5

chapter

Sampling and Sample Preparation

Rubén O. Morawicki
Department of Food Science,
University of Arkansas,
Fayetteville, AR 72704, USA
e-mail: rmorawic@uark.edu

S. Nielsen (ed.), *Food Analysis*, Food Science Text Series,
DOI 10.1007/978-3-319-45776-5_5,

5.1 INTRODUCTION

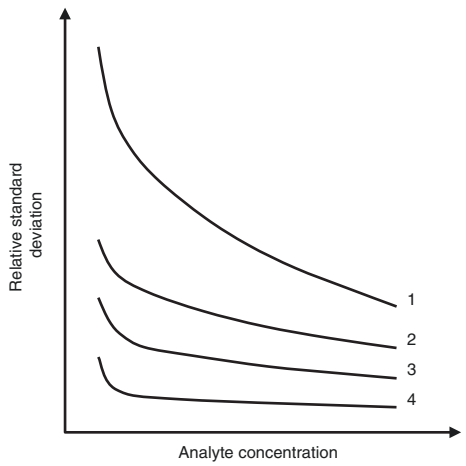
Quality attributes in food products, raw materials, or ingredients are measurable characteristics that need monitoring to ensure specifications are met. Some quality attributes can be measured online by using specially designed sensors and results obtained in real time (e.g., color of vegetable oil in an oil extraction plant). However, in most cases quality attributes are measured on small portions of material that are taken periodically from continuous processes or on a certain number of small portions taken from a lot. The small portions taken for analysis are referred to as **samples**, and the entire lot or the entire production for a certain period of time, in the case of continuous processes, is called a **population**. The process of taking samples from a population is called **sampling**. If the procedure is done correctly, the measurable characteristics obtained for the samples become a very accurate estimation of the population.

By sampling only a fraction of the population, a quality estimate can be obtained accurately, quickly, and with less expense and personnel time than if the total population were measured. The reliability of sampling is dependent more on the sample size than on the population size [1]. The larger the sample size, the more reliable the sampling. However, sample size is limited by time, cost, sampling methods, and the logistics of sample handling, analysis, and data processing. Moreover, in the case of food products, analyzing a whole population would be practically impossible because of the destructive nature of most analytical methods. Paradoxically, estimated parameters using representative samples (discussed in Sects. 5.2 and 5.3) are normally more accurate than the same estimations done on the whole population (census).

The sample actually analyzed in the laboratory can be of any size or quantity [2]. This **laboratory sample** has generally undergone preparation such as homogenization or grinding to prepare it for analysis and is much smaller than the sample actually collected. Sampling and associated problems are discussed in Sects. 5.2, 5.3, and 5.4, while preparation of laboratory samples for testing is described in Sect. 5.5.

Sampling starts the series of steps needed to make decisions about data collected: sampling, sample preparation, laboratory analysis, data processing, and interpretation. In each step, there is a potential for error that would compromise the certainty, or reliability, of the final result. This final result depends on the cumulative errors at each stage that are usually described by the variance [3, 4]. **Variance** is an estimate of the uncertainty. The total variance of the whole testing procedure is equal to the sum of the variances associated with each step of the sampling procedure and represents the **precision** of the process. Precision is a measure of the reproducibility of the data. In contrast, **accuracy** is a measure of how close the data are to the true value. The most efficient way to improve accuracy is to improve the reliability of the step with the greatest variance, and that is frequently the initial sampling step. Attention is often given to the precision and accuracy of analytical methods, with less attention given to the validity of sampling and sample preparation. However, sampling can often be the greatest source of error in chemical analysis in general, but especially a problem in food analysis because of the food matrix [5–7] (Fig. 5.1). Sample homogenization and other aspects of sample preparation are additional sources of potential error, even prior to the actual analysis.

As you read each section of the chapter, consider application of the information to some specific examples of sampling needs in the food industry: sampling for nutrition labeling (see Study Question 7 in this chapter), pesticide analysis (see also Chap. 33, Sect. 33.3), mycotoxin analysis (see also Chap. 33, Sect. 33.4),



5.1 figure

Typical relative standard deviation of error components for a nonhomogeneous food matrix. *1* Sampling; *2* homogenization; *3* sample preparation; *4* assay procedure (Used with permission from Lichon [6])

extraneous matter (see also Chap. 34), or rheological properties (see also Chap. 29). To consider sample collection and preparation for these and other applications subject to government regulations, you are referred also to the sample collection section of compliance procedures established by the Food and Drug Administration (FDA) and Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture (USDA) (see Chap. 3, Sect. 3.2.2.1 for FDA and Sect. 3.3 for USDA). Additional information is available on how the FDA does sampling linked to the Food Safety Modernization Act [8] and how USDA collects samples for the National Nutrient Databank [9, 10].

It should be noted that sampling terminology and procedures used may vary between companies and between specific applications. However, the principles described in this chapter are intended to provide a basis for understanding, developing, and evaluating sampling plans and sample handling procedures for specific applications encountered.

5.2 SELECTION OF SAMPLING PROCEDURES

5.2.1 Definition and Purpose of Sampling Plan

The **International Union of Pure and Applied Chemistry** (IUPAC) defines a sampling plan as: "A predetermined procedure for the selection, withdrawal, preservation, transportation, and preparation of the portions to be removed from a lot as samples" [11]. A sampling plan should be a well-organized document that establishes the goals of the sampling plan, the factors to be measured, sampling point, sampling procedure, frequency, size, personnel, preservation of the samples, etc. The primary aim of sampling is to obtain a sample, subject to constraints of size that will satisfy the sampling plan specifications. A sampling plan should be selected on the basis of the sampling objective, the study population, the statistical unit, the sample selection criteria, and the analysis procedures. Depending on the purpose of the sampling plan, samples are taken at different points of the food production system, and the sampling plan may vary significantly for each point.

5.2.2 Factors Affecting the Choice of Sampling Plans

Each factor affecting the choice of sampling plans (Table 5.1) must be considered in the selection of a plan: (1) purpose of inspection, (2) nature of population, (3) nature of product, and (4) nature of test method. Once these are determined, a sampling plan that will provide the desired information can be developed.

5.1 table Factors that affect the choice of sampling plans

Factors to be considered	*Questions*
Purpose of the inspection	Is it to accept or reject the lot?
	Is it to measure the average quality of the lot?
	Is it to determine the variability of the product?
Nature of the population	Is the lot large but uniform?
	Does the lot consist of smaller, easily identifiable su bplots?
	What is the distribution of the units within the population?
Nature of the product	Is it homogeneous or heterogeneous?
	What is the unit size?
	How consistently have past populations met specifications?
	What is the cost of the material being sampled?
Nature of the test method	Is the test critical or minor?
	Will someone become sick or die if the population fails to pass the test?
	Is the test destructive or nondestructive?
	How much does the test cost to complete?

