Macroanalytical Procedures Manual (MPM)

I. Introduction

September 2023

Author: Alan R. Olsen (Retired)
Editor: Monica Pava-Ripoll, Ph.D.
Co-editor: Ibrahim M. Ali, Ph.D.

Table of Contents

Editors' Preface

The Macroanalytical Procedures Manual (MPM) is revised as needed to update its content and incorporate new information. The MPM is updated cooperatively between FDA's Office of Regulatory Affairs (ORA) and the Center for Food Safety and Applied Nutrition (CFSAN).

Current editors would like to acknowledge all valuable contributions to the manual provided by previous editors:

- Content Editor: Alan R. Olsen, Microanalytical Entomology Specialist (Retired)
- Associate and Web Editor: George C. Ziobro, Ph.D., Research Chemist (Retired)
- Technical Editors:
    - Sherry A. Knight, Entomology Coordinator (Retired)
    - Richard Haynos, Entomology (Retired)

Additionally, we deeply appreciate and herein acknowledge the guidance of former Microanalytical Branch member, John C. Gecan (Retired).

We welcome feedback on the manual and its revisions. Please send feedbacks to the MPM Council at MPMCouncil@fda.hhs.gov.

I. Introduction

(1) Definition of Macroscopic Methods of Analysis

To consumers, "macroscopic" analysis of a product refers to an evaluation of the substance using the unaided senses (sight, smell, taste, or touch) of an individual. Every consumer in our society who exercises some judgment in the purchase of foods, cosmetics, and other goods, knowingly or unknowingly conducts some form of macroscopic examination to detect apparent or obvious defects. In the case of foods, this usually occurs upon purchase or utilization of the product. The examination could be as simple as a quick, perhaps subconscious, look at the product to make sure everything “looks good”, or as complex as a more in-depth inspection to look for specific defects. One of the most common examples of a consumer's macroscopic inspection is the careful shopper who squeezes and smells the produce before buying it.

To fulfill responsibilities for protecting the public health by ensuring the safety of our nation’s food supply and cosmetics, the U.S. Food and Drug Administration (U.S. FDA) conducts more systematic examinations to disclose not only apparent defects but also hidden defects. Over the years, standardized methods of macroscopic examination have evolved for determining filth, decomposition, and foreign matter in foods, drugs, and cosmetics and other products subject to the laws enforced by the U.S. FDA. These methods of analysis have been developed over time with the input from manufacturers, consumers, and regulatory authorities.

The objective of this manual is to compile and organize the standardized methods of macroscopic analysis which are useful in determining defects in various types of foods. Although in a general sense, the term "macroscopic" is not as broad as the term "macroanalytical," for the purposes of this manual, the terms are used interchangeably.

(2) Advantages and Limitations of Macroanalytical Methods

In general, macroscopic or macroanalytical methods for examination of food largely rely on the direct sensory information provided by the analyst as the primary means of detection and identification of defects. Although visual examinations are typically conducted with the naked eye, supplementary low power magnification (for example, up to 10x) is often used to confirm defects or describe them in greater detail.

There are several major advantages to the use of macroanalytical procedures. They are inexpensive and require little specialized equipment. They generally permit the analysis of a large quantity of product in a relatively short period of time; thus, allowing the analyst to assess the overall condition of the lot quite rapidly. The analyst can quickly identify and isolate those portions of the lot which may contain defects, limiting the amount of material which may need a more detailed microscopic evaluation.

Although macroscopic methods have many positive aspects, they may not be the method of choice for every analytical situation. In fact, the very features which add to their usefulness may also limit their application in some situations. Macroscopic procedures are suitable for defects that are visible to the naked eye. However, they are inadequate for defects that are concealed from the naked eye, such as those that are too small to detect, or those that are hidden due to processing or other factors. In these cases, microscopic techniques are necessary for the characterization and assessment of the contaminants or defects present in the sample.

Microscopic methods of analysis involve the detailed examination of a portion of the sample and provide a different type of information than macroscopic methods. They are used to describe and quantify defects on a different scale than macroscopic methods, and can identify "hidden" defects undetectable by gross evaluation of the sample. Microscopic methods, however, have their drawbacks; they are time-consuming, costly, and require specialized equipment.

It is apparent that macroscopic and microscopic methods of analyses for characterizing defects in foods tend to supplement each other, and together provide a comprehensive evaluation of defects in the product. It is important that the analyst realize the close association of the macroscopic and microscopic methods for use as a joint approach in solving analytical problems.

