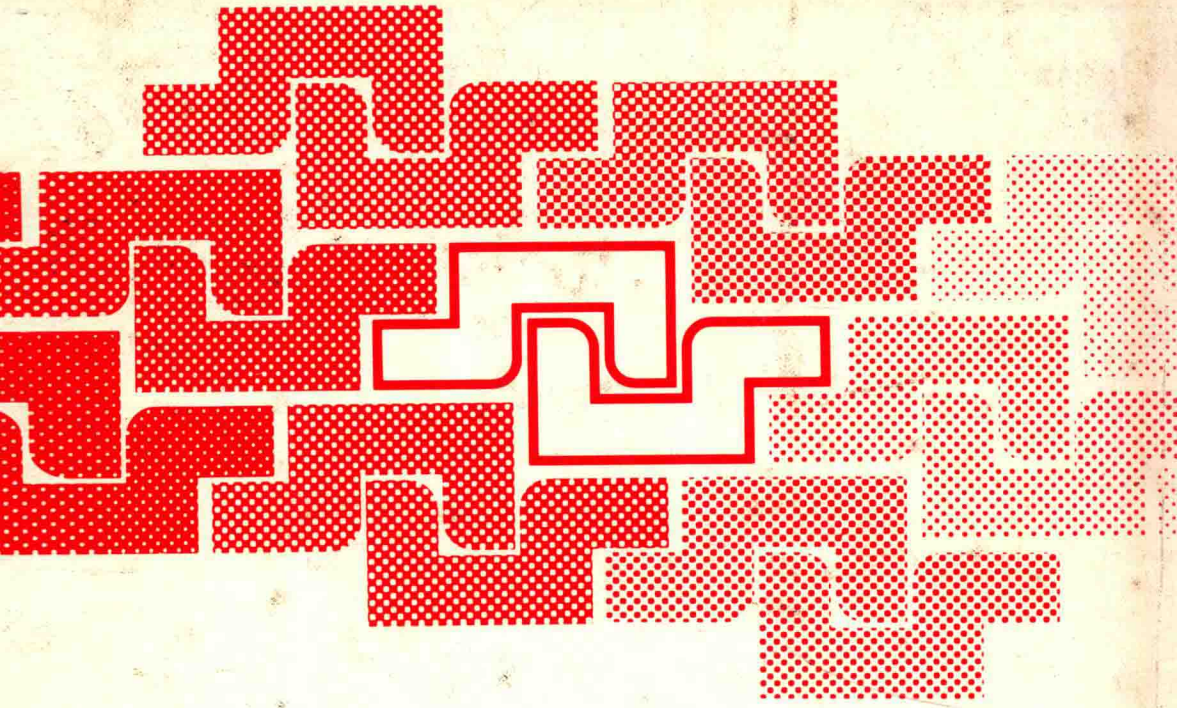


Methods for the Mycological Examination of Food

Edited by
A. D. King, Jr.
J. I. Pitt
L. R. Beuchat and
Janet E. L. Corry

NATO ASI Series

Series A: Life Sciences Vol. 122

Methods for the Mycological Examination of Food

Edited by

A. D. King, Jr.

United States Department of Agriculture
Albany, California

J. I. Pitt

Commonwealth Scientific and Industrial Research Organization
North Ryde, New South Wales, Australia

L. R. Beuchat

University of Georgia
Experiment, Georgia

and

Janet E. L. Corry

Ministry of Agriculture, Fisheries, and Food
London, England

Plenum Press
New York and London
Published in cooperation with NATO Scientific Affairs Division

Proceedings of a NATO Advanced Research Workshop on
Standardization of Methods for the Mycological Examination of Foods,
held July 11-13, 1984,
in Boston, Massachusetts

Library of Congress Cataloging in Publication Data

NATO Advanced Research Workshop on Standardization of Methods for the Mycological Examination of Foods (1984: Boston, Mass.)
Methods for the mycological examination of food.

(NATO ASI series. Series A, Life sciences; v. 122)
"Proceedings of a NATO Advanced Research Workshop on Standardization of Methods for the Mycological Examination of Foods, held July 11-13, 1984, in Boston, Massachusetts"—T.p. verso.
"Published in cooperation with NATO Scientific Affairs Division."
Includes bibliographies and index.
1. Food—Microbiology—Congresses. 2. Mycology—Technique—Congresses. 3. Food—Analysis—Congresses. I. King, A. D. II. North Atlantic Treaty Organization. Scientific Affairs Division. III. Series.
QR155.N38 1984 576'.163 86-25428
ISBN 0-306-42479-7

A Division of Plenum Publishing Corporation
233 Spring Street, New York, N.Y. 10013

Printed in the United States of America

Methods for the Mycological Examination of Food

NATO ASI Series

Advanced Science Institutes Series

A series presenting the results of activities sponsored by the NATO Science Committee, which aims at the dissemination of advanced scientific and technological knowledge, with a view to strengthening links between scientific communities.

