19

chapter

Analysis for Extraneous Matter

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S.S. Nielsen, *Food Analysis*, Food Science Texts Series, DOI 10.1007/978-1-4419-1478-1_19,
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19.1 INTRODUCTION

Analysis for extraneous matter is an important element both in the selection of raw materials for food manufacturing and for monitoring the quality of processed foods. The presence of extraneous material in a food product is unappealing and can pose a serious health hazard to the consumer. It also represents lack of good manufacturing practices and sanitary conditions in production, storage, or distribution. The presence of extraneous materials in the product ingredients may render the final product adulterated and not suitable for human food.

19.1.1 Federal Food, Drug, and Cosmetic Act

The **Federal Food, Drug, and Cosmetic Act** (FD&C Act) of 1938 with Amendments administered and enforced by the US Food and Drug Administration (FDA) (1) defines a food as **adulterated** "if it consists in whole or in part of any filthy, putrid, or decomposed substance, or if it is otherwise unfit for food [Section 402 [21 USC 342] (a)(3)]; or if it has been prepared, packed, or held under unsanitary conditions whereby it may have become contaminated with filth, or whereby it may have been rendered injurious to health" [Section 402 [21 USC 342] (a)(4)]. The filthy, putrid, or decomposed substances referred to in the law include the extraneous matter addressed in this chapter. In addition, extraneous matter includes adulterants that may be encountered in processing systems, such as lubricants, metal particles, or other contaminants (animate or inanimate) that may be introduced into a food intentionally or because of a poorly operated food processing system. These aspects are not covered in this chapter.

19.1.2 Good Manufacturing Practices

The **Current Good Manufacturing Practice in Manufacturing, Packing, or Holding Human Food** (cGMPs) was published in 1969 by the Food and Drug Administration (FDA) (21 CFR Part 110) to provide guidance for compliance with the FD&C Act (2) (see also Chap. 2). That regulation provides guidelines for operating a food processing facility in compliance with Section 402 (a)(4), and these guidelines have not been revised since 1986. Currently, the cGMPs are being amended to make the compliance guidelines more risk-based. Paramount to complying with the FD&C Act and cGMPs is the thorough inspection of raw materials and routine monitoring of food processing operations to ensure protection of the consuming public from harmful or filthy food products.

19.1.3 Defect Action Levels

Most of our foods are made from or consist in part of ingredients that are obtained from plants or animals and are mechanically stored, handled, and transported in large quantities. It would be virtually impossible to keep those materials completely free of various forms of contaminants. In recognition of that, the FDA (3) has established **defect action levels** (DALs) that reflect current maximum levels for natural or unavoidable defects in food for human use that present no health hazard. They reflect the maximum levels that are considered unavoidable under good manufacturing practices and apply mainly to contaminants that are unavoidably carried over from raw agricultural commodities into the food processing system. The manner in which foods are manufactured may lead to their contamination with extraneous materials if strict controls in processing are not maintained. This latter type of contamination leads to food safety issues, and DALs are not used to determine compliance. Other actionable levels of contaminants may be found in the FDA *Compliance Policy Guide* (*CPG*) *Manual* (4).

The most current information of FDA laws and regulations relevant to extraneous matter, including cGMPs, DALs, and CPGs, can be found on the Internet:

Federal Food, Drug, and Cosmetic Act (FD&C Act) – http://www.fda.gov/opacom/laws/fdcact/fdctoc.html

Current Good Manufacturing Practices (cGMPs) – http://www.gmp1st.com/fdreg.htm

Food Defect Action Levels (DALs) – http://www.cfsan.fda.gov/~dms/dalbook.html

Compliance Policy Guidance (CPG) – http://www.fda.gov/ora/compliance_ref/cpg/

19.1.4 Purposes of Analyses

The major purposes for conducting analyses for extraneous matter in foods are to ensure the protection of the consuming public from harmful or filthy food products, to meet regulatory requirements of the FD&C Act Sections 402 (a)(3) and 402 (a)(4), and to comply with DALs.

19.2 GENERAL CONSIDERATIONS

19.2.1 Definition of Terms

Terms used by AOAC International (AOAC Method 970.66) to classify or characterize various types of extraneous materials are defined as follows.

19.2.1.1 Extraneous Materials

Any foreign matter in product associated with objectionable conditions or practices in production, storage, or distribution; included are various classes of filth, decomposed material (decayed tissues due to parasitic or nonparasitic causes), and miscellaneous matter such as sand and soil, glass, rust, or other foreign substances. Bacterial counts are not included.

19.2.1.2 Filth

Any objectionable matter contributed by animal contamination such as rodent, insect, or bird matter, or any other objectionable matter contributed by unsanitary conditions.

19.2.1.3 Heavy Filth

Heavier material separated from products by sedimentation based on different densities of filth, food particles, and immersion liquids. Examples of such filth are sand, soil, insect and rodent excreta pellets and pellet fragments, and some animal excreta pellets.

19.2.1.4 Light Filth

Lighter filth particles that are oleophilic and are separated from product by floating them in an oil–aqueous liquid mixture. Examples are insect fragments, whole insects, rodent hairs and fragments, and feather barbules.

19.2.1.5 Sieved Filth

Filth particles of specific size ranges separated quantitatively from product by use of selected sieve mesh sizes.

