

**manuals  
of food quality control**

**7. food analysis:  
general techniques,  
additives,  
contaminants,  
and composition**



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**prepared by fao with the support of the  
swedish international development authority (sida)**

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## FOREWORD

The control of food safety and quality is an integral part of national programmes for development. National food control systems are designed to protect the health and welfare of the consumer, to promote the development of trade in food and food products, and to protect the interests of the fair and honest food producer, processor or marketer against dishonest and unfair competition. Emphasis is placed on the prevention of chemical and biological hazards which result from contamination, adulteration or simple mishandling of foods. Also important are the maintenance of general food quality and the control of the use of food additives and food processing procedures.

In order to establish a workable food control system, a national government must:

1. Enact food control legislation.
2. Promulgate regulations to enforce that legislation.
3. Create an agency to conduct the enforcement.
4. Establish food inspection and analysis staff within the agency or agencies concerned.
5. Provide physical facilities including a food control laboratory.

To assist the national governments of developing countries in this process, FAO, with the support of the Swedish International Development Authority (SIDA) has published the series Manuals of Food Quality Control. These are incorporated as part of the FAO Food and Nutrition Paper Series No. 14, and include:

No. 14/1	The Food Control Laboratory
No. 14/2	Additives, Contaminants, and Techniques (out of print)
No. 14/3	Commodities (out of print)
No. 14/4	Microbiological Analysis
No. 14/5	Food Inspection
No. 14/6	Food for Export
No. 14/7	Food Analysis: General Techniques, Additives, Contaminants, and Composition
No. 14/8	Food Analysis: Quality, Adulteration, and Tests of Identity

In addition, FAO, WHO and UNEP jointly have published many guidelines and other documents designed to further assist developing countries in forming adequate food control systems. These publications include:

Methods of Sampling and Analysis of Contaminants in Food -  
A Report of the Second Joint FAO/WHO Expert Consultation,  
Rome - 1978

Guidelines for Establishing or Strengthening National Food  
Contamination Monitoring Programmes - FAO Food Control  
Series No. 5 - 1979

Guidelines for the Study of Dietary Intakes of Chemical Contaminants - WHO Offset Publication No. 87 - 1985

Guide to Codex Recommendations concerning Pesticide Residues, Part 2 - Maximum Limits for Pesticide Residues, Second Preliminary Issue - Rome - 1985

Recommended Practices for the Prevention of Mycotoxins in Food, Feed and their Products - FAO Food and Nutrition Paper No. 10, Rome - 1979

Food Standards, Codes of Practice and Methods of Analysis Recommended by the Codex Alimentarius Commission - Joint FAO/WHO Food Standards Programme (several titles)

Food Additive Evaluations and Specifications of Purity and Identity - Reports and Monographs of the Joint FAO/WHO Expert Committee on Food Additives (several titles)

The above publications, and others, are available to persons and organizations. AO is also interested in receiving comments regarding this volume and suggestions for future improvement. Please send to:

The Chief  
Food Quality and Standards Service  
Food Policy and Nutrition Division  
Food Agriculture Organization of the  
United Nations  
Via delle Terme di Caracalla  
00100 Rome, Italy

AO wishes to acknowledge the generous support of the Swedish International Development Authority (SIDA), in the preparation of this volume, and the efforts of Mr. J. Weatherwax and Mr. P.G. Martin who were responsible for the preparation of the text.

#### **SPECIAL NOTE**

The methods and analytical procedures described in this Manual are designed to be carried out by properly trained personnel in a suitably equipped laboratory. In common with many laboratory procedures, the methods quoted frequently involve hazardous materials.

For the correct and safe execution of these methods it is essential that laboratory personnel follow standard safety procedures for the handling of hazardous materials.

While the greatest care has been exercised in the preparation of this information, FAO expressly disclaims any liability to users of these procedures for consequential damages of any kind arising out of or connected with their use.

The methods are also not to be regarded as official because of their inclusion in this Manual. They are simply methods which have been found by experience to be usable in the average laboratory.

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## 1. SCOPE OF THIS MANUAL OF FOOD ANALYSIS

This Manual includes discussions of analytical techniques as well as analytical methods generally applicable to all foods. Specific methods used only with certain foods are covered in the Manual, "Food Analysis: General Quality, Adulteration, Identity."

The techniques discussions cover all physical manipulations during analysis as well as how to effectively use analytical equipment, instruments and glassware. Proper technique is all important for analytical results to be consistent, reproducible and valid. The manipulation techniques shown will keep error at a minimum if followed exactly. It is therefore recommended that they be adopted, even if another technique has been previously used.

The general methods are those for additives, contaminant residues, and compositional (proximate) analysis in foods. The following precautions apply to all of the procedures:

1. Use only distilled water or the equivalent. (Deionized water is often suitable).
2. Use the best grade of reagent chemicals available and purify if necessary.
3. Follow the method instructions exactly, as many of the procedures are empirical.
4. Use all laboratory safety procedures and equipment.