The series is published by an international board of publishers in conjunction with the NATO Scientific Affairs Division

A Life Sciences **B Physics**	Plenum Publishing Corporation New York and London
C Mathematical and Physical Sciences	D. Reidel Publishing Company Dordrecht, Boston, and Lancaster
D Behavioral and Social Sciences **E Engineering and Materials Sciences**	Martinus Nijhoff Publishers The Hague, Boston, Dordrecht, and Lancaster
F Computer and Systems Sciences **G Ecological Sciences** **H Cell Biology**	Springer-Verlag Berlin, Heidelberg, New York, London, Paris, and Tokyo

Recent Volumes in this Series

Volume 115—Mechanisms of Secondary Brain Damage
edited by A. Baethmann, K. G. Go, and A. Unterberg

Volume 116—Enzymes of Lipid Metabolism II
edited by Louis Freysz, Henri Dreyfus, Raphaël Massarelli, and Shimon Gatt

Volume 117—Iron, Siderophores, and Plant Diseases
edited by T. R. Swinburne

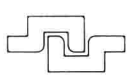
Volume 118—Somites in Developing Embryos
edited by Ruth Bellairs, Donald A. Ede, and James W. Lash

Volume 119—Auditory Frequency Selectivity
edited by Brian C. J. Moore and Roy D. Patterson

Volume 120—New Experimental Modalities in the Control of Neoplasia
edited by Prakash Chandra

Volume 121—Cyst Nematodes
edited by F. Lamberti and C. E. Taylor

Volume 122—Methods for the Mycological Examination of Food
edited by A. D. King, Jr., J. I. Pitt, L. R. Beuchat, and Janet E. L. Corry



Series A: Life Sciences

PREFACE

The desirability, indeed the necessity, for standardization of methods for the examination of foods for contaminant and spoilage mycoflora has been apparent for some time. The concept of a specialist workshop to address this problem was borne during conversations at the Gordon Research Conference on "Microbiological Safety of Foods" in Plymouth, New Hampshire, in July 1982. Discussions at that time resulted in an Organizing Committee of four, who became the Editors, and a unique format: all attendees would be expected to contribute and, in most cases, more than once; and papers in nearly all sessions would be presented as a set of data on a single topic, not as a complete research paper. Each session would be followed by general discussion, and then a panel would formulate recommendations for approval by a final plenary session. The idea for this format was derived from the famous "Kananaskis I" workshop on Hyphomycete taxonomy and terminology organized by Bryce Kendrick of the University of Waterloo, Ontario in 1969. Attendance would necessarily be limited to a small group of specialists in food mycology.

The scope of the workshop developed from answers to questionnaires circulated to prospective participants. To generate new data which would allow valid comparisons to be drawn, intending participants were given a variety of topics as assignments and asked to bring information obtained to the workshop. Because of time constraints and other factors, the Organizing Committee decided that some subjects which might be considered to be appropriate for a workshop of this type lay outside its scope. The most obvious of these was the question "Should we count fungi in foods?" We felt this was a philosophical question, for which we had neither the time needed nor the information available to provide the answer. We are aware that people are enumerating fungi in foods and that they will continue to do so. So the assumption was made that enumeration is a worthwhile system, and the major aim set was to standardize techniques currently in use. Given the information gained in this way, more fundamental questions can be tackled at some future date.

It was agreed to concentrate attention on dilution plating, a universally accepted procedure, and direct plating, in which particulate foods or commodities such as grains are incubated directly on media. It was also agreed that the estimation of fungi in foods by other methods, such as chitin or ATP assays, and consideration of the taxonomy of food spoilage fungi should be presented in review sessions without attempting to reach consensus. The microscopic counting of mold fragments and analysis for mycotoxins in foods were judged to be outside the scope of the workshop.

The First International Workshop on "Methods for Mycological Examination of Foods" was held in Boston on July 11-13, 1984, just two years after it was conceived. Twenty-six people attended from Australia, Denmark, England, Hungary, the Netherlands, Turkey and the United States. Some eighty formal

presentations in ten organized sessions, and seemingly endless formal and informal discussion sessions were held during a very full three-day program.