19.2.2 Diagnostic Characteristics of Filth

There are certain qualities characteristic to extraneous materials that serve as proof of presence of foreign or objectionable matter in food. Examples include specific diagnostic characteristics of **molds** (i.e., parallel hyphal walls, septation, granular appearance of cell contents, branching of hyphae, blunt ends of hyphal filaments, nonrefracted appearance of hyphae); diagnostic characteristics of **insect fragments** (i.e., recognizable shape, form, or surface sculpture, an articulation or joint, setae or setal pits, sutures), **rodent hairs** (i.e., pigment patterns and structural features), **feather barbules** (i.e., structural features); diagnostic characteristics of **insect-damaged grains** (IDK) and packaging materials; and chemical identification of **animal urine** and **excrement**. These diagnostic characteristics are outlined by AOAC International (formerly Association of Official Analytical Chemists) for positive identification of extraneous matter or filth (5).

The AACC International (formerly American Association of Cereal Chemists) publishes a methods book that includes a section on extraneous matter, containing descriptive material helpful in identifying insect and rodent contaminants (6). Several microscopic and radiographic illustrations are provided by the AACC International as authentic reference materials to help analysts to identify filth. AACC Method 28–95, "Insect, Rodent Hair, and Radiographic Illustrations," provides a series of colored pictures representative of insect fragments commonly found in cereal products and pictorial examples of rodent hair structure.

Kurtz and Harris (7) provide a virtual parts catalog of insect fragments with a series of micrographs. Gentry et al. (8), an updated version of the Kurtz and Harris publication, includes colored micrographs of common insect fragments. Also included in AACC 28–95 are radiographic examples of grain kernels that contain internal insect infestation. AACC Method 28-21A, "X-ray Examination for Internal Insect Infestation," provides an outline of the apparatus and procedure for X-ray examination of internal insect infestation in grain (9).

19.3 OFFICIAL AND APPROVED METHODS

There are various laboratory methods for separating (isolating) extraneous materials from foods and for identifying and enumerating them. The FDA and the AOAC International have published reference articles, books, and methods on analysis of extraneous materials. The most authoritative source, and that generally considered official by the FDA, is the *Official Methods of Analysis of AOAC International*, Chap. 16, "Extraneous Materials: Isolation" (5). This chapter includes methods for extraneous matter isolation in various food categories (Table 19-1). The AOAC International "Extraneous Materials: Isolation" chapter contains a subchapter dealing with **molds**. This includes identification of molds and methods for isolation of molds in fruits and fruit products and vegetables and vegetable products.

The AACC International (6) has established methods for isolating and identifying extraneous matter in cereal grains and their products (AACC Method 28-00, listed in Table 19-2). In most instances, the AACC methods are based on FDA or AOAC methods, but the format is slightly different. The AACC presents each procedure in an outline form that includes the scope, apparatus, and reagents required and the procedure in itemized steps while the AOAC uses a narrative paragraph form (Table 19-2).

19-1 table Official Methods of AOAC International for Analysis of Extraneous Materials

Section	Title
16	Extraneous materials: isolation
16.1	General
16.2	Beverages and beverage materials
16.3	Dairy products
16.4	Nuts and nut products
16.5	Grains and their products
16.6	Baked goods
16.7	Breakfast cereals
16.8	Eggs and egg products
16.9	Poultry, meat, and fish and other marine products
16.10	Fruits and fruit products
16.11	Snack food products
16.12	Sugars and sugar products
16.13	Vegetables and vegetable products
16.14	Spices and other condiments
16.15	Miscellaneous
16.16	Animal excretions
16.17	Mold
16.18	Fruits and fruit products
16.19	Vegetables and vegetable products

A valuable resource on analysis for extraneous matter is *Principles of Food Analysis for Filth, Decomposition and Foreign Matter*, FDA Technical Bulletin No. 1 (10). The FDA *Training Manual for Analytical Entomology in the Food Industry* (11) is prepared to facilitate the orientation of food analysts to the basic techniques they will need for filth analysis. A recent, more advanced resource is *Fundamentals of Microanalytical Entomology: A Practical Guide to Detecting and Identifying Filth in Foods* (12). Most chapter authors of this resource are, or have been, FDA personnel "involved in the forensic aspect of piecing together the etiological puzzles of how insect filth gets into processed food products" (12). The authors share their experience gained in gathering and developing evidence used to document violations of the law that the FDA is mandated to enforce.

19.4 BASIC ANALYSIS

Various methods for isolation of extraneous matter are suggested in Sect. 19.3, which define different types of filth: separation on the basis of differences in **density, affinity for oleophilic solvents, particle size; diagnostic characteristics** for identification of filth; and **chemical identification** of contaminants. Since all methods of analysis for extraneous matter for all categories of food cannot be discussed in this chapter, only the underlying principles of the methods are summarized below. Readers may need to refer to the specific AOAC methods cited for detailed instructions of the procedures.

19-2 table Approved Methods of the AACC International for Analysis of Extraneous Materials

Number	Title
28	Extraneous matter
28-01.01	Apparatus or materials for extraneous matter methods
28-02.01	Reagents for extraneous matter methods
28-03.02	Special techniques for extraneous matter methods
28-06.01	Cinder and sand particles in farina – counting method
28-07.01	Cinder and sand particles in farina – gravimetric method
28-10.02	Macroscopic examination of external contamination in whole grains
28-19.01	External filth and internal insect infestation in whole corn
28-20.02	Microscopic examination of external contamination in whole grains
28-21.02	X-ray examination for internal insect infestation
28-22.02	Cracking-flotation test for internal insects in whole grains
28-30.02	Macroscopic examination of materials hard to hydrate
28-31.02	Pancreatin sieving method, for insect and rodent filth in materials hard to hydrate
28-32.02	Sieving method, for materials hard to hydrate
28-33.02	Pancreatin nonsieving method for insect and rodent filth in materials easy to hydrate
28-40.01	Acid hydrolysis method for insect fragments and rodent hairs – wheat–soy blend
28-41.03	Acid hydrolysis method for extracting insect fragments and rodent hairs – light filth in white flour
28-43.01	Glass plate method, for insect excreta
28-44.01	Iodine method, for insect eggs in flour
28-50.01	Decantation method, for rodent excreta
28-51.02	Flotation method, for insect and rodent filth
28-60.02	Tween-versene method, for insect fragments and rodent hairs in rye flour
28-70.01	Defatting-digestion method, for insect fragments and rodent hairs
28-75.02	Sieving method, for light filth in starch
28-80.01	Flotation method, for insect and rodent filth in popped popcorn
28-85.01	Ultraviolet light examination, for rodent urine
28-86.01	Xanthydrol test, for urea
28-87.01	Urease-bromthymol blue test paper, for urea
28-93.01	Direction of insect penetration into food packaging
28-95.01	Insect, rodent hair, and radiographic illustrations