(3) Scope of the Macroanalytical Procedures Manual

This manual compile standardized macroanalytical procedures for identifying defects in food products. However, as discussed in section (2) above, macroscopic procedures are frequently interrelated with and supplemented by microscopic methods of analyses. Each provide a different type of information.; thus, the Macroanalytical Procedures Manual (MPM) will refer to microscopic methods in some situations. These microscopic methods may be grouped in two categories:

- Microanalytical methods published by the Association of Official Analytical Collaboration (AOAC) International (Chapter 16: Extraneous Materials). Where required, MPM sections simply refer to the applicable AOAC official method of analysis.
- Microanalytical methods published by the AOAC, but which may need to be modified for a particular situation. In these cases, special instructions are provided in this manual so that the microanalytical method can be modified as necessary. Reference is made to the appropriate section of the AOAC official method of analysis. Available guidance for method modification must be followed.

Thus, when using this manual, the analyst may be instructed to combine both macroscopic and microscopic methods of analysis. Information provided by microscopic methods will aid in interpreting and evaluating macroscopic findings and in determining the overall quality of the food.

(4) Legal Aspects

The methods described in this manual are part of FDA's regulatory program to ensure that food and other commodities are safe for human consumption. This regulatory program derives in part from Sections 402(a)(3) and 402(a)(4) of the Federal Food, Drug, and Cosmetic Act (FD&C Act) which deal with adulteration of food (U.S. Congress, 1934). Subchapter III of the same act prohibits the manufacture, sale, and distribution of adulterated foods. Pertinent rules, regulations, guidelines, advisory opinions, and other notices issued under the statutory requirements of the FD&C Act provide further details on implementation and enforcement of these sections. The "Applicable Documents" section for each method refers to appropriate information for the food product(s) covered by that method.

a. **Adulteration of Food**. Many of the defects in food and other commodities which are addressed by macroscopic methods are the result of attack by pests such as rodents, insects, birds, etc. These attacks encompass practically any living stage of animal or plant life which can directly or indirectly injure, cause disease, or result in damage to food or other material. Defilement of a food by pests or contamination by other sources of extraneous matter may render the food adulterated under section 402(a)(3) of the FD&C Act (21 CFR §342 (a)(3)). That section states that a food shall be deemed to be adulterated "if it consists in whole or in part of any filthy, putrid or decomposed substance, or if it is otherwise unfit for food." Thus, the presence of macroscopic defects such as insect-damage, mold, animal-contamination, rancidity, and dirty material may comprise sufficient grounds to consider the food adulterated. Moreover, under Section 402(a)(4) of the FD&C Act (21 CFR §342 (a)(4)), food shall be deemed adulterated "if it has been prepared, packed, or held under insanitary conditions whereby it may have become contaminated with filth, or whereby it may have been rendered injurious to health." Hence, to be adulterated, food need not be shown to contain filth or other contaminants; a demonstration that the food was prepared, packed, or held under conditions whereby it would, with reasonable

possibility, become contaminated is legally sufficient to prove adulteration and provide grounds for taking regulatory action against the food. Macroscopic examination of facility samples or exhibits collected during facility investigations/inspections may provide such evidence.

b. **Defect Action Levels**. Defect action level means a level of a non-hazardous, naturally occurring, unavoidable defect at which FDA may regard a food product "adulterated" and subject to enforcement action under section 402(a)(3) of the FD&C Act. To be equitably administered, each Defect Action Level must be coupled to an acceptable standardized method of analysis. This manual provides descriptions of such methods.

   The FDA Food Defect Levels Handbook (21 CFR 117.110) covers many of the products contained in this manual, and includes approximately 200 action levels for various types of defects in about 110 food products. These levels have been established in recognition that it is not now possible, and never has been possible, to grow, harvest, and process crops that are totally free of natural defects. Accordingly, through the years, the FDA has established levels for natural or unavoidable defects in certain foods consistent with the technological capabilities of the affected industry and with acceptable standards of safety and security.

   It should be noted that 21 CFR 117.110(a) clearly states that "The manufacturer, processor, packer, and holder of food must at all times utilize quality control operations that reduce natural or unavoidable defects to the lowest level currently feasible."

(5) Determination of Defects

Each of the methods contained in Chapter V: Macroanalytical Methods describes procedures to be followed in the examination of a particular food or commodity. Based on the results of this examination, further determination is made as to whether or not the levels represent limits at which FDA will regard the food product to be adulterated using the Defect Action Levels established by the FDA. Particular defects which are likely to result in a determination that a given lot or shipment is considered adulterated are described in Chapter V: Macroanalytical Methods' as a subsection of the method for the specific food product. This part of the introduction presents a discussion of two points which bear general relevance to the determination of defects by using macroanalytical methods.

