LECTURE 4
STANDARD SAMPLE PREPARATION PROCEDURES FOR FOOD ANALYSIS

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1 Introduction

Quality attributes in food products, raw materials, or ingredients are measurable characteristics that need monitoring to ensure that specifications are met. 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. Moreover, in the case of food products, analyzing a whole population would be practically impossible because of the destructive nature of most analytical methods.

2 Major steps in sampling procedure

The first step in any sampling procedure is to clearly define the population that is going to be sampled. By population we mean the entire lot or the entire production for a certain period of time, in the case of continuous processes. The population may vary in size from a production lot, a day's production, to the contents of a warehouse.

The ideal population 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 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 procedures can make the sample representative of the population or take heterogeneity into account in some other way.

The second step in the sampling procedure is distinguished as sample collection which means that a particular number of items is drawn from the population. The sample

must be drawn at random. 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 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.

Consequent steps in sampling procedure include sample preparation, laboratory analysis, data processing, and interpretation. If the procedure is done correctly, the measurable characteristics obtained for the samples become a very accurate estimation of the population. 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.

3 Sampling purposes

Most sampling is done for a specific purpose, and the purpose may dictate the nature of the sampling approach. Sampling purposes vary widely among different food industries; however, the most important categories include the following:

- Nutritional labeling
- Detection of contaminants and foreign matter
- Statistical process control
- Acceptance of raw materials and ingredients
- Release of lots of finished product
- Detection of adulterations (counterfeits)
- Microbiological safety
- Authenticity of food ingredients, etc.

Types of samples analyzed in a quality assurance program for food products are listed in Table 1.

Table 1. Types of samples analyzed in a quality assurance program for food products

Sample Type	Critical Questions
Raw materials	Do they meet your specifications? Do they meet required legal specifications? Are they safe and authentic? Will a processing parameter have to be modified because of any change in the composition of raw materials? Are the quality and composition the same as for previous deliveries? How does the material from a potential new supplier compare to that from the current supplier?
Process control samples	Did a specific processing step result in a product of acceptable composition or characteristics?

	Does a further processing step need to be modified to obtain a final product of acceptable quality?
Finished product	Does it meet the legal requirements? What is the nutritive value, so that label information can be developed? Or isthe nutritive value as specified on an existing label? Does it meet product claim requirements (e.g., “low fat”)? Will it be acceptable to the consumer? Will it have the appropriate shelf life? If unacceptable and cannot be salvaged (*собирать и использовать утильсырьё*), how do you handle it (trash? rework? seconds?)
Competitor’s sample	What are its composition and characteristics? How can we use this information to develop new products?
Complaint sample	How do the composition and characteristics of a complaint sample submitted by a customer differ from a sample with no problems?

4 The choice of sampling plans

A sampling plan is a well-organized document that establishes the goals of sampling, the factors to be measured, sampling points, frequency, size of sample, personnel, preservation of the samples, etc. Depending on the purpose of the sampling plan, samples are taken at different points of the food production system. The choice of a sampling plan is an important consideration, especially when monitoring food safety by measurement of fungal toxins, named mycotoxins, in food systems. The choice of a sampling plan is affected by:

- Purpose of the inspection;
- Nature of the product;
- Nature of the test method;
- Nature of the population being investigated (Table 2).

Table 2. Factors affecting the choice of a sampling plan

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 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?
Nature of the population being investigated	Is the lot large but uniform? Does the lot consist of smaller, easily identifiable sublots? What is the distribution of the units within the population?

Sampling plans are designed for examination of either attributes or variables. Codex Stan 234-1999 "Recommended methods of analysis and sampling" provides references for methods of sampling by commodity categories and names.

4.1 Inspection by attributes

For the inspection by attributes sampling is performed to decide on the acceptability of a population. based on whether the sample possesses acertain characteristic or not (e.g., presence of *Clostridium botulinum*). An individual unit can only be good or defective. The sample must be drawn at random.