The AOAC and the AACC methods for analysis of extraneous materials involve the use of one or more of the following basic methods: filtration, sieving, wet sieving, gravimetry, sedimentation/flotation, cracking flotation, heat, acid or enzyme digestion, macroscopic and microscopic methods, and mold counts.

19-1 figure Fisher USA Standard test sieve. Range of mesh sizes is available for various particle size separations.

19.4.1 Sieving Method

Separation is based on difference in particle size of product and contaminant using **standard test sieves** (Fig. 19-1). For instance, the insects are (larger) separated from spices (smaller) using a 20 mesh sieve, and wheat grains (larger) are separated from insects (smaller) using a 10 or 12 mesh sieve. Then the contaminant is identified using a **widefield stereomicroscope**.

19.4.2 Sedimentation Method

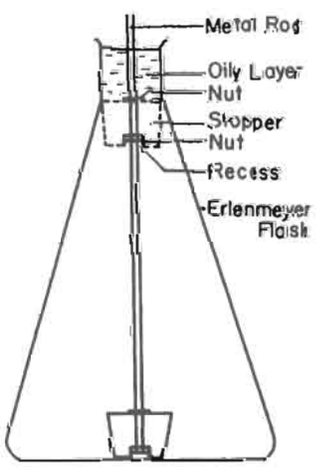
Separation is based on different densities of product, contaminant, and immersion fluid. Specific gravity of immersion solution (carbon tetrachloride/chloroform) allows heavier shell, sand, glass, metal, or excreta contaminants to settle; less dense product floats. Apparent lower **specific gravity** of internally infested wheat kernels, for instance, allows them to float while sound wheat kernels settle in 1.19 specific gravity solution. Contaminants are then identified using a microscope.

Analysis of high-fat-containing samples such as nuts requires defatting using petroleum ether prior to filth analysis (AOAC Method 968.33A). The chloroform and chloroform:carbon tetrachloride solvents allow pieces of shell, sand, and soil to settle at the bottom of the beaker on the basis of specific gravity and cause the defatted nut meats to float and be decanted. Essentially the same procedure is suggested to isolate pieces of rodent excreta from corn grits, rye and wheat meal, whole wheat flour, farina, and semolina in AOAC Method 941.16A. It should be noted that the use of the more toxic solvents such as carbon tetrachloride, chloroform, and petroleum ether is avoided in most contemporary analytical methods.

19.4.3 Flotation Methods

Flotation methods are designed to isolate microscopic filth by floating the filth upwards, typically in an **oil/water-phased system**. Insect fragments, mites, and hairs are lipophilic and likely to be in the oil phase, thus they float to the surface with the oils. Plant tissues and most related tissues are hydrophilic, and they tend to stay in the water phase. Therefore, separation is based on the principle of affinity for oleophilic solvents. Gravity further helps this process, and larger particles sink. To accomplish the separation of filth from food, a number of solution systems are used to ensure that the majority of the product sinks, while the oils with trapped filth float. The oil phase is trapped off with a **Wildman trap flask** (Fig. 19-2), filtered, collected on a filter paper, and examined microscopically to determine the amount and kinds of filth present (13).

Flotation is a common method used to determine insect fragments, rodent hairs, and other forms of light filth in wheat flour (AOAC Method 972.32). The acid digestion is used to break down the starch in the flour and allows the other flour constituents to more cleanly separate from the dilute acid solution. Although the AOAC method calls for digestion by autoclaving, AACC Method 28–41B provides for an alternative hotplate digestion, which might be more convenient for some laboratories. The oleophilic property of insect fragments, rodent hairs, and feather barbules allows them to be coated by the mineral oil and trapped in the oil layer for separation and collection on ruled filter paper. The heavier sediments of the digestion are washed and drained from the funnel. Fragments and rodent hairs are reported on the basis of 50 g of flour.



19-2 figure Wildman trap flask. Stopper on shaft is lifted up to neck of flask to trap off floating layer. [Adapted from (5), AOAC Method 945.75, Extraneous Materials in Products.]

