FOOD ANALYSIS: THEORY and PRACTISE

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This course covers the principles of chemical and instrumental methods for the qualitative and quantitative analyses of food components (moisture, protein, carbohydrate, lipids, minerals and vitamins), linking them to the basic chemical structures and properties of moisture, protein, carbohydrate, lipids, minerals and vitamins and their roles in food systems.

COURSE OBJECTIVES:

-Provide a knowledge on analytical methods developed for elucidating the composition of foods, their chemical and physical properties as well as for investigating food authenticity;

-Apply this knowledge with particular emphasis on application of analytical chemistry to foods by providing various selected examples for individual food commodity types.

It is intended to give some background information on principles of of methods developed for food analysis.

Fundamental principles and applications will be stressed rather than details of procedures.

Nothing can replace actual hands-on laboratory experience as a learning tool; however, this course is attempting to make a synopsis of both conventional and novel techniques and the theories underlying them.

COURSE OUTLINE

1.Introduction : Bodies engaged in food analyses, standards used, legislative aspects

2.Sampling and techniques : Related statistical concepts, types of samples and sampling, and sample handling

3.Proximate analysis for nutrition labelling : A compendium of all available methods for moisture, protein , lipid, carbohydrate analyses, as well as the analyses for quantifying and characterizing individual components of proteins, lipids and carbohydrates, the respective principles, procedures, applications, cautions, advantages and disadvantages of individual methods

4. Wet and dry ashing, low temperature plasma ashing, microwave ashing, principles and instrumentation, applications, post ashing procedures (alkalinity and solubility of ash)

5. Mineral analyses on ash: gravimetric, titrimetric, colrimetric methods, ion-selective electrodes, atomic absorption and atomic emission spectroscopies, ICP-AES

6. Analysis of vitamins (bioassays, microbiological assays, physico-chemical methods, UV-vIS spectrophotometry, fluorometry, liquid chromatography)

7. Enzymes: Determination of enzymic activity, applications of enzymes in other analyses as analytical aids

Student presentations on selected topics:

- 8. Contaminants : Mycotoxins, pesticides, animal drugs

- 9. Food additives: sulphurdioxide, food dyes (qualitative and quantitative)

- 10.Analyses for authenticity testing

- 11. Analysis of sensory attributes: Objective methods for color and texture analyses
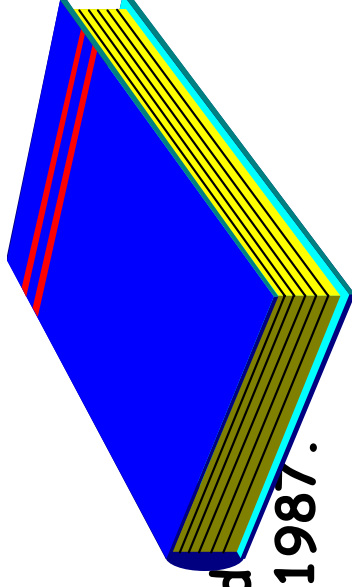
COURSE EVALUATION CRITERIA

Midterm Examination: 30%
Final Examination   : 50%
Assignment   : 20%

- **TEXTBOOK:**
- "Food Analysis, S. Nielsen, 1998.

Reference Book: Food Analysis, Theory and Practice, Y. Pomeranz and C.E. Meloan, 1987.

I. INTRODUCTION

Why Foods Are Analyzed?

1. For routine controls (Official Surveillance) on commodities on the food market, conducted for:

a) Health reasons-vital commodities, affect health directly; to see that they are wholesome and healthy

b) Economical reasons- for trade and quality aspects

c) Nutritional Labelling

Definition of " Adulteration":

All kinds of cheating; i.e. removal of a constituent that should be present; addition of a substance to increase bulk or weight, to make it appear better than it really is, to reduce its strength; partial or complete substitution of one product for another, or concealment of an inferior or damaged product.

Objectives are:
- Protecting consumers from adulteration

- Protecting producers from dishonest and unfair competition.
Individual examples of food adulteration include use of unpermitted additives and cases where a valuable constituent is deliberately removed

2. For quality controls by industrial firms.

3. Research purposes

4. For industrial development (both to improve the product quality and to formulate novel products).

FOOD SAFETY:

Only way to know for sure that a food is safe to eat is to run physical, chemical, biological and sensory analysis. This requires a "tripod" system:

1. You have to have official standards and specifications, where identity and quality criteria of foods are clearly defined and the analyzed samples have to conform to these. (TSE, ISO, Codex Alimentarius Standards and sometimes buyer technical specifications.)

2. Official methods of analysis:

(International and National Standardized Methods)

The methods of the following international associations form the basis of Turkish official methods given in TGKY and TS's:

AOAC-Association of Official Analytical Chemists

AACC-Association of Cereal Chemists

AOCS-Association of Oil Chemist Society

IUPAC-International Union of Pure and Applied Chemists, Food Chemicals Codex

AOAC = Association of Official Analytical Chemists.

- Publishes Official Methods of Analysis and the prestigious periodical " J. of AOAC".

- Organized in 1884 by USState & Federal Chemists.

- Initially covered fertilizer analyses only; now cover foods, drugs, cosmetic and agricultural products.

Every five years these are revised, by including newly developed methods, discarding plausible ones. Now their online versions are available upon purchase.

3. Legislations and officially authorized bodies to apply these.

For example, in European community, all exports and imports have to conform to EC regulations and standards . In USA there are the Code of Federal Regulations, USDA Standards, EPA Pesticide Regulations, Food Additives Amendment etc. In Turkey we have the TGKY.

Furthermore, there is need for also duly accredited laboratories for the results of analyses to be officially recognised.

In Turkey, we have relevant Turkish standards, Turkish Food Codex. (Previously GMT-Turkish Food Act).