Sampling plans by attributes are based on the hypergeometric (Gaussian), binomial, or Poisson statistical distributions and are provided by particular standards. Any sampling plan is distinguished by the sample size, the acceptance number and the rejection number. For example, ISO 5538:2004 "Milk and milk products -- Sampling -- Inspection by attributes" stipulates the following parameters: the sample size *n*, the acceptance number *Ac*, and the rejection number *Re* in the following way (fig.1):

Table 1 — Inspection Level I — AQL = 2,5 %

Lot size	Normal inspection			Tightened inspection			Reduced inspection		
	n	Ac	Re	*n*	Ac	Re	*n*	Ac	Re
Up to 150	5	0	1	8	0	1	2	0	1
151 to 500	20	1	2	32	1	2	8	0	2
501 to 1 200	32	2	3	32	1	2	13	1	3
1 201 to 3 200	50	3	4	50	2	3	20	1	4
3 201 to 10 000	80	5	6	80	3	4	32	2	5
10 001 to 35 000	125	7	8	125	5	6	50	3	6
35 001 to 150 000	200	10	11	200	8	9	80	5	8
150 001 to 500 000	315	14	15	315	12	13	125	7	10
Over 500 000	500	21	22	500	18	19	200	10	13

Figure 1. Sample size, the acceptance number and the rejection number as a function of a lot size (from ISO 5538:2004)

Note: AQL is the Acceptable Quality Limit, ranging from 2,5% to 10,0%.
(2,5%; 4,0%; 6,5%; 10%)

Example 1. For a lot size ranging from 151 to 500 items, the sample size n=20, Ac=1, Re=2 (see fig. 1). This means that if a sample of 20 units contains no defectives or 1 defective, the lot shall be accepted. If the sample contains 2 defectives or more, the lot shall be rejected.

The sample size is determined according to the lot size. Selection of a particular sampling plan (Normal, Tightened or Reduced Inspection) depends largely upon the nature of defects which are likely to appear. All the possible defects can be classified as critical, major and minor defects and are featured in Table 3.

Table 3. Classification of defects

Type of defect	Definition	Examples	Affect on Sampling Plan
Critical defects	make the population of products unacceptable	heavy metals and pesticide residues at a critically high level	Ac=0. It means that the whole lot is rejected
Major defects	make the product unfit for the sale or processing	composition defects, integrity of packaging, visible contamination with dirt	Sampling plans using AQL ≤6,5%.
Minor defects	Fail to comply with standard, but still fit for use and sale	Small abnormalities in appearance	Sampling plans using AQL ≤10%.

Critical defects form a special category. They make the population of products unacceptable. No percentage of critical defects is tolerable. The acceptance number *Ac* is, of course, always zero. If a critical defect is found, this does not mean that the defective item is put into a different box and the inspection continues. It means that the whole lot is rejected. Examples include heavy metals and pesticide residues at a critically high level. A defective is a unit which contains more than the critical level of the contaminant.

The contract, the standard or specification shall clearly define all critical, major or minor defects.

When using reduced inspection, if the acceptance number is exceeded but the rejection number is not reached, the population should be accepted but inspection should revert to normal inspection.

Example 2. For reduced inspection of a lot size ranging from 151 to 500 items the sample size n=8, Ac=0, Re=2 (see fig. 1). This means that if a sample of 20 units contains 1 defective, the lot shall be accepted, but further inspection should revert to normal inspection. If the sample contains 2 defectives, the lot shall be rejected.

4.2 Inspection by variables

For the inspection by variables 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, and each characteristic should be sampled for separately when possible.

Standard variable characteristics of food products can be stipulated in three different ways:

1. By the lower specification limit *L;*
2. By the upper specification limit *U;*
3. By double specification limits *L and U.*

For example, according to the US Code for Federal Regulation the content of cocoa fat in cocoa powder should be ≤22 % and ≥10 %. In this case the content of cocoa fat is stipulated by double specification limits: the lower specification limit *L* is 10 %, while the upper specification limit *U is* 22 %.