19.4.3.1 Cracking Flotation Method

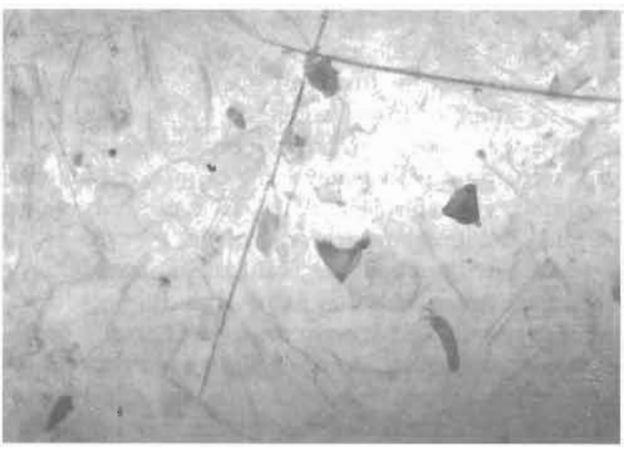
Internal infesting insects (such as in grains) can be determined using an oleophilic method. First any external insects are removed by sieving. Grain sample is coarsely cracked to free insects from kernels. Cracked grain sample is digested in 3–5% HCl solution and sieved with water to remove hydrolyzed starch and acid. The sample is transferred to a Wildman trap flask and boiled in 40% alcohol solution to deaerate. Tween 80 (polyoxyethylene sorbitan monooleate) and Na_4EDTA (tetrasodium salt of ethylenediaminetetraacetic acid) solutions are added to cause light bran particles to remain in solution during oil extraction of insect material. Light mineral oil is added to the solution to form a floating layer in which insect material is attracted due to its oleophilic nature. The oil layer is filtered through a ruled paper to collect the contaminating insects. The filter paper is examined microscopically.

19.4.3.2 Light Filth Floatation Method

Oleophilic filth is defined as light filth. Examples of light filth include insects, insect fragments, hairs, and feather barbules, which can be detected in a food product by separating them from the food in the oil phase of an oil/aqueous mixture. The analysis of light filth is accomplished through a series of steps, starting with a pretreatment that removes fats, oils, soluble solids, and fine particulate matter to enhance the wettability of the food. The second step requires mixing the food with a water and oil mixture. The food will remain in the aqueous phase and the light filth will rise to the top with the oil phase. In the third step, the extract with filth elements is poured onto a ruled filter paper using a filter flask and funnel (Fig. 19-3) and examined line by line under a stereomicroscope (Fig. 19-4). After identification and enumeration, the results are reported to provide the following information: (a) whole or equivalent insects (adults, pupae, maggots, larvae, cast skins), (b) insect fragments, identified, (c) insect fragments, unidentified, (d) aphids, scale insects, mites, spiders, etc. and their fragments, (e) rodent hairs (state the length of the hairs).

Insect fragments, rodent hairs, and other light filth can be isolated from flour samples by an acid hydrolysis method. Sediment products of the digestion are allowed to settle out in a separatory funnel and are drained away. The remaining oil layer is filtered through a ruled filter paper and the contaminants identified microscopically. However, certain grain products such as whole wheat flour contain amounts of bran particles that may result in excessive amounts of material being trapped in the oil layer, making it difficult to identify particles of filth.

19-3 figure Filter flask and funnel. Funnel has a collar (partially raised) that holds ruled filter paper in place on the funnel base for trapping filth for examination. Suction is applied with a water aspirator. (http://www.whatman.com)



19-4 figure Microscopic view of insect fragments and rodent hairs on a filter paper.

The "Tween-Versene Method for Insect Fragments and Rodent Hairs in Rye Flour" (AACC Method 28–60A) utilizes two chemical agents that tend to suppress bran accumulation in the heptane recovery layer. Tween 80 (polyoxyethylene sorbitan monooleate) is a nonionic agent that appears to have certain surface active properties that make it a useful adjunct to Na_4EDTA (tetrasodium salt of ethylenediaminetetraacetic acid). In the presence of Tween 80, Na_4EDTA appears to be

a depressor for food materials (such as bran and other light plant matter), which otherwise tend to float. It has been suggested that the chelating properties of Na_4EDTA may result in its adsorption onto the surfaces of food particles along with the surfactant Tween 80, thereby preventing an attraction of food particles to oils used to isolate light filth. By preventing plant material from being collected in the heptane layer that is trapped off, contaminants such as oleophilic insect parts (exoskeleton) that are contained in the separating oil are much easier to distinguish and identify. AACC Method 28–95 provides a description of insect fragments and rodent hair characteristics with illustrations (6).

19.4.4 Objectivity/Subjectivity of Methods

Insect parts, rodent hairs, and feather barbules in food products are generally reported as the total number of filth elements counted of each kind encountered per sample unit. They are identified on the basis of objective criteria. However, identifying insect fragments is not a simple task. Training and supervised practice are required to achieve competence and consistency. Some fragments are easily identified on the basis of structural shape and form. Mandibles, for example, are quite distinctive in their shape and configuration; certain species of insects can be determined on the basis of this one structure. In other instances, fragments may be mere chips of insect cuticle that have neither distinctive shape nor form but can be identified as being of insect origin if they have one or more of the characteristics given in Sect. 19.2.2. Experienced analysts should rarely misinterpret fragments.

Isolation of extraneous material from a food product so that it can be identified and enumerated can be a very simple procedure or one that requires a series of several rather involved steps. In the process of isolating fragments from flour by the acid hydrolysis method, for instance, the sample is transferred from the digestion container to the separatory container and then to the filter paper for identification and enumeration. At each of those transfers there is an opportunity for loss of fragments. Although the analyst may have made every effort to maintain the isolation "quantitative," there are opportunities for error. Both fragment loss and analyst variation are minimized by common use of standard methods and procedures and by proper training and supervised practice.

Another concern involves the significance of **insect fragment counts** (as well as particles of sand, pieces of rodent excreta, rodent hairs, etc.) in relation to **fragment or particle size**. Fragment counts are reported on a numerical basis; they do not reflect the total contaminant biomass that is present. A small fragment is counted the same as a large fragment. The size of the fragment may be a reflection of the process to which a common raw material (e.g., wheat) has been subjected; a more vigorous process produces more and smaller fragments than a less vigorous process. The state of insects may also be a factor. Dead (dried) forms produce greater numbers of fragments than live forms. These factors have been of concern to food processors for some time and have prompted the search for more objective means of determining insect contamination.