Authorized governmental organizations are:

- Ministries of Agriculture and of Health

- Municipalities

SOURCES OF INFORMATION

Sometimes the standards mentioned above are not sufficient and you have to develop your own method. For this, you have to search further in scientific literature.

In searching literature, first of all you have to define **keywords** for the individual subject. Then, proceed with the following sources:

A. General Sources

1. An advanced textbook -- theory and references allowing you to get even more depth and information, or a handbook.

2. Food organizations publish standard methods specific to certain types of foods.

   a. Standard Methods for Examination of Dairy Products published by the American Public Health Association

   b. American Association of Cereal Chemist's "Approved Methods of Analysis" for standard methods relating to dairy and cereal products.

   c. American Oil Chemists Society also publishes standard methods for testing oil and oil based products.

B. Computerized Library Searches

Nowadays there are modern information retrieval systems; examples include:

1. FSTA:

Food Science and Technology Abstracts on CD-ROMS, which start from 1969 and cover more than1200 journals published in 50 countries. Turkish Journals, Gida, and Gida Teknolojisi are included in this source.

2. Comprehensive Online Databases of:

1) USDA : United States of Department of Agriculture

2) Agricola : Include individual reports

3) FAO :PhD and master thesis on food -reletad issues.

General articles in the area - will contain methodology Abstracts from Chemical, Biological, and Agricultural journals - on-line through FSTA and require only a university email account (use your username and password to logon). You can also go to Central Library and search various databases using the CD-ROM equipment available in the reference area.

*These seldom go back further that 1990, but do not forget that these searches miss the logical development associated with reading whole articles (with citations), and miss reading the field - you get only your key word returns.

C. Reviews

You might consult reviews (i.e.Critical reviews in food science, surveys, or "Advances in food science", "Progress in food science") which describe the current available knowledge in the particular field. These can be assessed via:

1. Chemical Abstracts - This publication comes out annually and gives a bibliography of all chemical reviews.

2. "Analytical Chemistry" - every April - broad and complete coverage of analytical areas of chemistry.

3. "Advances in Food Research"- "Critical Reviews in Food and Nutrition", "Progress in Food and Nutrition Science", and "Recent Advances in Food Science" are good sources of review information.

D. Abstracts

1. **Chemical Abstracts** - publish over 200,000 abstracts per year from about 10,000 journals.

2. **Current Contents** - lists more than 1,000 journals in Agriculture and related areas.

3. **"Food Technology"** is published by the Institute of Food Technologists and often contains information that is very useful to the food analyst.

4. **Dairy Science Abstracts** publishes abstracts related to dairy products.

E. Theses

Thesis studies conducted on the subject are covered in "Dissertation Abstracts". Generally contain very good literature review and more detailed approaches to experiments than literature published in scientific journals. "Chemical Abstracts" can be used to search theses by subject.

F. Symposia

Proceedings of symposia contain individual articles that may have detailed methods section; but often are of space - variable quality and are not complete in their coverage of a subject.

G. Trade Publications

e.g. "Food Analysis" "Food Product Testing" "American Laboratory" - may contain some good information on advertisements in these periodicals - though be skeptic when reading the claims

QUALITY ASSURANCE AND QUALITY CONTROL IN LABORATORY EXPERIMENTS

TS EN ISO/IEC 17025 (2000)
"Deney ve Kalibrasyon Laboratuvarlarının Yeterliliği için Genel Şartlar"

Standard Laboratory Practices

- Sample handling before the laboratory
- Sample handling in the laboratory
- Instrumentation
- Reagents and standards
- Analytical procedures
- Standard operating procedures

Sample Handling Before the Laboratory

- Collection procedures
- Representativeness of sample collection
- Collection devices, e.g., pumps
- Containers, e.g., acid-washed bottles
- Preservatives, e.g., acid, cold room, freezing
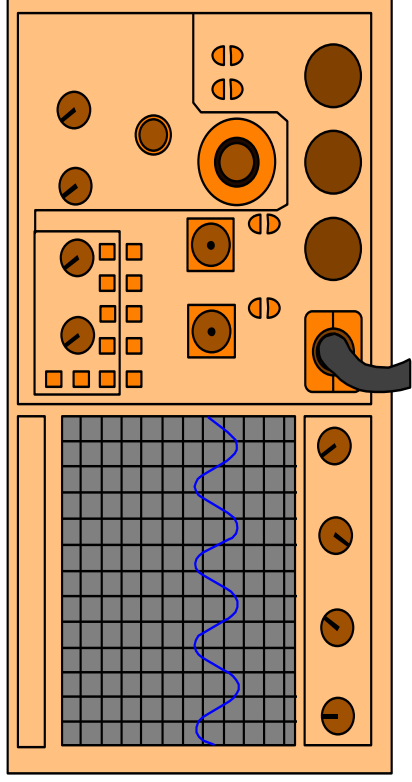- Transportation of samples
- Chain of custody

Sample Handling in the Laboratory

- Lab ID number
- Storage location
- Holding times
- Disposal
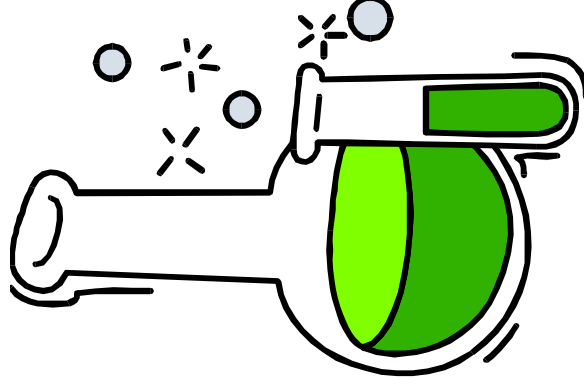
Instrumentation

- Is it appropriate for the task?
- Is it calibrated?
- Is it maintained?
- Is it clean before and after use?