- Sources of defects (field vs. storage)
- Discrimination between signs and symptoms of defects

For a more extensive discussion of defects in food, refer to Eisenberg (1981).

a. **Sources of Defects (Field vs. Storage)**. Insects, molds, birds, and rodents are the principal causative agents for most of the defects covered by macroanalytical methods. In general, these sources of defects may be classified as either "field"

or "storage" pests. In the field or orchard, a commodity crop is more likely to be susceptible to different pest attacks than it is after harvest or during storage. During its movement from farm to the processor and through distribution channels, environmental conditions surrounding the commodity change significantly. Because of the different habitats, different species of organisms challenge the product's integrity.

It is important to distinguish between these two types of defects, when possible. For example, the Method for Dried Fruits (Chapter V, Section 9.F: Method for Dried Fruits) specifies that field and storage insect infestation be reported separately. In many cases, these terms distinguish between pre- and post-harvest defects. Many of the defect action levels involve pre-harvest damage to crops from insects, fungi (molds), field rodents, birds, and other pests that are not completely avoidable under good agricultural practices. Processing of the commodity provides an opportunity to eliminate or control the extent of these defects through inspection, sorting, cleaning, and other steps to ensure production of an acceptable product. Because of this, field or orchard defects are different from defects which occur during processing under the close surveillance of the manufacturing establishment. Raw materials, other ingredients, and rework susceptible to contamination with pests, undesirable microorganisms, or extraneous material must comply with applicable FDA regulations for natural or unavoidable defects if a manufacturer wishes to use the materials in manufacturing foods. Likewise, raw materials, other ingredients and rework must be stored under conditions that will prevent spoilage and protect against contamination and minimize deterioration (21 CFR 117.80).

The distinction between field and storage defects, however, is not always sharply defined. In some instances, the same species of organism that attack the product in the field may continue attacking the food after harvesting or during storage. This becomes more evident when some practices involve leaving the crop in the field or the orchard for further drying and/or holding (for example, the sun-drying of fruits). Thus, it is susceptible to the same pests since conditions have not changed.

Macroanalytical methods of analysis therefore should include an identification of the sources of defects to the maximum extent possible. Such information is useful not only in evaluating the acceptability of the product for consumption but also in assessing responsibility for identifying weaknesses in quality control or preventive sanitation programs.

b. **Signs and Symptoms of Defects**. It may be convenient to understand the defects in foods covered in this Manual as comprising a combination of "signs" and "symptoms." "Signs" refer to the direct causal agents of the defect while "symptoms" are the adverse effects observable in the product material. For example, the sign could be the presence of the specific causal factor, such as a species of fungus, bacteria, virus, insect, rodent, bird, nematode, or other pests.

The symptom or observable adverse effect in the product may take various forms, such as different degrees and types of decomposition, tissue breakdown, lesions, or other abnormal conditions. In some instances, the symptom per se can conclusively identify the causal factor. For example, the circular, light brown, decayed lesions of "brown rot" on peaches are unique to *Monilinia fructicola* (Winter) Honey (Figure I-1).

Figure I-1. Peach showing brown rot, *Monilinia fructicola* (Winter) Honey. (Source: Photo Courtesy of Rebecca A. Melanson, Mississippi State University Extension, Bugwood.org)

In this case, the sign of fungus (or mold) is also present. It can be detected macroscopically and confirmed microscopically, if necessary. If the fruit is pulped, however, the symptoms may be completely masked, leaving only the microscopic sign as evidence of its presence. The MPM Method for Coffee Beans (Chapter V, Section 1.A) illustrates vividly the signs and symptoms associated with the two species of insects that commonly attack coffee beans in the field.
Sensitivity to sources of various defects as well as an understanding of the distinction between signs and symptoms of defects provide a basis for evaluating important clues. Accurate evaluation of these clues in turn improves the significance of analytical findings.

(6) Sampling Methods

The ultimate responsibility of the analyst is to provide analytical results that will eventually determine the acceptability or unacceptability of a given lot or shipment of food material based upon his or her examination. For reasons of time and cost, it is clearly impractical to examine every item in a shipment or lot. Thus, the analysis should focus on a detailed examination of material from a sample collected from the lot. Since decisions about regulatory action are therefore necessarily made by extrapolation, it is important that sampling techniques used are consistent with statistical theory.

At least two sampling techniques, representative and selective sampling, are used in conjunction with the methods in Chapter V: Macroanalytical Methods. These are discussed below.

a. **Representative Sampling**. Representative sampling or quota sampling is an objective sampling technique used when the sample of the material has been selected to maximize the probability that it contains the same proportion of defects as the entire lot, providing objective, reliable data to decide about the condition of the entire lot. To assure this, a representative sample must always be drawn by using random selection or random sampling. Random selection or sampling is sampling from a population such that each element in the population has an equal probability of being selected for inclusion in the sample. The concept of random "blind" sampling is to yield information about the average composition of the lot.