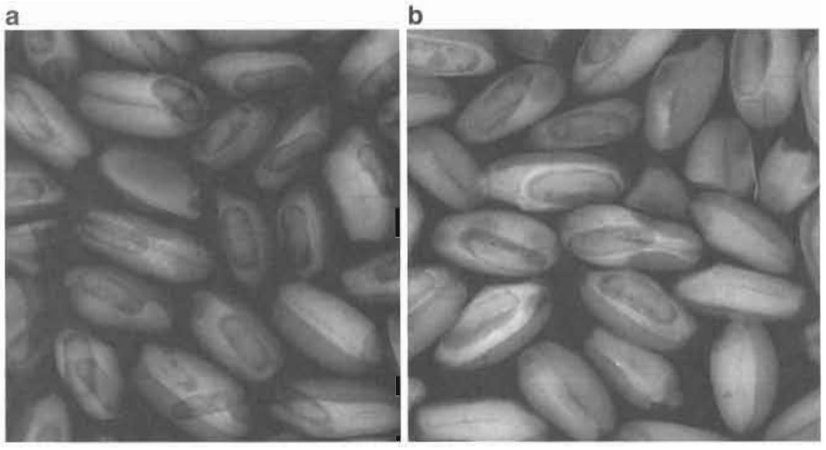
19.5 OTHER TECHNIQUES

19.5.1 Overview

Methods described in Sect. 19.3 are directed primarily at routine quality control efforts to determine if the level of natural or unavoidable defects is below the defect action level. To a certain extent, those routine methods can be used to identify the source of contaminants in processed foods. For example, the identification of certain insect fragments can indicate infestation in the raw commodity rather than in the processing system. However, other more sophisticated techniques offer opportunities to pinpoint the nature and source of other contaminants that may exist unavoidably or due to mistakes, accidents, material or equipment failures, or intentional adulteration.

The detection of insects in stored grain and the quantitation of insect parts present in grain products represent a serious and continuing problem for the grain industry. Approved methods of detection primarily involve visual and microscopic inspection and X-ray analysis, which require trained personnel and are time-consuming, difficult to standardize, and expensive. The assays for insect contamination are preferred to be highly specific, sensitive, rapid, and inexpensive. Moreover, it ideally should be employable by persons having minimal training, particularly in nonlaboratory settings such as at grain elevator and processing sites.

There are several attempts to develop rapid and efficient methods including the use of nuclear magnetic resonance, sound amplification, and infrared spectrometry as alternatives to presently used chemical techniques mentioned in preceding sections. Most of these techniques are expensive and challenging due to difficulty in quantification and identification of specific infestations. Immunological assays, which have found widespread use in clinical diagnostic settings and also in home use, have been explored to detect insect contamination. These methods are described below as they relate to detecting an infestation.



19-5 figure

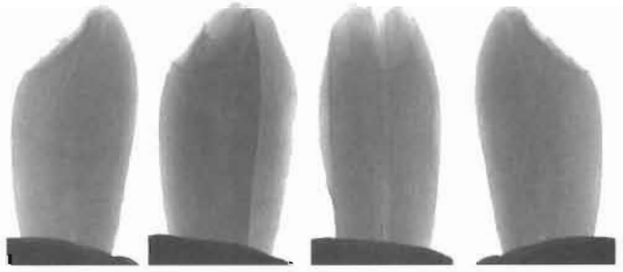
X-ray radiograph of infested wheat: (**a**) Lesser grain borer pupae, (**b**) Rice weevil pupae. (Courtesy of Moses Khamis.)

19.5.2 X-Ray Radiography

X-ray radiography is widely used as a test reference method (14). Grain processors use it as a means of inspecting wheat for internal insect infestation, which is the main source of insect fragments in processed cereal products (Fig. 19-5).The existing X-ray techniques enable the classification of at least four stages of insect development by measuring the area occupied by the insect, and an accurate classification is also possible based on visible insect morphology (15). The use of real-time digital imaging instead of X-ray radiographs to discriminate the infested kernels significantly shortened the X-ray procedures. Conventional film observations give, however, better accuracy (3% error rate) than the digital images (11.7%) for infestation by third-larval instars, while the error is less than 1% for both methods with a more advanced stage of larval development (16).

19.5.3 X-Ray Microtomography

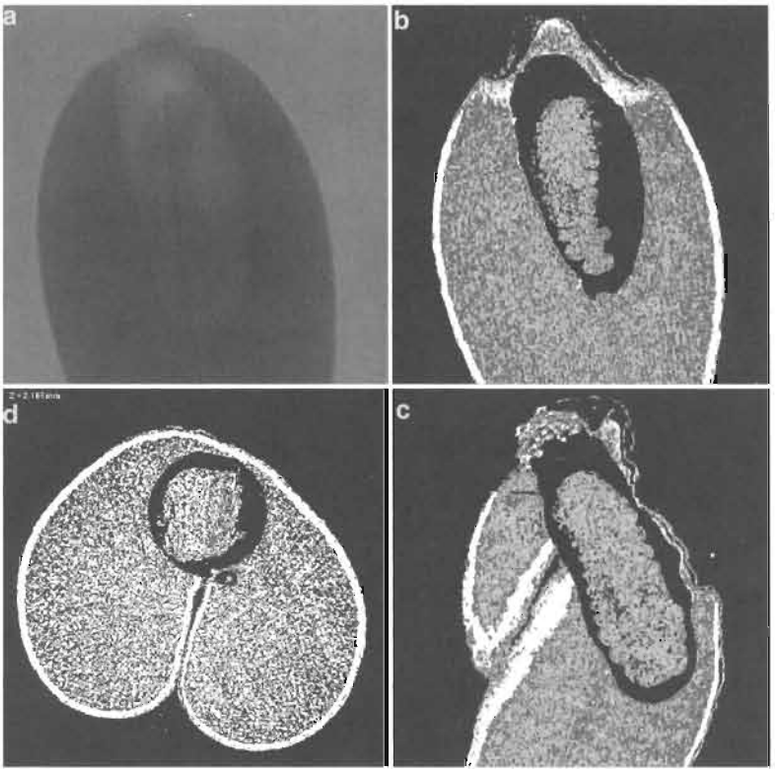
X-ray microtomography (XMT) is an emerging 3-D imaging technique that operates on the same basic principles as medical computed tomography (CT) scanners, but has much higher resolution. It is very effective in characterizing various internal structural features, which are not possible with conventional 2-D imaging methods. Conventional imaging techniques such as light microscopy, scanning electron microscopy (SEM), and digital video imaging have some limitations: They are destructive in nature, as sample preparation involves cutting to expose the cross section to be viewed. High-resolution XMT has a wide range of applications in science and engineering for which accurate 3-D imaging of internal structure of objects is crucial.