Reagents and Standards

- Purity
- Preparation
- Standardization
- Labeling
- Shelf life
- Disposal

Analytical Procedures

- *Watch one, do one, teach one*
- Plan ahead
- Start small
- Read the SOP and understand the principles
- Do not take shortcuts.
- Document everything.

Standard Operating Procedures

- Detailed instructions
- Specific for a given lab
- Hazards are known, understood, and respected
- Regular maintenance and updating or review
- Promote uniformity of procedure to minimize random variations and maximize comparability of data

QUALITY ASSESSMENT TECHNIQUES

- Replicates
- Random duplicates
- Spiked samples
- Standard analytes
- Internal test samples
- Interlaboratory comparison
- Control charts

Replicates

- Repetitive measurement on the same unknown sample
- Good assessment of precision
- Report average of results
- Time-consuming and costly

Random Duplicates

- Randomly select one sample per analytical batch

- For example, every 10-15 samples

Spiked Samples

- Known addition of analyte to a real sample
- Measure spiked sample and unspiked samples
- Compute % recovery
- Helps to account for matrix effects
- Helps to assess both accuracy and precision

Standard Analytes

- Pure materials made into standards to use regularly
- Used to calibrate instrumentation
- Assessment of accuracy
- Control chart

Internal Test Samples

- Real material
- Composition similar to unknowns
- Well-characterized material
- Control chart data

Method Validation
Interlaboratory Comparisons

Control Charts

- When to throw out an observation if there is no known source of error? A control chart can offer a relatively unbiased way of deciding if something is really wrong in the analysis.

- The charts document the precision of an analysis

- They allow us to identify trends in the data

Plotting Control Charts

- x-axis temporal scale: date, time sequence
- y-axis: analytical results, difference between results, deviation from true value, % recovery of theoretical value
- Use long-term means and calculated standard deviations for a standard sample.

Warning and Rejection Levels

- Warning levels: e.g., 1 standard deviation

- Rejection levels: e.g., 3 standard deviations

Rejection Criteria (typical)

- any point outside 3s
- two points in a row outside 2s
- four in a row outside 1s
- two in a row with a range of >4s
- ten in a row on the same side of the mean

Out-of-Control Data

- Reject the data set that is run with that control

- Correct the problem

- Repeat, if possible

- Use with disclaimer, if necessary

Components of Method Reliability(p.57-61)

The selected method should possess:

1. Reproducibility : depends on differences among different laboratories which perform the same analysis

2. Repeatability : depends on differences within the same laboratory

3. Freedom from systematic error

4. Specificity for analyzed property

5. Negligible limit of errors

Accuracy : The degree to which a mean estimate approaches the true value.

Precision : The degree to which two determinations yield consistent results.
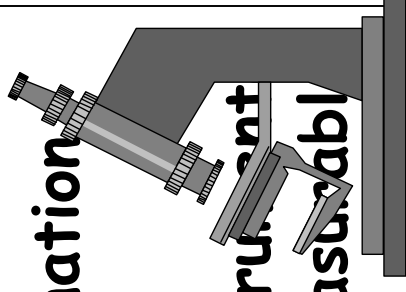
Sensitivity : Ratio between magnitude of instrument response and amount of analyte (smallest measurable difference between two samples)

For determining these properties, known amounts of the analyte at three different(low, intermediate, high) concentrations are spiked to 7 replicates each on blank samples, and analysed. $\bar{X}$, ranges of standard deviations and coefficients of variations are determined for each level to yield values for accuracy, precision, and sensitivity
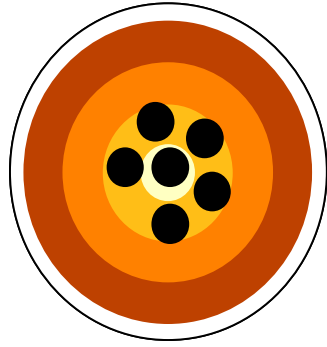
Accuracy and Precision
(Chapter 4)
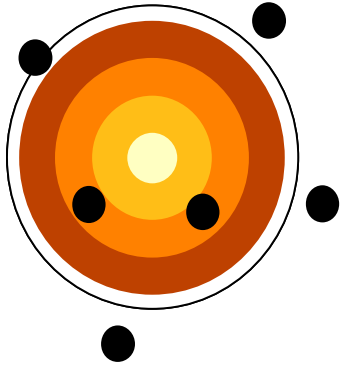
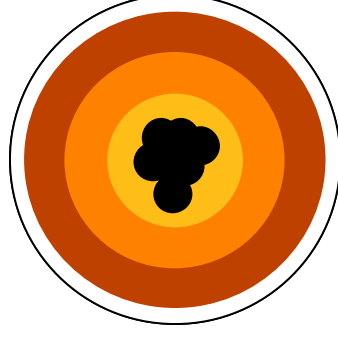**1.**
Accuracy:Poor
Precision:Poor
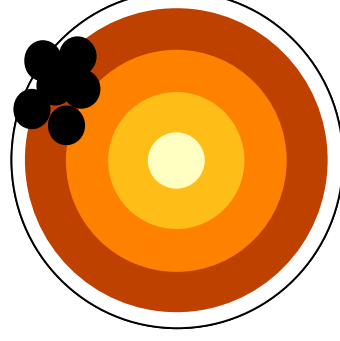
2.
Accuracy:Poor
Precision:Good

3.Accuracy:Good
Precision:Poor

4.
Accurate
Precise

- The "ERRORS"(any deviation from accuracy) might be either:

- "random-nonreproducible, uncontrollable"

- Or

- "systematic-repeats itself, and can be under control".

- For example, if results of analyses are always too high, that could be because the standards are of low potency. Such errors might also come from analyst's way of calculation or weighing, or from reagents and equipments, or environmental conditions-bright sunlight, high humidity, etc. So the analyst should be capable of thinking critically in assessing these situations (Read next slides in Turkish for learning more about errors).