Adapted from Puri et al. [2]

5.2.2.1 *Purpose of Inspection*

Most sampling is done for a specific purpose and the purpose may dictate the nature of the sampling approach. The two primary objectives of sampling are often to estimate the average value of a characteristic and determine if the average value meets the specifications defined in the sampling plan. Sampling purposes vary widely among different food industries; however, the most important categories include the following:

1. Nutritional labeling
2. Detection of contaminants and foreign matter
3. Acceptance of raw materials, ingredients, or products (acceptance sampling)
4. Process control samples
5. Release of lots of finished product
6. Detection of adulterations
7. Microbiological safety
8. Authenticity of food ingredients.

5.2.2.2 *Nature of Population and Product*

One must clearly define the population and understand the nature of the product that is going to be sampled to select an appropriate sampling plan. Populations for sampling are often defined in terms of being homogeneous or heterogeneous and being either discrete or continuous. The population and product can also vary greatly in size.

The ideal population and product would be uniform throughout and identical at all locations. Such a population would be **homogeneous**. Sampling from such a population is simple, as a sample can be taken from any location, and the analytical data obtained will be representative of the whole. However, this occurs rarely, as even in an apparently uniform product, such as sugar syrup, suspended particles and sediments in a few places may render the population heterogeneous. In fact, most populations and products that are sampled are **heterogeneous**. Therefore, the location within a population where a sample is taken will affect the subsequent data obtained. However, sampling plans and sample preparation can make the sample representative of the population or take heterogeneity into account in some other way. If the sample is heterogeneous, some additional questions become important [6]: What is the nature of the variation? Should samples be pooled or replicated? Should different portions of the sample be analyzed separately? Should the surface of the sample be tested?

Sampling from **discrete** (or compartmentalized) populations is relatively easy, since the population is split into multiple separate subunits (e.g., cans in a pallet of canned food, boxes of breakfast cereal in a truck, bottle of juice on a conveyer belt). The choice of the sampling plan is determined in part by the number and size of the individual subunits. Sampling is more difficult from a **continuous** population since different parts of the sample are not physically separated (e.g., potato chips on a conveyer belt, oranges in a semitruck) [6].

The population may vary in size from a production lot, a day's production, to the contents of a warehouse. Information obtained from a sample of a particular production lot in a warehouse must be used strictly to make inferences about that particular lot, but conclusions cannot be extended to other lots in the warehouse.

To use food analysis to solve problems in the food industry, it may be inadequate to focus just on the nature of the population and product to develop a sampling plan. For example, to collect samples to address a problem of variation of moisture content of packaged products coming off a production line, it would be important to examine each processing step. It would be necessary to understand where variation likely exists to then determine how to appropriately collect samples and data to analyze for variability.

One of the most challenging cases of accounting for the nature of the population, as it influences the sampling plan, is collecting samples to measure fungal toxins, named mycotoxins, in food systems. Mycotoxins are distributed broadly and randomly within a population and a normal distribution cannot be assumed [2]. Such distribution requires a combination of many randomly selected portions to obtain a reasonable estimate of mycotoxin levels. Methods of analysis that are extremely precise are not needed when determining mycotoxin levels, when sampling error is many times greater than analytical error [2]. In this case, sampling and good comminution and mixing prior to particle size reduction are more important than the chemical analysis itself. Additional information on sampling for mycotoxin analysis is provided in Chap. 33, Sect. 33.4.

5.2.2.3 *Nature of Test Method*

Procedures used to test samples collected vary in several characteristics that help determine the choice of sampling plan, e.g., cost, speed, accuracy, precision, and destructive vs. nondestructive. Low-cost, rapid, nondestructive tests that are accurate and precise make it more feasible to analyze many samples. However, limitations on any of these characteristics will make the nature of the test method a more important determinant of the sampling plan.

5.3 TYPES OF SAMPLING PLANS

5.3.1 Sampling by Attributes and Sampling by Variables

Sampling plans are designed for examination of either attributes or variables [4]. In **attribute sampling**, sampling is performed to decide on the acceptability of a population based on whether the sample possesses a certain characteristic or not. The result has a binary outcome of either conforming or nonconforming. Sampling plans by attributes are based on the hypergeometric, binomial, or Poisson statistical distributions. In the event of a binomial distribution (e.g., presence of *Clostridium botulinum*), the probability of a single occurrence of the event is directly proportional to the size of the sample, which should be at least ten times smaller than the population size. Computing binomial probabilities will allow the investigator to make inferences on the whole lot.

In **variable sampling**, sampling is performed to estimate quantitatively the amount of a substance (e.g., protein content, moisture content, etc.) or a characteristic (e.g., color) on a continuous scale. The estimate obtained from the sample is compared with an acceptable value (normally specified by the label, regulatory agencies, or the customer) and the deviation measured. This type of sampling usually produces data that have a *normal distribution* such as in the percent fill of a container and total solids of a food sample. In general, variable sampling requires smaller sample size than

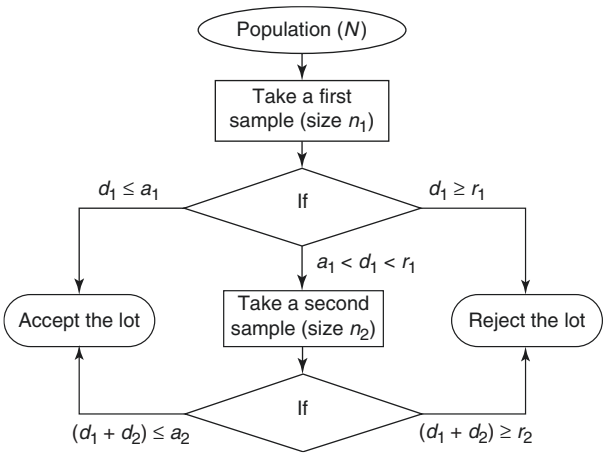
attribute sampling [1], and each characteristic should be sampled for separately when possible. However, when the FDA and the USDA's FSIS perform sampling for compliance of nutrition labeling, a composite of 12, and of at least six subsamples, respectively, is obtained and used for all nutrients to be analyzed.

5.3.2 Acceptance Sampling

Acceptance sampling is a procedure that serves a very specific role: to determine if a shipment of products or ingredients has enough quality to be accepted. **Acceptance sampling** can be performed by the food processor before receiving a lot of materials from a supplier or by a buyer who is evaluating the processor's output [6]. Acceptance sampling is a very broad topic that can be applied to any field; more specific literature can be consulted if needed.