Any sampling plan for inspection by variables is distinguished by the sample size *n*, and the acceptability constant of the sampling plan *k*. For example, ISO 8197:1988 "Milk and milk products -- Sampling -- Inspection by variables" stipulates the abovementioned parameters in the following way (fig.2):

Table A.1 – Inspection level 1 — AQL = 1 %

Lot size	Normal inspection		Tightened inspection		Reduced inspection	
	n	*k*	*n*	*k*	*n*	*k*
Up to 50	4	1,45	5	1,65	4	1,34
51 to 90	5	1,53	5	1,65	4	1,34
91 to 150	7	1,62	7	1,75	4	1,34
151 to 280	10	1,72	10	1,84	4	1,34
281 to 500	15	1,79	15	1,91	5	1,40
501 to 1 200	20	1,82	20	1,96	7	1,50
1 201 to 3 200	25	1,85	25	1,98	10	1,58
3 201 to 10 000	35	1,89	35	2,03	15	1,65
10 001 to 35 000	50	1,93	50	2,08	20	1,69
35 001 to 150 000	75	1,98	75	2,12	25	1,72
150 001 to 500 000	100	2,00	100	2,14	35	1,76
500 001 and over	150	2,03	150	2,18	50	1,80

Figure 2. Sample size, and the acceptability constant of the sampling plan *k* as a function of a lot size (from ISO 8197:1988)

The acceptability constant of the sampling plan *k* is to be compared with parameters Q_L and Q_U:

$$Q_L = \frac{\bar{x} - L}{s} \qquad (1)$$

$$Q_U = \frac{U - \bar{x}}{s} \qquad (2)$$

where *L* is the lower specification limit, *U is* the upper specification limit, *x* is an arithmetic mean, and s is the standard deviation.

The lot should be considered to comply with the requirements if

a) in the case of a lower specification limit:

$$Q_L \geq k$$

b) in the case of an upper specification limit:

$$Q_U \geq k$$

c) in the case of a double specification limit:

$$Q_L \geq k \text{ and } Q_U \geq k.$$

5 Acceptance sampling

Acceptance sampling is a very broad topic that can be applied to any field. Acceptance sampling serves a very specific role: to determine if a shipment of products or ingredients has enough quality to be accepted. Acceptance sampling can be used for evaluation of attributes or variables, or a combination of both. Acceptance sampling plans fall into the following categories: single, double, multiple, sequential, and skip plans.

5.1 Single sampling plans

In **single sampling plans**, the decision of accepting or rejecting a lot is based just on one sample of items taken at random. 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. That brings us to double sampling plans.

5.2 Double sampling plans

Fig. 3 shows an example of a **double sampling** plan which means that two samples are taken.

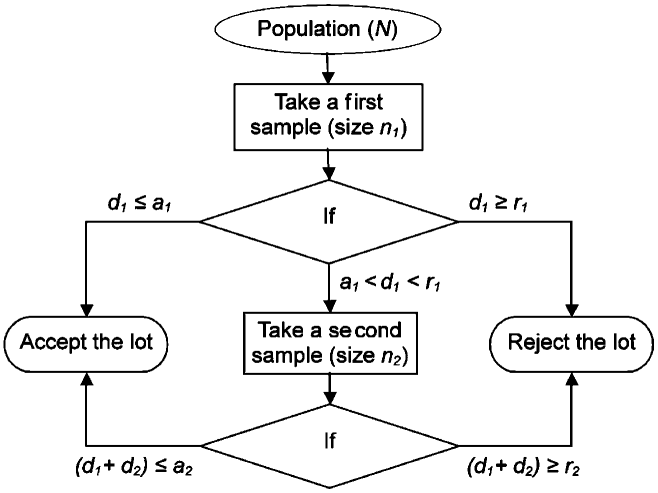

Figure 3. A double sampling plan

N is a population size; n_1 and n_2 are the sample size, a_1 and a_2 are acceptance numbers; r_1 and r_2, are rejection numbers; d_1 and d_2 are numbers of defectives/ nonconformities.

5.3 Multiple and sequential sampling plans

Multiple sampling plans are extensions of **double sampling plans** for which more than two samples are drawn to reach a conclusion. The ultimate extension of multiple sampling is sequential sampling. Under **sequential sampling** plan a sample is taken, and after analysis a decision of accepting, rejecting, or taking another sample is made. A sequential sampling graph is presented in Fig. 4. In this chart 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.