Record-Keeping and Data Processing

- Keep original field notes

- Data transfer

- Sample identity

- Computer processing

Computer Processing

- Save raw data and intermediate steps
- Save programs and macros (to retrace steps)
- Document data files, programs, macros, etc.
- Back-up frequently
- Store data in two places
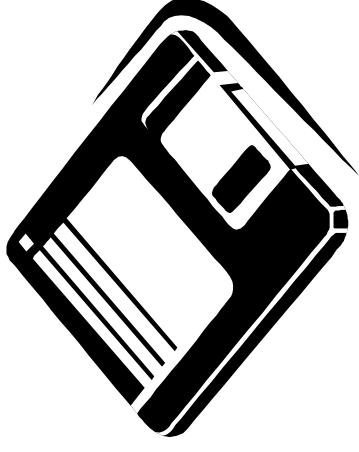
Field Notebooks

- Separate notebooks for different parts of project
  1. raw vs. processed data
  2. separate by type of measurement

- Loose-leaf, remove chronological data sheet to take to field

- Document reams of automated data (instead of including in notebook)
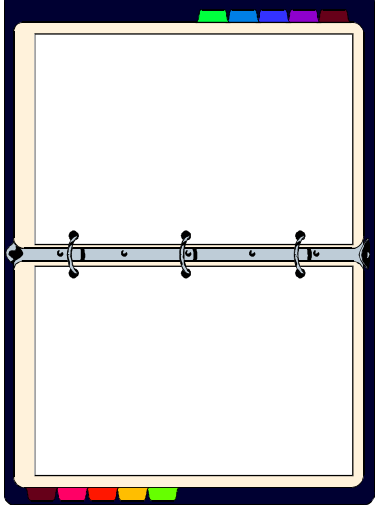
ERROR ASSESSMENT

- Systematic Errors

- Random Errors

- Error Analysis and Significant Figures
  (Measurement of Uncertainty:Ölçüm Belirsizliği)

Systematic Errors

- Operator error

- Instrument error

Random Errors

- Operator error
- Noise and drift in electronic circuits
- Temperature variations
- Building vibrations
- Passing traffic
- Humidity
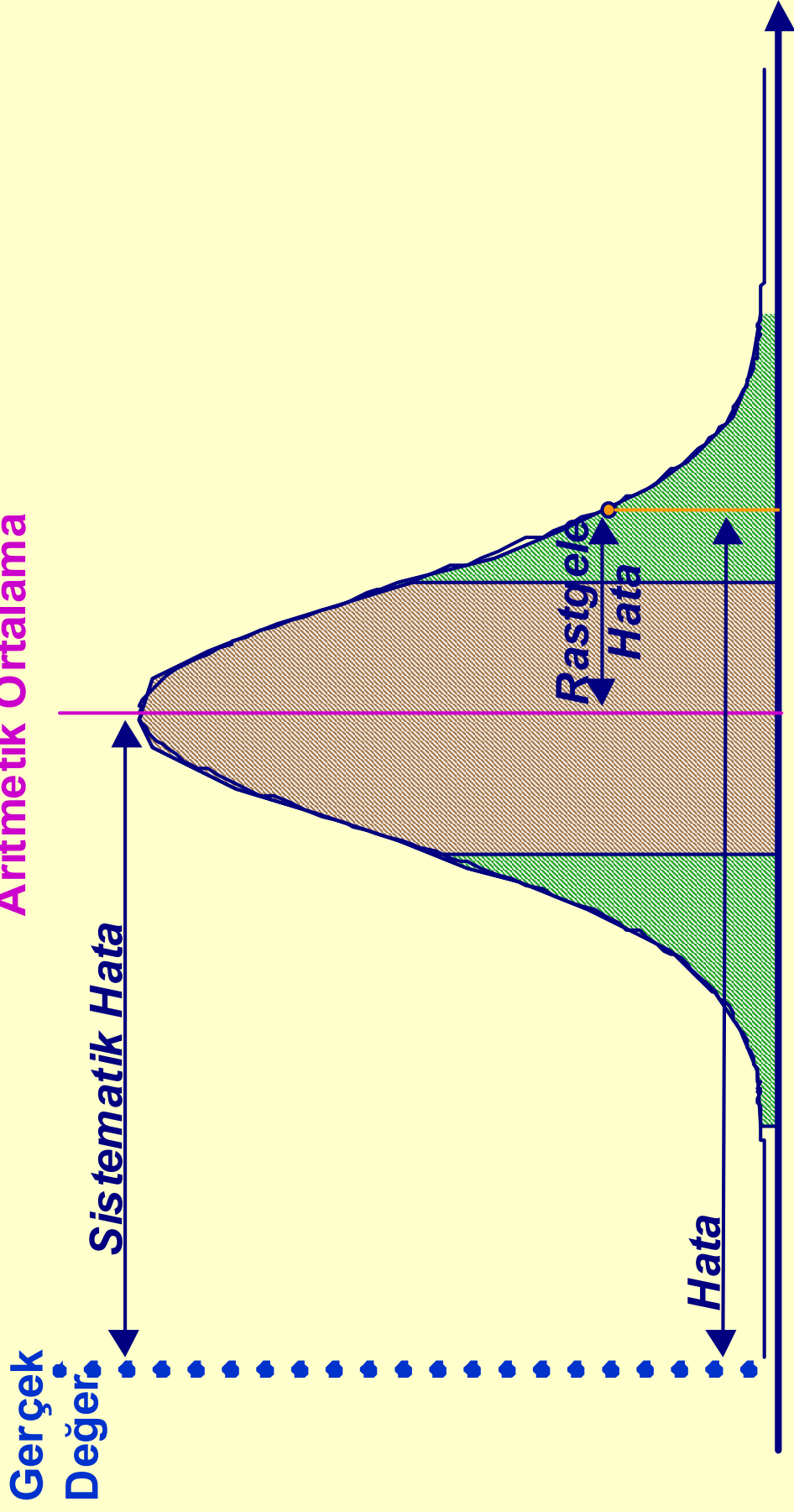
II. SAMPLING and Sample Preparation (Chapter 5)

SAMPLE

The sample should be representative of the whole material if the analysis of the sample is to have any meaning.