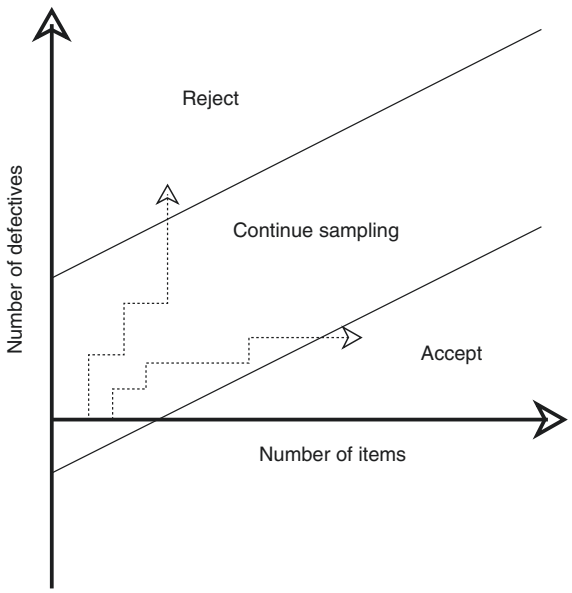
Lot acceptance sampling plans that may be used for evaluation of attributes or variables, or a combination of both, fall into the following categories:

1. *Single sampling plans.* The decision of accepting or rejecting a lot for this type of plan is based just on one sample of items taken at random. These plans are usually denoted as (n, c) plans for a sample size n, where the lot is rejected if there are more than c defective samples [12]. If results are inconclusive, a second sample is taken, and the decision of accepting or rejecting is made based on the combined outcome of both samples.
2. *Double sampling plans.* Similar to single sampling plan, but two samples are taken (Fig. 5.2).
3. *Multiple sampling plans.* These are extensions of double sampling plans and use more than two samples to reach a conclusion.
4. *Sequential sampling plans*. Under this plan, which is an ultimate extension of multiple sampling, a sample is taken, and after analysis a decision of accepting, rejecting, or taking another sample is made. Therefore, the number of total samples to be taken depends exclusively on the sampling process. In this chart of Fig. 5.3, the cumulative observed number of defective samples is plotted against the number of samples taken. Two lines—the rejection and acceptance lines—are drawn, thus dividing the plot in three different regions: accept, reject, and continue sampling. An initial sample is taken and the results plotted in the graph. If the plotted point falls within the parallel lines, then a second sample is taken, and the process is repeated until the reject or accept zones are reached [12]. Details about the construction of this plot are beyond the scope of this book, and particulars can be found in more specialized literature.
5. *Skip lot sampling.* Only a fraction of the submitted lots is inspected with this type of plan. It is a money-saving sampling procedure, but it can be implemented only when there is enough proof that the quality of the lots is consistent.



5.2 figure

Example of a double sampling plan with two points where the decision of acceptance or rejection can be made (Adapted from NIST/SEMATECH [13]). N, population size; n_1 and n_2, sample size; a_1 and a_2, acceptance numbers; r_1 and r_2, rejection numbers; d_1 and d_2, number of non-conformities, *Subindices* 1 and 2 represent samples 1 and 2, respectively



5.3 figure

Sequential sampling plan (Adapted from NIST/SEMATECH [13])

5.3.3 Risks Associated with Acceptance Sampling

There are two types of risks associated with acceptance sampling: producer's and consumer's risks [13].

The **consumer's risk** describes the probability of accepting a poor-quality population. This should happen rarely (<5% of the lots), but the actual acceptable probability (β) of a consumer risk depends on the consequences associated with accepting an unacceptable lot. These may vary from major health hazards and subsequent fatalities to a lot being of slightly lower quality than standard lots. Obviously, the former demands a low or no probability of occurring whereas the latter would be allowed to occur more frequently. The **producer risk** is the probability of rejecting (α) an acceptable product. As with consumer's risk, the consequences of an error determine the acceptable probability of the risk. An acceptable probability of producer's risk is usually 5–10%. Further discussion of sampling plans can be found in the following section.

5.4 SAMPLING PROCEDURES

5.4.1 Introduction

The reliability of analytical data is compromised if sampling is not done properly. As shown in Table 5.1, the use of the data to be obtained is one major factor determining the sampling procedure. Details for the sampling of specific food products are described in the *Official Methods of Analysis* of AOAC International [14] and in the *Code of Federal Regulations* (CFR) [15]. Two such examples for specific foods follow.

5.4.2 Examples

The AOAC Method 925.08 [14] describes the method for sampling flour from sacks. The number of sacks to be sampled is determined by the square root of the number of sacks in the lot. The sacks to be sampled are chosen according to their exposure. The samples that are more frequently exposed are sampled more often than samples that are exposed less. Sampling is done by drawing a core from a corner at the top of the sack diagonally to the center. The sampling instrument is a cylindrical, polished trier with a pointed end. It is 13 mm in diameter with a slit at least one third of the circumference of the trier. A second sample is taken from the opposite corner in a similar manner. The cores are stored for analysis in a clean, dry, airtight container that has been opened near the lot to be sampled. The container should be sealed immediately after the sample is added. A separate container is used for each sack. Additional details regarding the container and the procedure also are described below.

Title 21 CFR specifies the sampling procedures required to ensure that specific foods conform to the standard of identity. In the case of canned fruits, 21 CFR 145.3 defines a sample unit as "container, a portion of the contents of the container, or a composite mixture of product from small containers that is sufficient for the testing of a single unit" [15]. Furthermore, a sampling plan is specified for containers of specific net weights. The container size is determined by the size of the lot. A specific number of containers must be filled for sampling of each lot size. The lot is rejected if the number of defective units exceeds the acceptable limit. For example, out of a lot containing 48001–84000 units, each weighing 1 kg or less, 48 samples should be selected. If six or more of these units fail to conform to the attribute of interest, the lot will be rejected. Based on statistical confidence intervals, this sampling plan will reject 95% of the defective lots examined, that is, 5% consumer risk [15].

5.4.3 Manual Versus Continuous Sampling

To obtain a **manual sample**, the person taking the sample must attempt to take a "random sample" to avoid human bias in the sampling method. Thus, the sample must be taken from a number of locations within the population to ensure it is representative of the whole population. For liquids in small containers, this can be done by shaking prior to sampling. When sampling from a large volume of liquid, such as that stored in silos, aeration ensures a homogeneous unit. Liquids may be sampled by pipetting, pumping, or dipping. However, when sampling grain from a rail car, mixing is impossible, and samples are obtained by probing from several points at random within the rail car. Such manual sampling of granular or powdered material is usually achieved with triers or probes that are inserted into the population at several locations. Errors may occur in sampling [10], as rounded particles may flow into the sampling compartments more easily than angular ones. Similarly, hygroscopic materials flow more readily into the sampling devices than does nonhygroscopic material. Horizontal core samples have been found to contain a larger proportion of smaller-sized particles than vertical ones [16].