19-6 figure

Shadow images of a wheat kernel at various rotation angles.

Microtomographical techniques involve targeting the specimen with a polychromatic X-ray beam with high spatial coherence. The X-rays not absorbed by the specimen fall on specifically designed X-ray scintillators that produce visible light, which is then recorded by a charge-coupled device (CCD) camera. Scans are done by rotating the specimen between a fixed X-ray source and detector, around the axis perpendicular to the X-ray beam, while collecting radiographs of the specimen at small angular increments in the range 0–360°. The radiographs are reconstructed into a series of 2-D slices. The series of 2-D slices are then reconstructed into a 3-D image. The resulting XMT data can be visualized by 3-D rendering or 2-D slices derived from virtual model, using software that allows reconstruction of cross sections at various depth increments and along any desired orientation of the plane of cut (17). XMT is able to capture several features of the internal structures of grain kernels, which are not possible with the conventional imaging methods. Figure 19-6 is demonstration of shadow images



19-7 figure X-ray microtomography (XMT) images: (**a**) shadow image, (**b**) sagittal view, (**c**) axial view, and (**d**) coronal view of lesser grain borer in a wheat kernel.

taken at several step angles during scanning. A typical scan creates 200–400 of those images that are then used to create axial, sagittal, and coronal views shown in Fig. 19-7.

19.5.4 Electrical Conductance Method

The **electrical conductance** method is based on monitoring the conductance signals for each single kernel during milling in a Single Kernel Characteristics System (SKCS), which is commonly used for wheat hardness determination (15). This method is highly accurate for detecting older developmental stages of insects: the percentage of properly classified cases for small, medium, and large larvae and pupae is 24.5, 62.2, 87.5, and 88.6, respectively. The accuracy of this method depends also on insect species (rice weevil and lesser grain borer) and wheat type (soft or hard red winter wheat).

19.5.5 Impact-Acoustic Emission

Impact-acoustic emissions are used as a nondestructive, real-time method for detection of damaged grains and shelled nuts (18). Kernels are impacted onto a steel plate and the resulting acoustic signal is analyzed to detect damage using different methods: modeling of the signal in the time domain, computing time-domain signal variances and maximums in short-time windows, analysis of the frequency spectrum magnitudes, and analysis of a derivative spectrum. Features were used as inputs to a stepwise discriminant analysis routine, which selected a small subset of features for accurate classification using a neural network. Pearson et al. (18) reported that impact-acoustic emissions is a feasible and promising method for detection of IDK, sprout damage, and scab damage. More study is needed to improve accuracy on kernels infested with insects that have not yet emerged from the kernels. The computational cost of classifying a kernel using this technique is very low, allowing inspection of large numbers of wheat kernels very rapidly, ~40 kernels/s. Grain inspectors usually use a 100 g (3000 kernel) sample to inspect for IDK. This takes an inspector approximately 20 min to analyze manually, but can be accomplished in about 75 s with an acoustic system.

19.5.6 Microscopy Techniques

Microscopy techniques including light microscopy, fluorescence microscopy, and scanning electron microscopy (SEM) are used to study the structure/function relationships of food, but also can be applied to questions of extraneous matter. For example, SEM with energy dispersive spectroscopy (EDS)

can be used to determine the nature of metals in products that may be due to equipment failure or intentional adulteration due to tampering (19). Light microscopy in a polarized mode can be used to distinguish between plastics, glass, and other fiber or crystalline contaminants (20).

19.5.7 Near-Infrared Spectroscopy

Near-infrared spectroscopy (NIRS) is a relatively fast, accurate, and economical technique available to the grain industry for compositional analysis such as water, oil, fiber, starch, and protein in grains and seeds. It also has relatively recent applications in analysis of extraneous matter. NIRS has been used to identify several coleopteran species (21), and to detect parasitized weevils in wheat kernels (22) and external and internal insect infestation in wheat (23–25). Berardo et al. (26) reported that NIRS predicts the percentage of *Fusarium verticillioides* infection in maize kernels and the content of ergosterol and fumosin B1 in meals. In the same way, promising results were obtained when NIRS methodology was applied to detect scab-damaged kernels (27) and estimate deoxynivalenol, ergosterol, and fumonisin in single kernels of wheat (21) and corn (28).

Near-infrared spectroscopy used with a single kernel characterization system is able to detect later stages of internal insect infestation in wheat with a 95% confidence (25). In contrast to other procedures, this system is capable of being automated and incorporated into the current grain inspection process. NIRS also has been compared with the current standard insect fragment flotation method for its ability to detect insect fragments in flour (29). Fragment counts with both techniques were correlated; however, the flotation method was more sensitive below the FDA DAL of 75 insect fragments/50 g of wheat flour. NIR spectroscopy was able to predict accurately whether flour samples contained less than or more than 130 fragments/50 g.