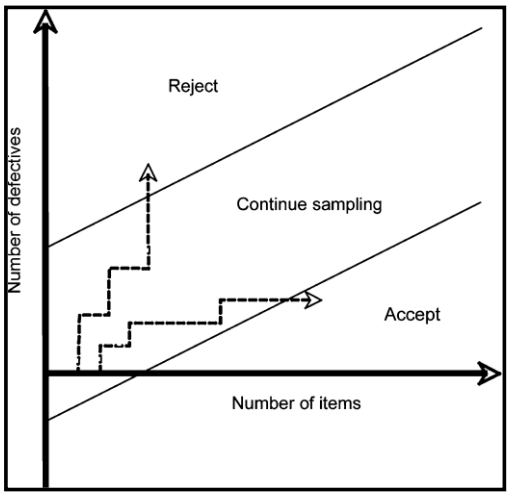

Figure 4. A sequential sampling graph.

Both multiple and sequential sampling plans reduce costs by rejecting low-quality lots or accepting high-quality lots quickly, while intermediate quality lots require further sampling.

6 Standard reference materials

A major consideration for determining method validity is the analysis of materials used as controls, often referred to as **standard reference materials** or **check samples**. Analyzing check samples concurrently with test samples is an important part of quality control. Standard reference materials can be obtained from particular national organizations: in Russia from National Service for reference materials, in the USA from the National Institute of Standards and Technology (NIST) and from US Pharmacopeia, in Canada from the Center for Land and Biological Resource Research, in Europe from the Institute for Reference Materials and Measurements (IRMM), and in Belgium from the Community Bureau of Reference (BCR).

Besides government related groups, numerous organizations offer check sample services that provide test samples to evaluate the reliability of a method.

For example, AACC International (the American Association of Cereal Chemists) offers check samples such as flours, semolina, and other cereal-based samples, for analyses such as moisture, ash, protein, vitamins, minerals, sugars, sodium, total dietary fiber, soluble and insoluble dietary fiber, and β-glucan. Samples also are available for testing physical properties and for microbiological and sanitation analyses.

Standard reference materials are important tools to ensure reliable data. However, such materials need not necessarily be obtained from outside organizations. Control samples internal to the laboratory can be prepared by carefully selecting an appropriate type of sample, gathering a large quantity of the material, mixing and preparing to ensure homogeneity, packaging the sample in small quantities, storing the samples appropriately, and routinely analyzing the control sample when test samples are analyzed.

7 Sample Preparation Management

Preparation of samples involves size reduction by grinding, mixing, etc. Collected samples should be sealed and a label attached, reproducing integrally the identification of product, the nature of the product and, at least, the identification number, name and signature (or initials) of the authorized person responsible for taking the samples.

Samples should be accompanied by a report, signed or initialed by the authorized sampling personnel and countersigned (as far as necessary or agreed by the parties concerned) by witnesses present.

The report should include at least the following information:

a) the place, date and time of sampling (the time only being required when agreed between the parties concerned);

b) the names and designations of the authorized sampling personnel and of any witnesses;

c) the precise method of sampling, including sample preparation and homogenization techniques;

d) the nature and number of units constituting the consignment, together with their batch code markings,

where available;

e) the identification number and any code markings of the batch from which the samples were taken;

f) the number of samples duly identified as to the batches from which they were taken;

g) if necessary, the place to which the samples are to be sent;

h) if possible, the name and address of the producer or trader or of the persons responsible for packing the product.

8 Conclusions

- Food quality is monitored at various processing stages but 100% inspection is rarely possible, or even desirable. Sampling is a vital process, as it is often the most variable step in the entire analytical procedure. To ensure that a representative sample of the population is obtained for analysis, standard sampling methods must be developed and implemented.
- The selection of the sampling procedure is determined by the purpose of the inspection, the food product, the test method, and the characteristics of the population.
- 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.
- Each sample must be clearly marked for identification and preserved during storage until completion of the analysis.

Discussion points

1. Identify at least three reasons you might need to determine certain chemical characteristics of a food product as part of a quality management program.
2. 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.
3. What precautions should be taken to ensure that the sample composition is not changed during preparation?

Resource materials

[1] ISO 707:2008 Milk and milk products -- Guidance on sampling
[2] ISO 5538:2004 Milk and milk products -- Sampling -- Inspection by attributes
[3] ISO 8197:1988 Milk and milk products -- Sampling -- Inspection by variables
[4] Codex Standard 234-1999 “Recommended methods of analysis and sampling”
[5] Codex Alimentarius Commission – http://www.codexalimentarius.net
[6] National Institute of Standards and Technology – http://www.nist.gov