Continuous sampling is performed mechanically. Figure 5.4 shows an automatic sampling device that is used to take liquid samples from a continuous production line. Continuous sampling should be less prone to human bias than manual sampling.

5.4.4 Statistical Considerations

5.4.4.1 *Probability Sampling*

Probability sampling plans prescribe the selection of a sample from a population based on chance. It provides a statistically sound basis for obtaining representative samples with elimination of human bias [2]. The probability of including any item in the sample is known and sampling error can be calculated. Several probability sampling methods are available to the researcher, and the most common ones are described in the next few paragraphs.

5.4 figure

An automatic liquid sampling device that uses air under high pressure to collect multiple 1.5 mL samples. The control box (*left*) regulates the sampling frequency (Courtesy of Liquid Sampling Systems Inc., Cedar Rapids, IA)

Simple random sampling requires that the number of units in the population be known and each unit is assigned an identification number. Then using a random selection process, a certain number of identification numbers are selected according to the sample size. The sample size is determined according to the lot size and the potential impact of a consumer or vendor error. The random selection of the individuals units is done by using random number tables or computer-generated random numbers. Units selected randomly (sample) are analyzed and the results can be considered an unbiased estimate of the population.

Systematic sampling is used when a complete list of sample units is not available, but when samples are distributed evenly over time or space, such as on a production line. The first unit is selected at random (random start) and then units are taken every *n*th unit (sampling interval) after that.

Stratified sampling involves dividing the population (size *N*) into a certain number of mutually exclusive homogeneous subgroups (size *N1*, *N2*, *N3*, etc.) and then applying random or another sampling technique to each subgroup. Stratified sampling is used when subpopulations of similar characteristics can be observed within the whole population. An example of stratified sampling would be a company that produces tomato juice in different plants. If we need to study the residual activity of polygalacturonase in tomato juice, we can stratify on production plants and take samples on each plant.

Cluster sampling entails dividing the population into subgroups, or clusters, and then selecting randomly only a certain number of clusters for analysis. The main difference between cluster sampling and stratified sampling is that in the latter, samples are taken from every single subgroup, while in cluster sampling only some randomly clusters selected are sampled. The clusters selected for sampling may be either totally inspected or subsampled for analysis. This sampling method is more efficient and less expensive than simple random sampling, if populations can be divided into clusters. Going back to the tomato juice example, when using cluster sampling, we would consider all processing plants, but we would select randomly just a few for the purpose of the study.

Composite sampling is used to obtain samples from bagged products such as flour, seeds, and larger items in bulk. Small aliquots are taken from different bags, or containers, and combined in a simple sample (the composite sample) that is used for analysis. Composite sampling also can be used when a representative sample of a whole production day in a continuous process is needed. In this case, a systematic approach is used to take equal aliquots at different times, and then a representative sample is obtained by mixing the individual aliquots. A typical example of composite sampling is the sampling plan mandated by the FDA and FSIS for nutritional labeling. They require a composite of 12 samples with at least six subsamples taken and analyzed for compliance with nutrition labeling regulations [17].

5.4.4.2 *Nonprobability Sampling*

Randomization is always desired. However, it is not always feasible, or even practical, to take samples based on probability methods. Examples include in preliminary studies to generate hypothesis, in the estimation of the standard deviation so a more accurate sampling plan can be designed, or in cases for which the bulkiness of the material makes inaccessible the removal of samples. In these cases, nonprobability sampling plans may be more economical and practical than probability sampling. Moreover, in certain cases of adulteration such as rodent contamination, the objective of the sampling plan may be to highlight the adulteration rather than collect a representative sample of the population.

Nonprobability sampling can be done in many ways, but in each case the probability of including any specific portion of the population is not equal because the investigator selects the samples deliberately. Without the use of a methodology that gives every element of the population the same chance to be selected, it is not possible to estimate the sampling variability and possible bias.

Judgment sampling is solely at the discretion of the sampler and therefore is highly dependent on the person taking the sample. This method is used when it is the only practical way of obtaining the sample. It may result in a better estimate of the population than random sampling if sampling is done by an experienced individual, and the limitations of extrapolation from the results are understood [2]. **Convenience sampling** is performed when ease of sampling is the key factor. The first pallet in a lot or the sample that is most accessible is selected. This also is called "chunk sampling" or "grab sampling." Although this sampling requires little effort, the sample obtained will not be representative of the population and therefore is not recommended. **Restricted sampling** may be unavoidable when the entire population is not accessible. This is the case if sampling is from a loaded boxcar, but the sample will not be representative of the population. **Quota sampling** is the division of a lot into groups representing various categories, and samples are then taken from each group. This sampling method is less expensive than random sampling but also is less reliable.

5.4.4.3 *Mixed Sampling*

When the sampling plan is a mixture of two or more basic sampling methods that can be random or nonrandom, then the sampling plan is called **mixed sampling**.

5.4.4.4 *Estimating the Sample Size*

Sample size determination can be based on either **precision analysis** or **power analysis**. Precision and power analyses are done by controlling the confidence level (type I error) or the power (type II error). For the purpose of this section, the precision analysis will be used and will be based on the confidence interval approach and the assumptions that the population is normal.

The confidence interval for a sample mean is described by the following equation:

$$\bar{x} \pm z_{\alpha/2}\frac{\mathrm{SD}}{\sqrt{n}} \tag{5.1}$$

where:

$\bar{x}$ = sample mean
$z_{\alpha/2}$ = z-value corresponding to the level of confidence desired
SD = known, or estimated, standard deviation of the population
n = sample size

In Eq. 5.1, $z_{\alpha/2}\frac{\mathrm{SD}}{\sqrt{n}}$ represents the maximum error (E) that is acceptable for a desired level of confidence. Therefore, we can set the equation $E = z_{\alpha/2}\frac{\mathrm{SD}}{\sqrt{n}}$ and solve for n:

$$n = \left(\frac{z_{\alpha/2}\mathrm{SD}}{E}\right)^2 \tag{5.2}$$

The maximum error, E, in Eq. 5.2 can be expressed in terms of the accuracy (γ) as $E = \gamma \times \bar{x}$. Then Eq. 5.2 can be rearranged as

$$n = \left(\frac{z_{\alpha/2}\mathrm{SD}}{\gamma \times \bar{x}}\right)^2 \tag{5.3}$$

Now, we have an equation to calculate the sample size, but the equation is dependent on an unknown parameter: the standard deviation. To solve this problem, we can follow different approaches. One way is to take few samples using a nonstatistical plan and use the data to estimate the mean and standard deviation. A second approach is using data from the past or data from a similar study. A third method is to estimate the standard deviation as 1/6 of the range of data values [13]. A fourth method is to use typical coefficients of variation (defined as $100 \times [\text{standard deviation} / \text{population mean}]$) assuming we have an estimation of the population mean.