19.5.8 Enzyme-Linked Immunosorbent Assays

To develop an optimal immunological assay for an insect contamination of foodstuffs, antibodies are required that are directed against an insect-specific antigen, preferably protein, likely to be present in any life stage of the contaminating insect or in insect remains. Antigens and antibodies are two key parts of any immunoassay (see also Chap. 17).

For an immunoassay with broad specificity it is required to use an insect-specific protein such as myosin. Myosin is ubiquitous in insects; it is present in large quantities in adult insect tissue and is also present in appreciable quantities in other life stages (30). An **enzyme-linked immunosorbent assay** (ELISA) method has been developed to measure quantitatively the amount of insect material in a sample (30). It is also possible to develop an immunoassay specific for a particular species of insect contamination using antibodies having a unique species specificity. Kitto et al. (31) developed such techniques (patented in 1992) for detecting the amount of insect contamination in foodstuffs. The method comprises the following steps:

1. Preparing an aqueous solution or suspension of a homogenized grain sample
2. Substantially affixing at least a portion of solution or suspension to a solid surface
3. Applying to solid surface a specifically binding insect antigen (or antibody) and enzyme to form an antibody-enzyme conjugate, resulting in formation of a colored product when the enzyme reacts with a substrate
4. Washing unbound conjugate from the solid surface
5. Incubating the solid surface with an enzyme substrate under conditions allowing colored product to be formed when enzyme is present
6. Correlating amounts of color formed with an amount of insect contamination

Recent research (32) showed that the myosin in fourth instars of the lesser grain borer developing within kernels of wheat degraded within the first 2 weeks when larvae were killed with phosphine, a fumigant commonly used to manage insect infestations in stored grain. Myosin degradation resulted in underestimating insect fragment estimates by about 58%.

19.6 COMPARISON OF METHODS

A number of methods that have been developed to detect insects in commodity samples (Table 19-3) are described here in general terms:

1. Density separation based on infested kernels being lighter weight and floating in a liquid
2. Staining kernels to detect weevil egg plugs
3. Detection of carbon dioxide or uric acid produced by the internally feeding insects
4. Detection of insects hidden inside kernels using near-infrared spectroscopy (NIRS)
5. Detection by use of nuclear magnetic resonance (NMR)
6. Detection by X-ray images and digital image analysis techniques

19-3 table **Insect Detection Methods Applicable for Commodity Samples (Adapted from (35))**

Test Method	*Applicability*	*Comments*
Visual inspection	Whole grains, milled products	Qualitative, only high-level infestation detected
Sampling and sieving	Whole grains, milled products	Commonly practiced, hidden infestation not detected
Heat extraction	Whole grains	Adults and larvae detected
Acoustics	Whole grains	*Feeding sounds*: Active stages detected *Impact-acoustic emissions*: Nondestructive, real time; detect insect, sprout, and scab damage
Breeding out	Whole grains	Time consuming
Imaging techniques		
X-ray method	Whole grains	Non-destructive, highly accurate, able to detect both live and dead insects inside grain kernels; cannot detect insect eggs, prohibitive capital cost
Near infrared spectroscopy	Whole grains, milled products	Rapid, sensitive, can be automated, no sample preparation; cannot detect low levels of infestation, sensitive to moisture content, calibration of equipment is complex and frequent
Nuclear magnetic resonance	Whole grains	Less sensitive
Serological techniques	Whole grains, milled products	Highly sensitive, species specific; shows infestation from unknown past to till date
Uric acid determination	Whole grains, milled products	Shows infestation from unknown past to till date
CO_2 analysis	Whole grains	Simple, time consuming; indicates current level of infestation; not suitable for grains having >15% moisture
Specific gravity methods	Whole grains	Simple and quick, not suitable for oats and maize
Cracking and floatation method	Whole grains	Variable results noted
Fragment count	Whole grains, milled products	Highly variable results noted; shows infestation from unknown past to till date
Staining techniques		
Egg plugs	Whole grains	Specific for *Sitophilus* spp.
Ninhydrin method	Whole grains	Eggs and early larvae not indicated

7. Acoustical sensors to hear sounds from insects feeding inside kernels
8. Enzyme-linked immunosorbent assays (ELISA) to detect myosin in insect muscle

Some of the recent methods have been developed by adapting the single-kernel characterization system (SKCS), computed tomography (CT), acoustic-impact emissions, and use of a electrical conductive roller mill (15,33,34).

The choice of method depends on several factors: (a) type of infestation (inside or outside food grains, in the surrounding premises or inside bulk grain), (b) required level of inspection (macroscopic vs. microscopic, qualitative vs. quantitative), (c) availability of equipment and facilities, and (d) required sensitivity (35). Most of the methods aim to detect the presence of live insects directly or indirectly. External insects are detected by visual inspection, sampling, sieving, and heat-extraction methods, while internal (hidden) insects are detected by radiography, staining techniques to identify egg plugs, and near-infrared and fragment count methods. Determination of uric acid or CO_2 level serves as an indirect way of detecting and

estimating internally feeding insects, and these methods may be suitable if infestations are restricted to one insect species. Depending on some storage conditions, grain may contain molds and insects, and in such cases CO_2 produced by molds may interfere in accurately detecting or estimating insects. Fragment count and ELISA methods can be used for the detection of both living and dead insects. In general, problems encountered with these detection methods are that the most accurate methods, such as X-ray and computed tomography (CT), are laborious and expensive, while rapid, automated methods may not be suitable for detecting eggs and young larvae (32).