Sample should be a composite that adequately samples from each of the populations within the product to be analyzed.

Sampling is the process of drawing or selecting containers or sample units from a lot or production. As a result of sampling, information is obtained by which an estimate can be made to accept, reject or negotiate the merchandise in question. Sampling procedures which contain both sample size and acceptance criteria are commonly referred to as "acceptance sampling".

There are many types of acceptance sampling systems in use today. A plan that is suitable for one product or type of inspection may be entirely unsuitable for another product or inspection system. The plan selected is determined to a large extent by the degree to which it satisfies the needs of the user.

- In developing acceptance sampling plans, initial consideration should be given to quality evaluation of the end product. This requires opening of containers with resultant loss of products. This type of inspection is referred to as "destructive sampling". Not only is the loss of product an important consideration, but also destructive sampling is generally quite time consuming,

- Consequently, both inspection time and economic loss of product through destructive inspection are significant limiting factors in developing sampling plans for quality evaluation of processed foods. Sample size must necessarily be relatively small in order to make the plan practical in application.

RISKS

The aim of any sampling plan should be to accept more "good" lots and reject more "bad" lots. Since probability and chance are involved, decisions will, of necessity, involve an element of risk. This risk factor has to be accepted as a part of any sampling procedure. One method of reducing the buyer's risk of accepting deliveries of non-conforming quality is to increase sample size. In other words, the larger the sample, the less risk involved in accepting "bad" lots.

"Inspection level" is the term indicating the relative amount of sampling and inspection performed on lots of a given product or class of products.

The validity of conclusions drawn from analysis depends very highly on sampling.

Population : Any finite group or collection having a property that distinguishes items which belong from which don't belong.

Sample: A piece or item that shows the quality of the population from which it was taken.

Sampling: Selection from a population of a finite number of samples for analysis.

Aliquot (Test portion): That quantity of sample sufficient for measuring the property of interest.

Steps in sampling are:

1. Identification of population
2. Identification of sampling scheme
3. Collection of .-representative samples
4. Reduction to aliquot size

In order to be truly representative, sampling should conform to statistical norms. However, a general rule is:

$$n = c\sqrt{N}$$

Here,

n: Number of samples taken from population,

N: The population (# of individual units in population)

For example, if there are 2200 boxes each containing 24 sausages, that makes 52800 units. There are tables for each specific type of commodity.

(i.e. if weight of unit is<1kg, take 229 samples, if w>1kg, take 48 samples.)

C: A factor that is <1 for homogenous properties, and c>1 for heterogeneous samples. In less than 28 observations we can cover the range "±3s".

Official Sampling Plans :
These include:

1. Inspection Levels ;For normal trading purposes Level I is recommended. In the case of dispute or controversy, i.e. for Codex referee purposes, Level II is recommended.

2. Sample Sizes in relation to lot size and container size; and

3. Acceptance Numbers .
A sample is drawn at random from the lot according to the appropriate schedule in the Sampling Plans.
Each sample unit is examined according to the requirements of the individual Codex Standard and classified as either "acceptable" or as "defective". Based on the total number of "defectives " in the sample, the lot either "meets" or "fails" the requirements of the Codex standard.

•Plans apply, according to the following criteria:

- Meets if the number of "defectives" is equal to, or less than, the acceptance number of the appropriate plan.

- Fails if the number of "defectives" exceeds the acceptance number of the appropriate plan.

• **Next slides give a tabular presentation appropriate for acceptance sampling of prepackaged foods where an AQL* of 6.5 has been accepted for certain product characteristics.**

•AQL* :Acceptable Quality Level. This characteristic is defined as "the maximum percent defective units in lots that will be accepted most of the time (approximately 95percent of the time)". Lots or production containing more defective material will be accepted less often –the ratio of rejection to acceptance increasing as the sample size increases and as the percent defective material in the lot increases.

Summary of The Turkish Food Codex Sampling Plans

A. (For routine controls- LEVEL I)

A.1. IF NET WEIGHT IS 1 KG or LESS:

(N)*	(n)**	(c)***
<4800	6	1
4801-24000	13	2
24001-48000	21	3
48001-84000	29	4
84001-144000	48	6
144001-240000	84	9
>240000	126	13

Here,

* N: The population

**n= Number of samples taken from population

***c= acceptable number of nonconforming samples

A.2.IF NET WEIGHT IS > 1 KG ,but < 4,5 KG :

(N)	(n)	(c)
<2400	6	1
2401-15000	13	2
15001- 24000	21	3
24001-42000	29	4
42001-72000	48	6
72001-120000	84	9
>120000	126	13

A.3.IF NET WEIGHT is >4,5 :

(N)	(n)	(c)
<600	6	1
601-2000	13	2
2001-7200	21	3
7201-15000	29	4
15001-24000	48	6
24001-42000	84	9
>42000	126	13

B. (For suspicious samples)LEVEL 2

B.1. IF NET WEIGHT IS 1KG or LESS:

(N)	(n)	(c)
<4800	13	2
4801-24000	21	3
24001-48000	29	4
48001-84000	48	6
84001-144000	84	9
144001-240000	126	13
>240000	200	19

B.2. IF NET WEIGHT IS > 1 KG but< 4,5 KG :

(N) (n) (c)

<2400	13	2
2401-15000	21	3
15001-24000	29	4
24001-42000	48	6
42001-72000	84	9
72001-120000	126	13
>120000	200	19

B.3.IF NET WEIGHT IS > 4,5 KG :

(N) (n) (c)

<600	13	2
601-2000	21	3
2001-7200	29	4
7201-15000	48	6
15001-24000	84	9
24001-42000	126	13
>42000	200	19

In using the Sampling Plans , the following information shall be known:

a Container size (net weight in kg or lb)

b Inspection Level

c Lot size (N)

d Requirements of the Codex Standard with respect to product quality (i.e. classification of defectives and requirements for acceptance of the lot).