If the estimated sample is smaller than 30, then the Student's t distribution needs to be used instead of the normal distribution by replacing the $z_{\alpha/2}$ with the parameter t, with n-1 degrees of freedom. However, the use of the Student's t-test distribution comes with the additional cost of introducing another uncertainty into Eq. 5.3: the **degrees of freedom**. For the estimation of the t-score, we need to start somewhere by assuming the degrees of freedom, or assuming a t-score, and then calculating the number of samples, recalculating the t-score with n-1 degrees of freedom, and calculating the number of samples again. For a level of uncertainty of 95%, a conservative place to start would be assuming a t-score of 2.0 and then calculating the initial sample size. If we use a preliminary experiment to estimate the standard deviation, then we can use the sample size of the preliminary experiment minus one to calculate the t-score.

Example: We want to test the concentration of sodium in a lot of a ready-to-eat food product with a level of confidence of 95%. Some preliminary testing showed an average content of 1000 mg of sodium per tray with an estimated standard deviation of 500. Determine the sample size with an accuracy of 10%.

Data: Confidence level = 95% => $\alpha = 0.05$ => $z = 1.96$; $\gamma = 0.1$; $\bar{x} = 1000$; SD = 500

$$n = \left(\frac{z_{\alpha/2}\mathrm{SD}}{\gamma \times \bar{x}}\right)^2 = \left(\frac{1.96 \times 500}{0.1 \times 1000}\right)^2 = 96 \text{ trays}$$

5.4.5 Problems in Sampling and Sample Storage

No matter how reliable our analytical technique is, our ability to make inferences on a population will always depend on the adequacy of sampling techniques. **Sampling bias**, due to nonstatistically viable convenience, may compromise reliability. Errors also may be introduced by not understanding the **population distribution** and subsequent selection of an inappropriate sampling plan.

Unreliable data also can be obtained by nonstatistical factors such as poor **sample storage** resulting in sample degradation. Samples should be stored in a container that protects the sample from moisture and other environmental factors that may affect the sample (e.g., heat, light, air). To protect against changes in moisture content, samples should be stored in an airtight container. Light-sensitive samples should be stored in containers made of opaque glass or the container wrapped in aluminum foil. Oxygen-sensitive samples should be stored under nitrogen or an inert gas. Refrigeration or freezing may be necessary to protect chemically unstable samples. However, freezing should be avoided when storing unstable emulsions. Preservatives (e.g., mercuric chloride, potassium dichromate, and chloroform) [1] can be used to stabilize certain food substances during storage. To help manage the issue of proper storage conditions for various samples, some laboratories use color-coded sample cups to ensure each sample is stored properly.

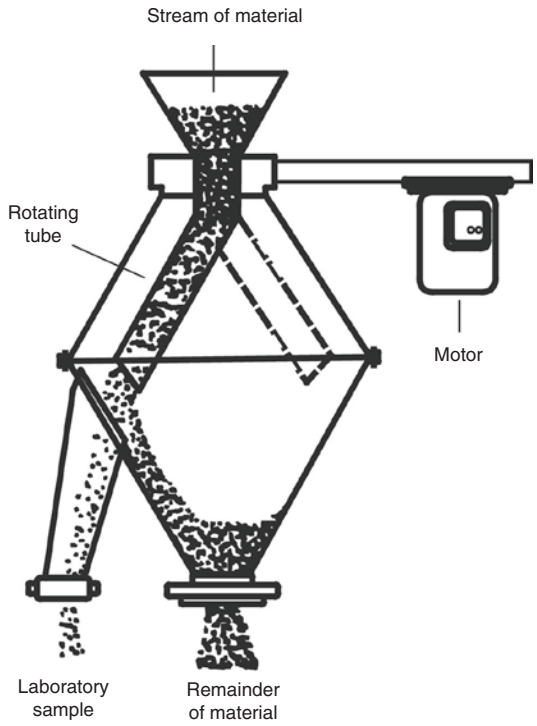
Mislabeling of samples causes mistaken sample identification. Samples should be clearly identified by markings on the sample container in a manner such that markings will not be removed or damaged during storage and transport. For example, plastic bags that are to be stored in ice water should be marked with water-insoluble ink.

If the sample is an official or **legal sample**, the container must be sealed to protect against tampering and the seal mark easily identified. Official samples also must include the date of sampling with the name and signature of the sampling agent. The chain of custody of such samples must be identified clearly.

5.5 PREPARATION OF SAMPLES

5.5.1 General Size Reduction Considerations

If the particle size or mass of the sample is too large for analysis, it must be reduced in bulk or particle size [1]. To obtain a smaller quantity for analysis, the sample can be spread on a clean surface and divided into quarters. The two opposite quarters are combined. If the mass is still too large for analysis, the process is repeated until an appropriate amount is obtained. This method can be modified for homogeneous liquids by pouring into four containers and can be automated (Fig. 5.5). The samples are thus homogenized to ensure negligible differences between each portion [2].



5.5 figure A rotating tube divider for reducing a large sample (ca. 880 kg) of dry, free flowing material to a laboratory size sample (ca. 0.2 kg) (Courtesy of Glen Mills, Inc., Clifton, NJ)

AOAC International [14] provides details on the preparation of specific food samples for analysis, which depends on the nature of the food and the analysis to be performed. For example, in the case of meat and meat products [14], it is specified in Method 983.18 that small samples should be avoided, as this results in significant moisture loss during preparation and subsequent handling. Ground meat samples should be stored in glass or similar containers, with air- and watertight lids. Fresh, dried, cured, and smoked meats are to be bone free and passed three times through a food chopper with plate openings no more than 3 mm wide. The sample then should be mixed thoroughly and analyzed immediately. If immediate analysis is not possible, samples should be chilled or dried for short-term and long-term storage, respectively.

A further example of size reduction is the preparation of solid sugar products for analysis as described

in AOAC Method 920.175 [14]. The method prescribes that the sugar should be ground, if necessary, and mixed to uniformity. Raw sugars should be mixed thoroughly and rapidly with a spatula. Lumps are to be broken by a mortar and pestle or by crushing with a glass or iron rolling pin on a glass plate.

5.5.2 Grinding

5.5.2.1 *Introduction*

Grinding is important both for sample preparation prior to analysis and for food ingredient processing. Various mills are available for reducing particle size to achieve sample homogenization [17]. To homogenize moist samples, bowl cutters, meat mincers, tissue grinders, mortars and pestles, or blenders are used. However, mortars and pestles and mills are best for dry samples. Some foods are more easily ground after drying in a desiccator or vacuum oven. Grinding wet samples may cause significant losses of moisture and chemical changes. In contrast, grinding frozen samples reduces undesirable changes. The grinding process should not heat the sample, and therefore the grinder should not be overloaded because heat will be produced through friction. For especially heat-sensitive sample, grinders can be cooled with liquid nitrogen and then ground samples are stored at −80 °C. Contact of food with bare metal surfaces should be avoided if trace metal analysis is to be performed [18].