19.7 ISOLATION PRINCIPLES APPLIED TO FOOD PROCESSING

Examination of stored-product insects often requires extracting them from the commodity. An intensive summary of literature survey on insect extraction and detection methods can be found in reference (36). Isolation principles, such as particle size and density, discussed in the preceding sections are designed to identify extraneous materials in finished food products, monitoring quality, and compliance with DALs. In addition, some of these principles of isolation are used in a **proactive** way during processing to prevent extraneous matter from being incorporated into finished food products.

Wheat that contains hidden internal insect infestation is the primary source of insect fragments in processed cereal products. The current DAL for internal insect infestation in wheat is **32 IDK** per 100 g of wheat (3). IDK are those **visually** determined to have insect tunneling or emergence holes. Most processors rely on much lower levels of IDK ($\leq$6 IDK/100 g) to produce flour that meets customer tolerances and the FDA's DAL for insect fragments in flour. In addition, to prevent adulteration of flour with filth, entoleters and infestation destroyers in the milling process break up insect-damaged kernels, and these broken kernels along with the insect fragments are aspirated out of the milling stream. As previously indicated, **X-ray radiography** is used by some as a means for selecting grains for processing or for research purposes to age-grade internally developing stages of stored-grain insects. More recently, NIR spectroscopy has provided a new tool for assessing internal insect infestation in wheat. By selectively milling only wheat that has minimal or no evidence of internal insect infestation, grain processors can effectively limit insect fragments in their products. In like manner, bakers and other users of processed grain products can selectively monitor for insect fragments in their raw materials using one of the approved methods for extraction and enumeration of fragments or by sending samples to a private laboratory for fragment analysis.

Most food processing systems that deal with agricultural products generally apply some type of cleaning operations as an initial step. In flour milling, for example, wheat is passed through a system called the "cleaning house," which consists of a series of machines that apply the principles of particle size and density separation. Sieves remove contaminants larger than wheat kernels as well as finer contaminants such as sand. In addition, air (aspiration) is used to remove plant material that is lighter than the grain. Current equipment to remove stones and other dense materials the same size as grain kernels use air passed upward through an inclined, tilted table. This causes the grain to "float" off the side of the table and the heavier material to continue and "tail" over the end of the table. In earlier systems, grains were passed through washers in which water separated the grain from heavier material (such as stones) much like fluming of potatoes or fruit does. Impact with rotating disks and steel pegs (entoleters and infestation destroyers) or grinding operations are used prior to milling to break open kernels of wheat containing internal insect infestation. As a means of reducing insect fragments in the finished product, this process is followed by aspiration to lift out any light insect contaminants released in the operation.

As a final step in wheat milling, flour is generally passed through sieves fine enough to remove insect eggs and any other contaminants that might be present. This is to assure that when flour leaves the mill it is free of any viable form of insect contamination (37). Where flour is used in large quantities, such as commercial bakeries, prior to use, flour is again sieved to ensure that no contamination has occurred in transport and storage of the flour.

Metal contamination has been a major concern of all food processors. Although metal detection methods are not specifically among the isolation techniques represented in AOAC International or AACC International methods manuals, they serve the purpose of isolating contaminants from food products. Magnets of various types have been used on raw materials and processing systems to prevent the passage of metal into handling and processing equipment where both equipment damage and product contamination are concerns. **Metal detectors** are employed in many food processing operations and on finished product packaging lines to detect ferrous and nonferrous metal fragments and to prevent contaminated products from entering consumer food channels.

Recent X-ray technology suggests that X rays may have an advantage over other methods for detecting metal and that they can also be used to detect glass,

wood, plastic, and bone chips in foods. Detection of these extraneous materials also can be automated with rejection systems in packaging lines (38).

19.8 SUMMARY

Extraneous matter in raw ingredients and in processed foods might be unavoidable in the array of foods that are stored, handled, processed, and transported. DALs are established for amounts considered unavoidable and of no health hazard. A variety of methods are available to isolate extraneous matter from foods. Those methods largely prescribed by AOAC International employ a series of physical and chemical means to separate the extraneous material for identification and enumeration. Major concerns in the analysis of food products for extraneous matter are the objectivity of methods and the availability of adequately trained analysts. Some "principles" of isolation are applied in a proactive way in food processing operations.

Currently available methods (both macroscopic and microscopic) show varying degrees of efficiency in analysis of extraneous matter and filth in foods. Some techniques are time-consuming, require trained personnel, and are difficult to implement in real time. Some techniques have not been found feasible to be implemented in food inspection systems because of their cost, unreliability, and the varying degrees of success obtained in detecting infestations. Macroscopic and microscopic procedures 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 complementary methods for use as a joint approach in solving analytical problems.

19.9 STUDY QUESTIONS

1. Indicate why the FDA has established DALs.
2. Explain why practicing cGMPs has no impact on DALs.
3. List three major reasons for conducting analysis for extraneous matter in foods.
4. What two resources provide methods for separating extraneous matter from cereal grains and their products?
5. There are several basic principles involved in separating (isolating) extraneous matter from foods. List five of these principles and give an example of each principle.
6. Briefly describe the major constraint(s) to currently accepted methods for analyses of extraneous matter in foods.
7. Explain how some of the more recent analytical techniques can assist in identifying sources of extraneous matter in foods.
8. What are some likely sources of error with the various analytical methods?

19.10 ACKNOWLEDGEMENT

This contribution is paper number 10-062-B of the Kansas Agricultural Experiment Station, Kansas State University, Manhattan, KS 66506.

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Food Analysis

Fourth Edition

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ISBN 978-1-4419-1477-4 e-ISBN 978-1-4419-1478-1
DOI 10.1007/978-1-4419-1478-1
Springer New York Dordrecht Heidelberg London

Library of Congress Control Number: 2010924120

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