The following steps are taken in INSPECTION

a The appropriate inspection level is selected as follows :

Inspection Level I - Normal sampling

Inspection Level II - Disputes (Codex referee purposes sample size), enforcement or need for better lot estimate.

·b Determine the lot size (N), i.e. number of primary containers or sample units.

·c Determine the number of sample units (sample size (n)) to be drawn from the inspection lot, consideration being giving to container size , lot size , and inspection level.

·d Draw at random the required number of sample units from the lot giving proper consideration to code or other identifying marks in selection of the sample .

·e Examine the product in accordance with the requirements of the Codex Standard. Classify any container or sample unit which fails to meet the specified quality level of the standard as a defective on the basis of the classification of defectives contained in the Codex Standard.

·f Refer to the appropriate Sampling Plan

It is not necessary to restrict the sample size to the minimum corresponding to the appropriate lot size and Inspection Level. In all cases a larger sample may of course be drawn.

Statistical concepts are influenced greatly by particle size and homogeneity. Sometimes, food contamination may be very heterogenic. The attribute is in some cases unevenly distributed in food throughout the sample.

Macroheterogenity implies differing concentrations among units of lots

Microheterogenity implies differing concentrations among parts of units

OJECTIVES OF SAMPLING

Food samples are collected for:

1. Commercial transactions like imports, exports or local purchases

2. Routine quality controls-both official and auto controls:

    -by official authorities-to see that they conform to predetermined specifications.

    -by plant personnel for auto-controls (or for surveillance of processes).

3. Complaint samples from customers/ consumers

4. Investigating competitor's samples

5. Research and development -should be based on statistical experimental designs

The information on the following should be included in an experimental or a sampling plan:

-the objective of the investigation including information on the components or organisms to be determined;

-the parties involved (client, person taking the sample, laboratory, etc.)

-the nature of the sample, sampling location and time of sampling;

-the number of samples, the way which they are to be taken, packed and transported (requirements on sterility, containers and equipment, sampling model, etc.):

-any requirements regarding the pre-treatment of samples and the selection of analytical methods;
-the time and cost requirements (for the entire investigation, for the person taking the sample, for the laboratory);
-any possible legal requirements and international agreements which have to be observed;
-the requirements on documentation (on sampling, reports from the laboratory, the client's own summary); and
-the quality assurance aspects of the investigation (the client's own activities, any requirements on the person taking the sample, the laboratory and any others involved).

Sampling should be carried out aiming at ensuring that the sample is representative of the consignment to be investigated.

Established information which will help in the consideration of the development of sampling plans and procedures are the following:

1. The instructions on Sampling for the Codex Commodity Committees on the Application of the General Principles of Codex Sampling Procedures. This document was developed by the Codex Committee on Methods of Analysis and Sampling and is currently undergoing revision in the light of the new role and importance of the Codex Alimentarius Commission.

2. The Code of Practice on Sampling for Analysis or Examination prepared under the UK Food Safety Act 1990.

3. International Commission on Microbiological Specifications for Foods.

TYPES OF SAMPLING

1. Random Sampling: The bulk of the sample material is divided into a number of segments and samples are selected according to a predetermined pattern. For example, sampling plan may be based on generated random numbers. You have to give equal chance for each batch for selection (i.e. you either select from Random numbers or by drawing numbers from a bag or taking every n'th sample- i.e. for official quality controls)

- 2. Systematic Sampling: You should have a predetermined pattern to base your experimental design. Applications are In investigating certain property as effected by certain processing parameters.You have to draw samples from various points of process to test changes in composition with changes in parameters of process (mostly research and development purposes). You can base your samples on preset design types like "central composite design".

Subsampling: With large initial sample size, you will have to decrease the sample-size till you reach the required aliquot sample size.

- For this purpose, after spreading the whole sample on a wide surface, use quartering with a straight edge and mixing only the two of the opposite quarters.

- Sometimes, samples from different batches can be admixed to form a "composite-sample", which(in Turkish "paçal") is an admixture prepared by adding one subsample to another (of 2 or more portions of same size, weight or volume)

"Laboratory Samples":

In general glass and metal containers are preferred to plastic and paper containers. Plastic and paper containers do not permit transfer of water vapour or air in or out. Hermetically sealed jars are far better.

Some samples are :

Labile: That which is easily decomposed

Thermolabile: destroyed easily by heat(especially vitamins, enzymes and fatty acids).

For these, you have to minimize the time of experiment. Sometimes you may have to put them in refrigerated containers or a freezer. Ideally you should freeze all the samples if you wont analyze them immediately, but this is quite expensive.

Laboratory Sample Sizes

Average or optimal food sample size is 250 gr for unpacked foods, but this is very much correlated with the particle size of units of population. For example:

- Spice samples <100 gr
- Fruits (apples) = 1 kg
- Hazelnuts = 250 gr

Also, for microbiological analysis of heterogeneously contaminated samples(i.e. Moulds)>500 g of sample is required.

Sample Preparation for Nutritional Labelling

- Most analytical experiments are very expensive. You should minimize the number of samples you take and take care that no changes occur from sampling to analysis stage

- The ideal samples for Nutrient Analysis should consist of   a composite of 12 sub-samples (consumer units) randomly chosen - 1 from each of 12 cases.

Guidelines for sample handling

1-Proper Sample identification (Date, who took the sample-the inspector or the analyst-, the population it represents, temperature should be specified in a distinctive manner.)