To break up moist tissues, a number of slicing devices are available: bowl cutters can be used for fleshy tubers and leafy vegetables, while meat mincers may be better suited for fruit, root, and meat [19]. Addition of sand as an abrasive can provide further subdivision of moist foods. Blenders are effective in grinding soft and flexible foods and suspensions. Rotating knives (25000 rpm) will disintegrate a sample in suspension. In colloidal mills, a dilute suspension is flowed under pressure through a gap between slightly serrated or smooth-surfaced blades until they are disintegrated by shear. Sonic and supersonic vibrations disperse foods in suspension and in aqueous and pressurized gas solution. The Mickle disintegrator sonically shakes suspensions with glass particles, and the sample is homogenized and centrifuged at the same time [19]. Alternatively, a low shear continuous tissue homogenizer is fast and handles large volumes of sample.

Another alternative is **cryogenic grinding** or **cryogrinding**. This method is ideal for biological samples and materials that are sensitive to oxygen or temperature. However, most materials are suitable for this technique. Cryogrinding can be performed manually with a mortar and pestle after freezing the sample with liquid nitrogen. The mortar and pestle have to be prechilled with liquid nitrogen before adding the material. Also, there are several brands of specialized grinding equipment with an integrated cooling system that perform the cryogenic freezing and grinding automatically.

5.5.2.2 *Applications for Grinding Equipment*

Mills differ according to their mode of action, being classified as a **burr**, **hammer**, **impeller**, **cyclone**, **impact**, **centrifugal**, **or roller mill** [19]. Methods for grinding dry materials range from a simple pestle and mortar to power-driven hammer mills. Hammer mills wear well and they reliably and effectively grind cereals and dry foods, while small samples can be finely ground by ball mills. A ball mill grinds by rotating the sample in a container that is half filled with ceramic balls. This impact grinding can take hours or days to complete. A chilled ball mill can be used to grind frozen foods without predrying and also reduces the likelihood of undesirable heat-initiated chemical reactions occurring during milling [19]. Alternatively, dry materials can be ground using an ultracentrifugal mill by beating, impacting, and shearing. The food is fed from an inlet to a grinding chamber and is reduced in size by rotors. When the desired particle size is obtained, the particles are delivered by centrifugal force into a collection pan [19]. Large quantities can be ground continuously with a cyclone mill.

5.5.2.3 *Determination of Particle Size*

Particle size is controlled in certain mills by adjusting the distance between burrs or blades or by screen mesh size/number. The **mesh number** is the number of square screen openings per linear inch of mesh. The final particles of dried foods should be 20 mesh for moisture, total protein, or mineral determinations. Particles of 40 mesh size are used for extraction assays such as lipid and carbohydrate estimation.

In addition to reducing particle size for analysis of samples, it also is important to reduce the particle size of many food ingredients for use in specific food products. For example, rolled oats for a grain-based snack bar may have a specified granulation size described as 15% of the oats maximum passes through a #7 US standard sieve. A higher granulation (i.e., more smaller particles) would mean more fines and less whole oats in the finished bar. This would result in higher incidences of snack bar breakage.

There are a variety of methods for measuring particle size, each suited for different materials. The simplest way to measure particle sizes of dry materials of less than 50 μm in diameter is by passing the sample through a series of vertically stacked sieves with increasing mesh number. As the mesh number increases, the apertures between the mesh are smaller and only finer and finer particles pass through subsequent sieves (see Table 5.2). Sieve sizes have been specified for salt, sugar, wheat flour, cornmeal, semolina, and cocoa. The sieve method is inexpensive and fast,

5.2 table US standard mesh with equivalents in inches and millimeters

US standard mesh	Sieve opening	
	Inches	Millimeters
4	0.1870	4.760
6	0.1320	3.360
7	0.1110	2.830
8	0.0937	2.380
10	0.0787	2.000
12	0.0661	1.680
14	0.0555	1.410
16	0.0469	1.190
18	0.0394	1.000
20	0.0331	0.841
30	0.0232	0.595
40	0.0165	0.400
50	0.0117	0.297
60	0.0098	0.250
70	0.0083	0.210
80	0.0070	0.177
100	0.0059	0.149
120	0.0049	0.125
140	0.0041	0.105
170	0.0035	0.088
200	0.0029	0.074
230	0.0024	0.063
270	0.0021	0.053
325	0.0017	0.044
400	0.0015	0.037

but it is not suitable for emulsions or very fine powders [20]. A standard in the industry is the W.S. Tyler® **Ro-Tap® Sieve Shaker** (Fig. 5.6).

To obtain more accurate size data for smaller particles (<50 μm), characteristics that correlate to size are measured, and thus size is measured indirectly [21]. **Surface area** and **zeta potential** (electrical charge on a particle) are characteristics that are commonly used. Zeta potential is measured by an electroacoustic method whereby particles are oscillated in a high-frequency electrical field and generate a sound wave whose amplitude is proportional to the zeta potential. Optical and electron microscopes are routinely used to measure particle size. Optical microscopes are interfaced with video outputs and video-imaging software to estimate size and shape. The advantage of the visual approach is that a three-dimensional size and detailed particle structure can be observed.

A widespread technique to measure particle size distribution uses a principle known as **light scattering** or **laser diffraction**. When a coherent light source, such as laser beam, is pointed to a particle, four interactions of the beam with the particle can take place: reflection, refraction, absorption, and diffraction. **Reflection** is the portion of light that is rejected by the particle. **Refraction** is the light that goes through transparent or translucent materials and exits the particle

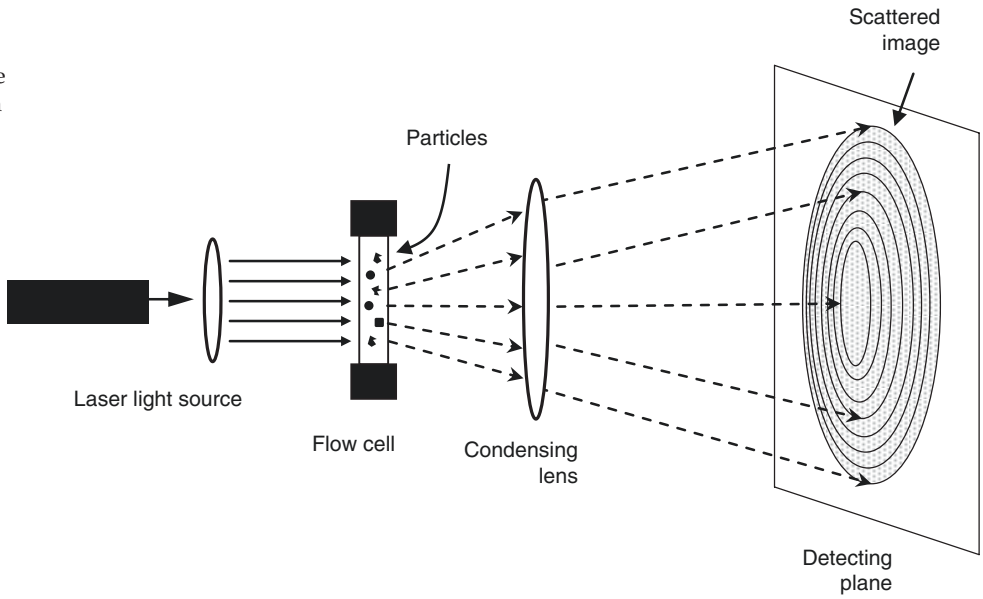
5.6 figure W.S. Tyler® Ro-Tap® Sieve Shaker (Courtesy of W.S. Tyler®, Mentor, OH)