The reference citations at the end of appropriate sections gives both the references listed in the text, as well as some general references which may provide background information.

The first edition of this Manual was written in 1977 by Mr. Peter G. Martin presently of Lyne, Martin and Radford, Public Analysts, Reading, Berkshire, England. The present revised edition has been prepared with Mr. Martin's support and assistance by Mr. John R. Weatherwax, retired Laboratory Director for the United States Food and Drug Administration, Los Angeles, California, USA.

## 2. SAMPLE PREPARATION TECHNIQUES

### 2.1 SUBSAMPLING

The way in which a sample is taken for analysis is the first of a series of potential sources of error in food analysis. Some liquid foods are reasonably homogeneous, but solid and semi-solid foods are always heterogeneous. It must be assumed that the attribute for which the food is being examined, is unevenly distributed throughout the sample.

The laboratory usually has no control over the field sampling of the food product and must assume that the portion received for analysis is representative of the lot of food sampled. (Note that field sampling is covered in detail in Chapter 3 of FAO Food and Nutrition Paper 14/5, Manual of Food Quality Control #5 - Food Inspection). The laboratory sample as received may be bagged, packaged, tinned or bottled and most often includes multiple units. The laboratory analyst must first decide what type of subsampling should be done on the sample received. The analyst may make the subsamples representative or selective depending on what is to be determined in the analysis. If the analyst merely wishes to prove that something exists without worrying about its relationship to the whole, then a selective sample is taken. An example would be spotty decomposition or mould contamination. The sample could be inspected visually or by odour and only the suspect parts removed for examination. Most analyses, however, involve comparison to a standard for the whole, so that representative portions must be taken.

The taking of a representative sample is obviously the more difficult of the two. A liquid food (e.g. milk) generally need only be well mixed or shaken before subsampling. Semi-solid foods are those containing a solid material plus a large portion of free liquid. Examples include many canned foods. In the event that the solid or the liquid are to be analyzed individually, they are separated using a sieve or filter and individually mixed for subsampling. When both solid and liquid phases are to be analyzed as a unit, it is often advisable to blend or otherwise homogenize the two before subsampling.

Solid samples can be of three general types, namely finely divided (e.g. whole cereal grains or flour), an aggregate (e.g. solid mixtures such as sausage), or a whole unit (e.g. an entire fruit). Finely divided dry products can be mixed for subsampling using commercial portioning equipment such as a Jones Divider, or by spreading the sample over a large surface, quartering with a straight-edge and mixing opposite quarters. The two mixed halves can be recombined and the process repeated one or more times to make the subsample portion even more representative. An aggregate solid sample is probably the most difficult as it consists of different food materials usually with different physical properties. The challenge is to take a subsample having a composition representing an average of the food sampled. This most often requires that the aggregate food be chopped or ground before mixing and subsampling. A discussion of this can be found in Section 2.3. The whole unit sample can be most easily subsampled by taking a representative portion of the food. This could be a quarter of a fruit, a piece of loin from a whole fish or other similar sectioning.

In summary, a selective subsample consists only of suspect portions and ignores the remainder of the sample. A representative subsample, however, must as best possible represent an average of the whole sample.

### 2.2 COMPOSITING

A composite is defined as an admixture of two or more portions of a substance. A composite is formed by first subsampling two or more portions of the same food. An example would be subsampling several individual cans from the same food lot. These subsamples are then combined and mixed so that a portion taken of the composite would be representative of the whole. A composite is simply a

physical attempt to average the normal variation between individual sample units or portions. It is most useful when the analytical result must be compared to a standard or requirement involving the entire food product.

As the composite is to be representative, the subsamples of the individual sample units must not only be taken correctly (see Section 2.1), but must all be approximately the same size, weight or volume. Given correct subsampling, the only remaining problem is to make the composite reasonably uniform and representative. This may involve chopping and grinding as well as physical mixing.

### **2.3 CHOPPING, GRINDING, MIXING**

The well equipped food analysis laboratory should have a variety of sample preparation equipment including mechanical choppers, mincers, grinders, blenders and a hammer or similar mill.

The type of mechanical processing equipment selected will depend on the food product to be treated. The analyst must also keep in mind that mechanical grinders, mills, etc. usually generate heat during the processing. This can possibly change the sample composition, such as for fatty foods where the heat may be sufficient to partially melt the fat. In such cases, hand chopping and mixing may be the best procedure. In other instances, the sample may have to be frozen before grinding. The analyst must judge the best method for himself, depending on the kind of food and the substance for which it is to be analyzed.