   Another important element in representative sampling is size. The larger the sample size, the higher the probability that the representative sample contains the same proportion of defects as the entire lot. If perfect certainty were required, clearly the entire lot should be sampled. Statistical theory allows the analyst to work with relatively small representative samples while maintaining quite high levels of certainty that determinations made on the basis of the examination of a sample are accurate reflections of the condition of the entire lot.

   Questions regarding sample size and levels of certainty in the methods provided in this manual have been resolved in advance by agency statisticians applying the concepts of acceptance sampling. A regulatory sample usually consists of a specific number of subsamples. The minimum number of subsamples and sample sizes required for the analysis have been incorporated into the procedures in Chapter V: Macroanalytical Methods. Some procedures usually call for a fixed sample size whereas other procedures allow for the examination of the material in an iterative fashion using a sequential sampling plan. For sequential sampling, the decision rules indicating when to accept, reject, or continue with the analysis are built into the procedure. The sample preparation section of each procedure provides guidance as appropriate. For example, in Chapter V Section 3.A: Method for Wheat, Corn, Popcorn Kernels, and Rice, the procedure for determination of insect-damaged wheat kernels recommends using dividers (rotary splitter, stationary splitter (Jones Divider), or fractional shoveling) to mix and reduce the size of the analytical portion.

b. **Selective Sampling**. Selective sampling or purposive sampling is a subjective sampling technique where materials are drawn to confirm a suspected defect. Unlike representative sampling where material is drawn at random to assess the general condition of a lot, selective sampling is deliberately biased to confirm the presence of suspected defects because representative sampling could result in the dilution of the contaminant to a point below the practical limits of measurement of a macroscopic method. Macroscopic examination of import

samples, on the wharf, factory samples, or exhibits of defiled food material submitted for macroscopic examination are usually drawn by using selective sampling. For example, inspection of a food manufacturing facility may disclose damaged bags of dried beans with characteristic fluorescent rodent urine stains on the surface of the bag. The fluorescent material, often caked, and the adjacent beans are the best sample for use in laboratory verification of the presence of urine or related defects. Another instance where selective sampling would be advisable is when a shipment of bulk food items is in the cargo hold of a ship that is believed to have previously contained a toxic ore; the portion of the shipment taken from the lower part of the cargo hold would be the appropriate sample. Similarly, in the case of cocoa beans in a consignment, any bags that have been affected by water are more susceptible to mold attack than sound bags. Thus, a sample selected from the wet bags is the appropriate material to be sent to the laboratory for examination to confirm adulteration.

Because sampling is a practical necessity, an understanding of sampling techniques is important to the methods described in this manual. The two sampling techniques discussed here, representative and selective sampling, each play a significant part in application of these macroanalytical procedures to determine whether a lot meets regulatory standards. More information regarding sampling can be found in the Investigations Operations Manual (IOM).

(7) General Comments

a. **Scientific names**: Occasionally, scientific names of organisms change because of nomenclature research, or other reasons. The latest updates on nomenclature for scientific name changes may not be included in the current version of this manual.

b. **Method Citation**: The method used to analyze the sample should be cited using the chapter number and corresponding title for method/procedure instead of the page number from the printed 1984 edition. For example, when analyzing a sample of rice, cite the method(s) as follow:

> MPM V3A (4): Procedure: Determination of Extraneous Materials in Wheat, Corn, Popcorn Kernels, and Rice.
>
> MPM V3A (9): Procedure: Determination of Insect-Damaged Rice.
>
> MPM V3A (10): Procedure: Determination of Decomposition in Rice Due to Molds and Other Factors.

References Cited in Section

Eisenberg, W. V. (1981). Sources of Food Contaminants. In Gorham, J.R. (Ed.) *FDA Technical Bulletin No. 1: Principles of Food Analysis for Filth, Decomposition, and Foreign Matter* (pp 11-25). Washington, D.C.: U.S. Government Printing Office.

U.S. Congress. (1934) United States Code: Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 301-392 Suppl. 5. [Periodical] Retrieved from the Library of Congress, https://www.loc.gov/item/uscode1934-006021009/.

Additional Information

Olsen, A. R., Sidebottom, T. H., & Knight, S. A. (Eds.). (1996). *Fundamentals of Microanalytical Entomology: A Practical Guide to Detecting and Identifying Filth in Foods*. CRC Press.

Sharma, G. (2017). *Pros and cons of different sampling techniques*. International Journal of Applied Research. 3 (7) 749-752.

Taherdoost, H. (2016). *Sampling methods in research methodology; How to choose a sampling technique for research*. International Journal of Academic Research in Management. 5 (2) 18-27.

Revision History

Version No.	Purpose of change	Date
V0	New process	1984
V1	Electronic version	1998
V2	Include 508-compliance guidelines; reflect changes in 21CFR 117.110; added the following sections: 'General Comments', 'References Cited in Section', and 'Additional Information'.	2023