with an unchanged wavelength, at a different angle, and generally with a lower intensity. Some of the light can be **absorbed** by the particle and re-irradiated at a different frequency, which can display as fluorescence or heat. The last interaction, **diffraction**, is the result of light interacting with the edges of the particle. This interaction makes the light spread out, or scatter, and produce wave fronts around the particle that act as secondary spherical waves. This phenomenon is similar to Young's double-slit experiment, which readers can find in a simple Google search.

The secondary waves generated at the edge of the particles interact with each other, in some areas constructively and others destructively, thus producing interference patterns that correlate with the particle size. In general, larger particles scatter light at smaller angles and with a higher intensity. Smaller particles, on the other side, scatter light at wider angles and with less intensity. These scatter patterns can be correlated with particle size by using mathematical algorithms.

Besides size, shape and optical properties affect the angle and intensity of the scattered light. When particles are capable of transmitting light, the refractive index plays a role in particle size determination. Therefore, light scattering instruments take advantage of the refractive index to improve accuracy, especially for small particles.

A typical laser diffraction particle analyzer has a sample port, a medium for the sample transport, a flow cell, a laser beam, a detector, electronics, and software. The sample is introduced into the port, which contains some dispersion system to avoid particle agglomeration. The sample is then carried by a liquid, for wet samples, or compressed air, for dry samples, to a flow cell. As the sample goes through



5.7 figure Basics of an instrument to measure particle size distribution by light scattering (laser diffraction) (Adapted from Shimadzu Corporation [22])

the flow cell, a laser beam irradiates the sample. This results in diffraction patterns that after passing through a condensing lens are projected on a detector where the diffraction angles and intensities are measured. This information is then transformed into a particle size distribution by the instrument electronics and a computer software algorithm (Fig. 5.7). Results are displayed as frequency distributions with particle size on the x-axis and frequency on the y-axis.

Determination of particle size by light scattering has many advantages, such as:

- Wide dynamic range—from the nanometer to the several millimeters range
- Suitable for wet and dry materials—such as solid in liquid suspensions, dry powders, liquid-liquid emulsions, and pastes
- Fast measurement—in the order of seconds
- No calibration needed
- Easy to use and interpret results
- Proficient at detecting mixes of large and small particles
- Technique covered by ISO standard 13320:2009

The most important disadvantages are the following:

- Assumption that particles are spherical, which introduced error in particles that deviates a lot from sphericity.
- Particles agglomeration.
- Results are expressed in volume basis, which gives more weight to large particles.
- Results cannot be compared with other size determination methods.

For particles in the nanoscale range, a widely used technique for particle analysis is **dynamic light scattering**. These instruments determine particle size and even the molecular weight of large molecules in solution. This is achieved by measurement of frequency shifts of light scattered by particles due to Brownian motion.

Understanding of the principles of the instrument used to obtain size data is vital to appreciate the limitations of each method. For example, data obtained from the same sample using sieves and light scattering will differ [21]. Sieves separate particles using square holes, and therefore they distinguish size in the smallest dimension, independent of shape. However, light scattering techniques assume that the particle is spherical, and data are derived from the average of all dimensions. Particle size measurement is useful to maintain sample quality, but care must be taken in choosing an appropriate method and interpreting the data.

5.5.3 Enzymatic Inactivation

Food materials often contain enzymes that may degrade the food components being analyzed. Enzyme activity therefore must be eliminated or controlled using methods that depend on the nature of the food. Heat denaturation to inactivate enzymes and freezer storage (−20 to −30 C) for limiting enzyme activity are common methods. However, some enzymes are more effectively controlled by changing the pH or by salting out [19]. Oxidative enzymes may be controlled by adding reducing agents.

5.5.4 Lipid Oxidation Protection

Lipids present particular problems in sample preparation. High-fat foods are difficult to grind and may

need to be ground while frozen. Unsaturated lipids are sensitive to oxidative degradation and should be protected by storing under nitrogen or vacuum. Antioxidants may stabilize lipids and may be used if they do not interfere with the analysis. Light-initiated photooxidation of unsaturated lipids can be avoided by controlling storage conditions. In practice, lipids are more stable when frozen in intact tissues rather than as extracts [19]. Therefore, ideally, unsaturated lipids should be extracted just prior to analysis. Low-temperature storage is generally recommended to protect most foods.

5.5.5 Microbial Growth and Contamination

Microorganisms are present in almost all foods and can alter the sample composition. Likewise, microorganisms are present on all but sterilized surfaces, so sample cross-contamination can occur if samples are not handled carefully. The former is always a problem and the latter is particularly important in samples for microbiological examination. Freezing, drying, and chemical preservatives are effective controls and often a combination of these is used. The preservation methods used are determined by the probability of contamination, the storage conditions, storage time, and the analysis to be performed [19].

5.6 SUMMARY

Food quality is monitored at various processing stages but 100% inspection is rarely possible or even desirable. To ensure a representative sample of the population is obtained for analysis, sampling and sample reduction methods must be developed and implemented. The selection of the sampling procedure is determined by the purpose of the inspection, the nature of the population and product, and the test method. Increasing the sample size will generally increase the reliability of the analytical results, and using t-test techniques will optimize the sample size necessary to obtain reliable data. Multiple sampling techniques also can be used to minimize the number of samples to be analyzed. Sampling is a vital process, as it is often the most variable step in the entire analytical procedure.

Sampling may be for attributes or variables. Attributes are monitored for their presence or absence, whereas variables are quantified on a continuous scale. Sampling plans are developed for either attributes or variables and may be single, double, or multiple. Multiple sampling plans reduce costs by rejecting low-quality lots or accepting high-quality lots quickly, while intermediate-quality lots require further sampling. There is no sampling plan that is risk-free. The consumer risk is the probability of accepting a poor-quality product, while the vendor risk is the probability of rejecting an acceptable product. An acceptable probability of risk depends on the seriousness of a negative consequence.