The aims of the Workshop were in large measure accomplished. Agreement was reached on the standardization of a wide range of methods and media suitable for particular aspects of isolating and enumerating fungi in foods. In this Proceedings are published the edited manuscripts, the general discussion following various sessions and the recommendations approved at the final plenary session. Thus a reasonably complete picture is provided of the proposed standard methods and the data on which they are based.

This workshop was sponsored by grants obtained from the United States National Science Foundation and the Australian Department of Science and Technology under the United States - Australia Cooperative Science Program and from the North Atlantic Treaty Organization as an Advanced Research Workshop. Additional support was received from Difco Laboratories, Detroit, Michigan, Oxoid Ltd., Basingstoke, England and the Pillsbury Company, Minneapolis, Minnesota. We express our gratitude to these sponsors for making the workshop possible.

Special thanks are expressed to Ailsa D. Hocking, Commonwealth Scientific and Industrial Research Organization, North Ryde, New South Wales and Donald E. Conner, University of Georgia, Experiment, Georgia, for their assistance in arranging the workshop and recording its proceedings.

A. D. King, Jr.
J. I. Pitt
L. R. Beuchat
J.E. L. Corry

CONTENTS

CHAPTER 1

SAMPLE PREPARATION

Sample preparation is the first step in the mycological analysis of foods. It is obvious that sample preparation needs to be standardized if comparable results are to be obtained in different laboratories.

Responses to questionnaires returned by participants prior to the workshop indicated that a variety of sample preparation techniques were in use. For instance, about equal numbers of laboratories used stomaching or blending, spread or pour plating, and incubating plates upright rather than inverted. A wide variety of diluents were used for dilution plating and at least nine different techniques were reported for surface disinfection in preparation for direct plating of food pieces or grains. Responses from most people indicated that they used plastic Petri dishes and incubation temperatures of about 25°C. Their replies thus resulted in the assignment of a series of experiments that were reported at the workshop and are described in this chapter.

Equally important but not considered here is sampling, i.e., the selection of samples for analysis. The organizers believe that this subject is adequately handled by the ICMSF publication "Microorganisms in Foods. 2. Sampling for Microbiological Analysis: Principles and Specific Applications" (University of Toronto Press 1974). In consequence, sampling was not addressed at this workshop.

Effect of Sample Size on Variance of Mold and Yeast Counts from Hard Cheese and Skim Milk Powder

Because of intrinsic variability in the distribution of microbes in foods, results of enumeration procedures cannot be representative unless the sample size examined is as large as may conveniently be handled (Kilsby & Pugh 1981). The relevance of this observation to enumeration of molds was demonstrated by Jarvis et al. (1983) for cereal products. This investigation extends our observations to dairy products (cheese and skim milk powder).

Materials and methods

Sample. Sufficient quantities of cheese and skim milk powder were used to provide replicate 10- and 50-g subsamples of cheese and 2- and 10-g subsamples of milk powder.

Media. DRBC and DG18 media were supplied by Oxoid and prepared according

to instructions. The glycerol added to DG18 medium was BDH Analar grade.

Diluent. The diluent used was 0.1% peptone (Oxoid L37) in distilled water.

Sample size. For hard cheese, 10-g and 50-g samples were examined in duplicate. For skim milk powder, 2-g and 10-g samples were examined in duplicate.

Examination of samples. Samples were blended in a Stomacher using 10 volumes of diluent. Appropriate dilutions (0.1 ml) were spread-plated in triplicate. Plates were incubated upright at 25°C for 5 days and mold and yeast colonies were counted.

Statistical analysis. After logarithmic transformation, the mean colony count ($\bar{x}$) and variance (s^2) were derived by normal methods. Estimates of log average counts (α) were derived using the equation, $\log \alpha = \bar{x} + \ln s^2/2$ where ($\bar{x}$) = estimate of the true mean log count (μ) and s^2 = estimate of the log population variance (s^2) as described by Kilsby & Pugh (1981).

Results and discussion

The data are summarized in Tables 1 and 2. Previous experience (Kilsby & Pugh 1981; Jarvis et al. 1983) suggested that as sample size was increased the mean log propagule (or colony) count would also increase and the variance would decrease. This phenomenon was observed also in three of the four comparisons made in the present investigation but not for the fourth investigation (mold propagule count on milk powder). The reason for this discrepancy cannot be identified specifically but could well have arisen through chance effects.

Comparison of the mean log propagule or colony counts with the derived log average counts (log α) indicates a closer comparability for the derived yeast counts than for mold counts. A similar situation was observed in previous studies. The data in Table 3, derived from the data of Jarvis et al. (1983), show similar variability in derived log average counts. This may indicate that the primary distribution of fungal propagules follows an even more disparate system than do bacterial or yeast colony counts.

