organisms should not be recovered from any of thirty sample units examined (n = 30, c = 0, m = 0).

Mesophilic aerobic bacteria: Mesophilic aerobic bacteria should not be recovered from any of five sample units examined when the test is carried out according to the method described in a number exceeding one million per gramme, nor in a number exceeding 50,000 per gramme from three or more of the five sample units examined. (n = 5, c = 2, m =  $5 \times 10^4$ , M =  $10^6$ ).

Coliform bacteria: Coliform bacteria should not be recovered from any of five sample units examined, when the test is carried out according to the method described, in a number exceeding 1,000 per gramme, nor in a number exceeding ten per gramme from three or more of the five sample units examined. (n = 5, c = 2, m = 10, M =  $10^3$ ).

4.2 Other Egg Products

Salmonellae: Salmonella organisms should not be recovered from any of ten sample units examined when the test is carried out according to the method described. (n = 10, c = 0, m = 0).

In products intended for special dietary purposes, salmonella organisms should not be recovered from any of thirty sample units examined (n = 30, c = 0, m = 0).

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SUMMARY STATUS OF WORK  
(prepared by the Secretariat)

Code/Paper	Status Step	To be dealt with by	Document ALINORM App.	Working paper for next session
General Principles of Food Hygiene	9	Governments	CAC/RCP 1-1969	
Revision of General Principles	4	Governments		CX/FH 77/3 *
Canned Fruit and Vegetable Products	9	Governments	CAC/RCP 2-1969	
Dried Fruits	9	Governments	CAC/RCP 3-1969	
Desiccated Coconut Dehydrated Fruits and Vegetables including Edible Fungi	9	Governments	CAC/RCP 4/5-1971	
Tree Nuts	9	Governments	CAC/RCP 6-1972	
Fresh Fish <u>3/</u>	9	Governments	CAC/RCP 9-1976 *	
Canned Fish <u>3/</u>	9	Governments	CAC/RCP 10-1976 *	
Meat Hygiene <u>1/</u>	9	Governments	CAC/RCP 11-1976 *	
Processed Meat Products <u>2/</u>	9	Governments	CAC/RCP 12-1976 *	
Ante-Mortem and Post-Mortem Inspection <u>1/</u>	9	Governments	CAC/RCP 13-1976 *	
Poultry Processing	9	Governments	CAC/RCP 14-1976 *	
Egg Products	9	Governments	CAC/RCP 15-1976 *	
Molluscan Shellfish <u>3/</u>	7	11th FFP (14th FH)		*
Frozen Fish <u>3/</u>	7	11th FFP (14th FH)	ALINORM 78/13 Appendix IV (amendments only)	ALINORM 78/18 Appendix... *
Processing of Froglegs	5	12th CAC 2nd Joint FAO/ WHO Expert Consult. on Microb. Spec.	ALINORM 78/13 Appendix II	
Microbiological Specifications for Egg Products	5 (8)	12th CAC	ALINORM 78/13 Appendix VI	
Peanuts (Groundnuts)	4	14th FH	ALINORM 78/13 Appendix III	
Low Acid Canned Foods	4	14th FH	ALINORM 78/13 Appendix V (Sections I, II and III)	CX/FH 77/4 * (Sections IV and V)
Foods for Infants and Children	4	14th FH		CX/FH 77/5 *
Guidelines for Development and Application of Microbiological Specifications for Foods		2nd Joint Consult. on Microb. Spec.		*
Harmonization of Definitions (Background paper)		14th FH		*
Acidified Low Acid Canned Foods	pro-posed			

- \* To be distributed in due course  
1/ Elaborated independently by the Codex Committee on Meat Hygiene  
2/ Elaborated independently by the Codex Committee on Processed Meat Products  
3/ Elaborated in collaboration with the Codex Committee on Fish and Fishery Products