Sampling plans are determined by whether the population is homogeneous or heterogeneous. Although sampling from a homogeneous population is simple, it rarely is found in practical industrial situations. Sampling from heterogeneous populations is most common, and suitable sampling plans must be used to obtain a representative sample. Sampling methods may be manual or continuous. Ideally, the sampling method should be statistically sound. However, nonprobability sampling is sometimes unavoidable, even though there is not an equal probability that each member of the population will be selected due to the bias of the person sampling. Probability sampling is preferred because it ensures random sampling and is a statistically sound method that allows calculation of sampling error and the probability of any item of the population being included in the sample.

Each sample must be clearly marked for identification and preserved during storage until completion of the analysis. Official and legal samples must be sealed and a chain of custody maintained and identified. Often, only a portion of the sample is used for analysis and sample size reduction must ensure that the portion analyzed is representative of both the sample and population. Sample preparation and storage should account for factors that may cause sample changes. Samples can be preserved by limiting enzyme activity, preventing lipid oxidation, and inhibiting microbial growth/contamination.

5.7 STUDY QUESTIONS

1. As part of your job as supervisor in a quality assurance laboratory, you need to give a new employee instruction regarding choosing a sampling plan. Which general factors would you discuss with the new employee? Distinguish between sampling for attributes vs. sampling for variables. Differentiate the three basic sampling plans and the risks associated with selecting a plan.
2. Your supervisor wants you to develop and implement a multiple sampling plan. What would you take into account to define the acceptance and rejection lines? Why?
3. Distinguish probability sampling from nonprobability sampling. Which is preferable and why?
4. (a) Identify a piece of equipment that would be useful in *collecting* a representative sample for

analysis. Describe precautions to be taken to ensure that a representative sample is taken and a suitable food product that could be sampled with this device. (b) Identify a piece of equipment that would be useful for *preparing* a sample for analysis. What precautions should be taken to ensure that the sample composition is not changed during preparation?

5. For each of the problems identified below that can be associated with collection and preparation of samples for analysis, state one solution for how the problem can be overcome:
   (a) Sample bias
   (b) Change in composition during storage of sample prior to analysis
   (c) Metal contamination in grinding
   (d) Microbial growth during storage of product prior to analysis

6. The instructions you are following for cereal protein analysis specify grinding a cereal sample to 10 mesh before you remove protein by a series of solvent extractions:
   (a) What does 10 mesh mean?
   (b) Would you question the use of a 10-mesh screen for this analysis? Provide reasons for your answer.

7. You are to collect and prepare a sample of cereal produced by your company for the analyses required to create a standard nutritional label. Your product is considered "low fat" and "high fiber" (see information on nutrient claims and FDA compliance procedures in Chap. 3). What kind of sampling plan will you use? Will you do attribute or variable sampling? What are the risks associated with sampling in your specific case? Would you use probability or nonprobability sampling, and which specific type would you choose? What specific problems would you anticipate in sample collection, storage, and preparation? How would you avoid or minimize each of these problems?

REFERENCES

1. Horwitz W (1988) Sampling and preparation of samples for chemical examination. J Assoc Off Anal Chem 71: 241–245
2. Puri SC, Ennis D, Mullen K (1979) Statistical quality control for food and agricultural scientists. G.K. Hall and Co., Boston, MA
3. Harris DC (2015) Quantitative chemical analysis, 9th edn. W.H. Freeman and Co., New York
4. Miller JC (1988) Basic statistical methods for analytical chemistry. I. Statistics of repeated measurements. A review. Analyst 113: 1351–1355
5. Lichon, MJ (2000) Sample preparation for food analysis, general. In: Meyers RA (ed) Encyclopedia of analytical chemistry: applications, theory and instrumentation. John Wiley, New York
6. Lichon MJ (2004) Sample preparation, ch. 44. In: Nollet LML (ed) Handbook of food analysis, 2nd edn. Vol 3. Marcel Dekker, New York, pp. 1741–1755
7. Mälkki Y (1986) Collaborative testing of methods for food analysis. J Assoc Off Anal Chem 69(3):403–404
8. FDA (2016) Sampling to protect the food supply (last updated 5/5/2016) http://www.fda/gov/Food/ComplianceEnforcement/Sampling/ucm20041972.htm
9. Pehrsson PR, Haytowitz DB, Holden JM, Perry, CR, Beckler DG (2000) USDA's national food and nutrient analysis program: food sampling. J Food Comp Anal. 13:379–389
10. Pehrsson P, Perry C, Daniel M (2013) ARS, USDA updates food sampling strategies to keep pace with demographic shifts. Procedia Food Sci 2:52–59
11. IUPAC (1997) Compendium of chemical terminology, 2nd edn. (the "Gold Book"). Compiled by McNaught AD Wilkinson A. Blackwell Scientific, Oxford. XML online corrected version: http://goldbook.iupac.org (2006-) created by Nic M, Jirat J, Kosata B; updates compiled by Jenkins A. ISBN 0–9678550–9–8. doi:10.1351/goldbook
12. Weiers RM (2007) Introduction to business statistics, 6th edn. South-Western College, Cincinnati, OH
13. NIST/SEMATECH (2013) e-Handbook of statistical methods, chapter 6: process or product monitoring and control. http://www.itl.nist.gov/div898/handbook/
14. AOAC International (2016) Official methods of analysis, 20th edn., (On-line). AOAC International, Rockville, MD
15. Anonymous (2016) Code of federal regulations. Title 21. US Government Printing Office, Washington, DC
16. Baker WL, Gehrke CW, Krause GF (1967) Mechanism of sampler bias. J Assoc Off Anal Chem 50: 407–413
17. Anonymous (2016) Code of federal regulations. 21 CFR 101.9 (g), 9 CFR 317.309 (h), 9 CFR 381.409 (h). US Government Printing Office, Washington, DC
18. Pomeranz Y, Meloan CE (1994) Food analysis: theory and practice, 3rd edn. Chapman & Hall, New York
19. Cubadda F, Baldini M, Carcea M, Pasqui LA, Raggi A, Stacchini P (2001) Influence of laboratory homogenization procedures on trace element content of food samples: an ICP-MS study on soft and durum wheat. Food Addit Contam 18: 778–787
20. Kenkel JV (2003) Analytical chemistry for technicians, 3rd edn. CRC, Boca Raton, FL
21. Jordan JR (1999) Particle size analysis. Inside Lab Manage 3(7): 25–28
22. Shimadzu Corporation (2016) Particle Size Distribution Calculation Method. Lecture on Partical Analysis – Hands-on Course http://www.shimadzu.com/an/powder/support/practice/p01/lesson22.html [Accessed on June 29, 2016]