2-Reserving duplicate samples (şahit numune)

3-Securing seals for samples of official signficance, so that the container cannot be opened without breaking the seal.

4-Securing against probable damage (ie, insects, moulds, etc) by fumigation.

5-Securing against chemical & microbiological spoilage of labile components(Low To or frozen storage for thermolabiles, at low moisture,in dark places, under N^2 gas, with enzyme inactivation)

Pretreatments for Preserving Samples:

1. Clean to remove any surface contamination .

2. Dry for enzyme inactivation; by heat or by chemicals Ex: to shift to the optimal pH, add $(NH_4)_2SO_4$.

3. Storing in hermetic containers-glass and metals are preferred to paper and plastics. For avoiding any contact with air, keep under N_2 or dissolve in solvents to cut off O_2, and add antioxidants or preservatives for additional precaution. For samples susceptible to microbiological attack, use presterilized "asceptic" sample containers, and carry in ice-bags(0-10°C) .

4. Freeze or use dry ice wrapped in a paper and placed in chamber for thermolabile components (thermal treatments) In case of freezing, care should be taken in thawing very slowly, and not losing away any thawed liquids.

5. Pesticide applications may help against risk of insect infestation. In some cases where food materials may deteriorate due to parasites, you have to apply pesticides (first on a blotting paper, then in a closed container) . Fumigants may also be used for avoiding insect infestation, (CHCl3 or paradichloro benzene).

6. Grind to desired particle size

Homogenization of Laboratory Samples

You have to homogenize samples before analyses. For this purpose, first of all you have to decide on the basis of reporting your results.

Is it going to be on the edible portion (hazelnut→ only inner part)? If so, then get rid of inedible portion. However, if you are going to analyse apples for pesticides, you should not remove the skin part, because the pesticides are on the outer part and apples may be consumed with skins. Also of help for homogenizing are apparatus for:

-Mechanical grinding (mills)
-Blending; grating; stirring etc.

Ideal particle size for most analysis are (0.5-1 mm) or in mesh size (20/40 mesh).

- 20 mesh means there are 20 openings per 1 linear inch of a screen, this corresponding to ~o.1mm particle or diameter.

- 40 mesh corresponds to 40 openings per linear inch or ~0.06mm particle diameter.

20/40 mesh means that sample passes through sieve with mesh size 20, but is retained on sieve with mesh size 40.

In most cases, optimum particle size is indicated in the analysis method.

GENERAL ANALYTICAL STAGES

- Analysis might or might not destroy the initial composition.

- Both destructive and non-destructive techniques are available for most analyses

I. CLEAN-UP: Removal of other interfering substances.

II. CONCENTRATION or Dilution to within detection limits: Sometimes you have to bring in detection limits by direct dilution or by concentration.

III. DETERMINATION

I. CLEANUP: Many separation clean-up techniques are available. These are:

1. Extraction-Partitioning of materials between two phases depending on relative solubilities of analyte in the two phases. Examples: Solid-liquid and Liquid-liquid Extractions. In choosing the solvent system, the solvent selectivity and relative solubility is important.

In supercritical gas extractions, Supercritical CO_2 has increased solubility at lower temperatures;It is preferred because it is a nontoxic and practical gas with low boiling point(thus producing no heat damage) and is very environmental friendly. It is being used for recovering antioxidants and vegetable oils. Within some analytical methods, we also apply separatory funnels (ayirma hunisi) for liquid liquid extractions.

2. Distillation- Redistributes molecules between phases. All liquids have a specific vapor pressure that is constant at a given temperature. When the temperature ($T°$) is raised to that point so that vapour pressure of substance equals the external pressure, the substance boils.

Examples are "Fractional distillation" for samples with rather narrow boiling point range, and "Steam distillation" which are applied especially for heterogeneous liquid mixtures consisting of substances not soluble in each other like water. i.e. volatile water-insoluble materials can be thus separated from nonvolatiles.

3. Adsorption: Depends on the affinity or property of binding on solid surfaces. Example is

Column chromatography, involving selective desorbing by properly selected solvent systems (gases in liquid in GC, liquids in liquid in HPLC). All chromatographic systems apply adsorption/desorption principles.

Other clean-up techniques include "Crystallization", "Filtration", "Derivatization".

II. CONCENTRATION to Within Detection Limits

. In most cases it is done by evaporating in rotary vacuu
evaporators. The high temperatures might be harmful fo
thermolabile components; then you have to make it unde
vacuum and at lower temperatures.

III. DETERMINATION

. Quantification is realized by titration, or gravimetry, or
by computer controlled integration. But the method used
has to be validated and confirmed by recovery trials,
where a known amount of reference grade standard is
added to the blank and replicate analyses are performed
in automated methods.

 User-programmable laboratory robots are available now
Supervisory minicomputers are used for database
management and report generation.

Good Laboratory Practice
(İyi Laboratuar Uygulaması)

- Laboratuvarların test yaparken uymaları gereken koşulları ve organizasyonu belirleyen yönetim sistemidir.

- Amacı: Yapılan analitik ölçümlerin kalitesini güvence altına almak

GLP Ana Prensipler-2

- Standard Çalışma Prosedürlerine göre çalışma (SOP)
- Test ve kontrol konularının tanımlanmış olması
- Yazılı ve onaylı protokollara göre çalışma
- Ölçüm sonuçlarının rapor olarak sunulması
- Ölçüm sonuçlarına istendiğinde erişebilme

Ölçüm Doğruluğu
(Accuracy of Measurement)

Ölçüm sonucu ile ölçülen büyüklüğün gerçek değeri arasındaki yakınlık derecesi.