Table 1. Effect of sample size on mean log and log average propagule count of molds

Sample	Sample size (g)	No. of tests (n)	Mean $\log_{10}$ count ($\bar{x}$) and (range)	Variance (s^2)	$\log_{10}$ ave. count (log α)
Cheese	10	11	5.81 (5.00-6.27)	0.413	5.98
	50	12	6.05 (5.27-6.63)	0.126	6.20
Milk powder	2	11	3.52 (3.14-3.71)	0.028	3.55
	10	11	3.20 (2.69-3.65)	0.158	3.38

Table 2. Effect of sample size on mean log and log average propagule count of yeasts

Sample	Sample size (g)	No. of tests (n)	Mean $\log_{10}$ counts ($\bar{x}$) and (range)	Variance (s^2)	$\log_{10}$ ave. count (log α)
Cheese	10	8	5.96 (5.47–6.53)	0.113	6.09
	50	12	6.06 (5.77–6.64)	0.075	6.15
Milk powder	2	11	3.16 (2.0–4.12)	0.740	4.01
	10	7	3.55 (2.0–4.04)	0.520	4.15

Table 3. Effect of varying sample size on propagule counts in a dry cereal product (Data of Jarvis et al. 1983)

Sample size (g)	No. of replicates (n)	Mean log cfu/g ($\bar{x}$)	Variance (s^2)	Log average count (log α)
2	6	3.74	1.96	6.00
50	12	4.16	0.14	4.33
250	12	4.53	0.14	4.70

Conclusions

Increasing sample sizes generally gives a higher degree of reproducibility and a lower variance than can be achieved with small samples. However, the constant "derived log average count" concept of Kilsby & Pugh (1981) which can be applied in control analyses for bacteria and yeasts appears not to be applicable to mold propagules.

References

JARVIS, B., SEILER, D. A. L. OULD, ANGELA, J. L. & WILLIAMS, A. P. 1983 Observations on the enumeration of moulds in food and feeding stuffs. Journal of Applied Bacteriology 55, 325–336.

KILSBY, D. C. & PUGH, M. E. 1981 The relevance of the distribution of micro-organisms within batches of food to the control of microbiological hazards from foods. Journal of Applied Bacteriology 51, 345–354.

B. JARVIS
N. SHAPTON

Evaluation of Blending, Stomaching and Shaking for the Mycological Analysis of Dried Foods

Homogenization by blending is a common method of preparing food samples for microbiological analysis, especially for those laboratories following reference methods such as the U. S. Food and Drug Administration Bacteriological Analytical Manual (1980). The limitations of blending include production of aerosols, purchase and maintenance of blender jars, and decreased recovery of microorganisms due to rising temperatures during blending (Emswiler et al. 1977).

The Colworth Stomacher was developed as an alternative sample homogenizer. Tuttlebee (1975) described the mode of action of the instrument and the benefits derived from "stomaching" samples. Comparative studies on the recovery of bacteria from stomached and blended samples have been published (Tuttlebee 1975; Emswiler et al. 1977). Andrews et al. (1978) evaluated the use of the Stomacher for microbiologial analysis of thirty categories of food. They concluded that the efficiency of the Stomacher is food-specific.

Many of the previous comparative studies evaluated the effects of these homogenization methods on aerobic plate counts (APC) and recovery of specific bacteria. In this study, the effects of three sample preparation methods on enumeration of fungi from dried foods was determined.

Materials and methods

Experimental design. The experiment was planned using the randomized complete block design (RCBD, Dowdy & Wearden 1983) shown in Table 1. This design permitted simultaneous yet independent evaluations of the effects of sample preparation methods and diluents on enumeration of fungi from dried foods. The results of the test on diluents will be reported later in this chapter.

Cultures. The molds used in this study were Aspergillus flavus, A. ochraceus, another Aspergillus sp. and two Penicillium spp. Five Petri dishes of PDA (Difco) were surface-inoculated with each mold. The cultures were incubated at 25°C until heavy sporulation occurred.

Table 1. Randomized complete block design to evaluate the effects of sample preparation methods and diluents on enumeration of fungi from dried foods

Design component	Effect tested	Description
Treatments	Sample preparation methods	Blending Stomaching Shaking
Blocks	Diluents	0.1% peptone Phosphate-buffered water
Replicate observations	Sampling error	3 subsamples per treatment x block evaluation 3 plates at countable dilution per subsample