Ölçüm Doğruluğu $(x - \tau)$

$\tau =$ gerçek değer

$x =$ ölçüm sonucu

" Gerçeklik + Kesinlik "

Hata
(*Error*)

Ölçüm sonucundan, ölçülen büyüklüğe ait gerçek değerin çıkartılması ile elde edilen değer.

$$\text{Hata} = x - \tau$$

$\tau = $ **gerçek değer**

x = ölçüm sonucu

Hata Çeşitleri

- **Prosedür hataları**

  Tartım, Titrasyonda son noktanın tam anlaşılamaması, tam çözememe, emisyon spektroskopisinde kaynaktan gelen osilasyonlar, interferans, vs

- **Ölçüm hataları**
  - Sistematik hatalar
  - Rastgele hatalar

Rastgele Hata
(Random error)

Tekrarlanabilirlik koşulları altında aynı ölçüm sonsuz sayıda yapıldığında, her bir ölçüm değerinin sonsuz sayıdaki ölçümün ortalamasından çıkartılması ile elde edilen değerdir.

$$\text{Rasgele Hata} = X_i - X_{ort}$$

Sistematik Hata
(Systematic error)

Tekrarlanabilirlik koşulları altında gerçekleştirilen, aynı ölçülen büyüklüğe ait birbirini izleyen sonsuz sayıdaki ölçümün ortalamasından, ölçülen büyüklüğün gerçek değerinin çıkartılması ile elde edilen değerdir. Genelde belirsizlikle ifade edilir.

Sistematik Hata = $X_{ort} - \tau$

X_{ort} = ölçümün ortalaması τ = gerçek değer

Bağıl Hata
(*Relative Error*)

Ölçüm hatasının ölçülen büyüklüğün gerçek değerine bölünmesi ile elde edilen değer.

Eğilim
(Bias)

Ölçüm metodunun sistematik hatasıdır, ulaşılmak istenen değerden sapmayı gösterir. Sistemin offset'idir.

Uygun bir Referans Maddesi ile belirlenebilir.

- Metod
- Laboratuar
- Personel
- Matriks

Gerçek Değer
(*True value*)

Ele alınan belli bir büyüklüğün tanımına karşılık gelen ve ancak ideal bir ölçüm ile elde edilecek bir hipotetik değerdir.

$$x = \tau + \Delta + \delta = \mu + \delta$$

x = ölçüm sonucu τ = gerçek değer

Δ = eğilim, bias δ = rastgele hata

μ = beklenen değer

Kesinlik
(Precision)

Aynı ölçüm koşullarında yapılan ölçüm sonuçlarının birbirine yakınlığıdır ve sistemin tekrarlanabilirliğinin bir ölçüsüdür.

Tekrarlanabilirlik

(Repeatability)

Aynı ölçüm koşulları altında gerçekleştirilen, aynı ölçülen büyüklüğe ait birbirini izleyen ölçüm sonuçları arasındaki yakınlık derecesi.

Tekrar Gerçekleştirilebilirlik
(Reproducibility)

Farklı ölçüm koşulları altında gerçekleştirilen, aynı ölçülen büyüklüğe ait biribirini izleyen ölçüm sonuçları arasındaki yakınlık derecesi.

Duyarlılık
(Hassasiyet)
(Sensivity)

Bir ölçüm cihazının elektronik yanıt sinyalindeki değişimin bunu yaratan etki sinyalindeki değişime oranı.

Seçicilik
(Specificity)

Metodun sadece amaçlanan maddeyi ölçümü ölçme yeteneği

Metodun Sağlamlığı
(Ruggedness-Robustness)

Bir metodu uygularken yapılan ufak tefek operasyonel değişikliklerin sonucu etkileme durumudur.

Ölçüm Belirsizliği
(Uncertainty)

Ölçüm sonucu ile beraber yer alan ve ölçülen büyüklüğe makul bir şekilde karşılık gelebilecek değerlerin dağılımını karakterize eden parametredir.

Ölçüm hataları genelde belirsizlik içinde ifade edilir.

Belirsizliğin Nedenleri

- Numune alma (temsili numune)
- Numune alma veya numune hazırlama sırasındaki bulaşmalar
- Ekstraksiyon verimi
- Matriks etkileri ve etkileşimler
- Çevresel koşulların ölçüm işlemine etkilerinin bilinmemesi
- Volümetrik ekipmanların, terazilerin belirsizliği
- Cihazların çözünürlüğü
- Analog cihazların okunması
- Ölçüm metodundaki yuvarlama ve varsayımlar

Kalibrasyon
(Calibration)

Belli koşullarda ölçüm sisteminin gösterdiği değer ile ölçülen büyüklüğün gerçek değeri arasındaki bağıntıyı bulmak için yapılan işlemlerdir.

Kalibrasyon Eğrisi
(Calibration Curve)

Ölçüm sinyalinin ölçümü yapılan büyüklüğe karşı değerinin grafiksel gösterimidir.

Tayin Limiti
(Limit of Determination- Quantitation)

Bir ölçüm sistemi ile analite ait belirlenebilen en düşük değerdir. Kalibrasyon aralığının en düşük değeridir.

Kör örnekle yapılan ölçümlerin standart sapmasının 6 katı alınarak hesaplanır. .

Belirleme Limiti
(Limit of detection)

Ölçüm sistemi ile belli bir örnek içerisinde belirlenebilen en düşük miktardır.

Kör örnekle yapılan ölçümlerin standard sapmasının 3 katı alınarak bulunur.

Validasyon
(Validation)

Bir sistemin belirlenen özel amaçlara uygunluğunun objektif olarak test edilerek onaylanmasıdır.

Metod Validasyonu: Bir metodun performansını belirlemek için yapılan sistematik test / ölçüm ve istatistik değerlendirme çalışmalarıdır.

Belirsizliğin Nedenleri

- Numune alma (temsili numune)
- Numune alma veya numune hazırlama sırasındaki bulaşmalar
- Ekstraksiyon verimi
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