The moisture content of a food also plays an important role in determining the food processing procedure or equipment to use. Dry foods can generally be milled, while moist foods can be chopped, minced or ground. Very moist and liquid foods can be blended. The home food processors now available are very useful for many products.

If no mechanical processing equipment is available, then of course hand processing must be done. The tools used include knives, graters and choppers. When a sample is processed by hand, it must be sufficiently finely divided to permit proper mixing and later subsampling of the mixture.

The analyst must always keep in mind that proper sample preparation is not only to gain a representative portion for analysis, but is also to prevent change in the sample which may result in a biased analytical result.

### **2.4 FREEZING AND THAWING**

Freezing is often the only way to prevent a change in a food before analysis or for reserve storage. Examples include foods for decomposition analysis or foods which were sampled while frozen. It was mentioned in Section 2.3 that some foods must be frozen before grinding or other processing.

The single most important problem in handling frozen food samples is proper thawing before analysis. Thawing must take place in such a manner that the composition of the food remains unchanged. Thawing should be done slowly without heat and in a closed container to prevent moisture loss by drying or gain by condensation. Any separated liquid must be mixed back in the thawed product before subsampling for analysis.

### **2.5 RESERVE STORAGE**

The reserve portion of a food sample must be maintained in storage so that there is very little or no change from the original analysis. Ideally, the reserve portion analyzed at a future time will give a result equivalent to the original.

The storage container is very important. The container should protect the product against moisture loss or gain, and physical damage such as attack by vermin. In some cases the container must be hermetically sealed to prevent air oxidation.

For dry storage at room temperature, glass or metal containers with appropriate closures are usually sufficient. Rigid plastic containers would be a second choice. Plastic and paper bags are not as useful for dry storage because they are easily breached by insects or mechanically. The analyst should also keep in mind that commercially canned foods can deteriorate in storage and should check such stored items periodically.

Glass, rigid plastic, or thick plastic bags can be used for frozen storage. If glass or rigid plastic is used, space must be allowed in the container for expansion of the ice so as not to break the container. If thin plastic bags or paper is used, the product may lose moisture due to drying while frozen.

It is not advisable to store a reserve portion in a refrigerator for any great length of time, unless the container is air-tight. Even if the stored food does not otherwise deteriorate, it may become mouldy unless protected. Preservatives may be added only if they do not affect the desired analysis.

The amount of food product to be kept as a reserve depends on any legal as well as analytical requirements. A reasonable reserve would be that amount necessary to do three analyses. More should be kept if storage space is available.

The above reserve storage precautions also apply to overnight or longer storage while the analysis is being performed.

### 3. HANDLING TECHNIQUES

#### 3.1 SOURCES OF ERROR

Error is defined in one dictionary as a "...deviation from accuracy or correctness; a mistake...." Most experienced analytical chemists will agree that possible sources of error in analysis seem to be unlimited. On a practical level, however, most error is controllable.

Many texts divide error into two categories, namely random and systematic. Random errors are considered to be unsuspected and nonreproducible errors which are beyond control. Their very randomness, however, permits use of the techniques of statistics and probability to evaluate analytical results. On the other hand, systematic errors are those which arise from definite (and often known) causes and which are controllable. Systematic errors result in bias in the analysis result. This bias can be positive or negative and when considering the effect of a known error it is useful to determine its direction. This is especially true when an attempt is made to find the major source of error which may have caused an incorrect result. For example, if the result is incorrect and is unusually high, look for sources of contributing errors which might be something like a low potency standard which would give a calculated high analytical result.

Systematic error can be grouped into five general sources:

1. **Analyst** - this includes all human error such as incorrect handling technique, calculation blunders and simple errors in judgement.
2. **Equipment and glassware** - includes malfunctions, incorrect calibrations or use, etc.
3. **Reagents and standards** - includes contamination, low potency, interferences, etc.
4. **Environment** - includes the effects of temperature, humidity, light, etc.
5. **Method** - includes specificity, applicability, sensitivity, etc.

Note that all of the above systematic errors are controllable. The key to control, of course, is the analyst. A properly trained analyst who thinks critically about the analysis and who considers and corrects sources of error, will provide the most accurate analytical result.

The following sections (3.2 - 3.6) discuss some of the physical and handling aspects of analysis and controlling possible error.

#### 3.2 WEIGHING AND SOLID TRANSFERS

The first step in most analyses is weighing the sample portion taken for analysis. Even liquid foods are generally weighed rather than portioned volumetrically.

There are two common weighing techniques used in a laboratory. These are direct and by difference. To weigh directly, a container tare weight is determined, the sample is added and the container plus sample reweighed. This gives an accurate sample weight but the major drawback is the necessity to quantitatively transfer the sample from the tared container to another receptacle. This can be done most easily by rinsing using an appropriate solvent. The technique to do this using a glass rod is illustrated in Figure 3.1.

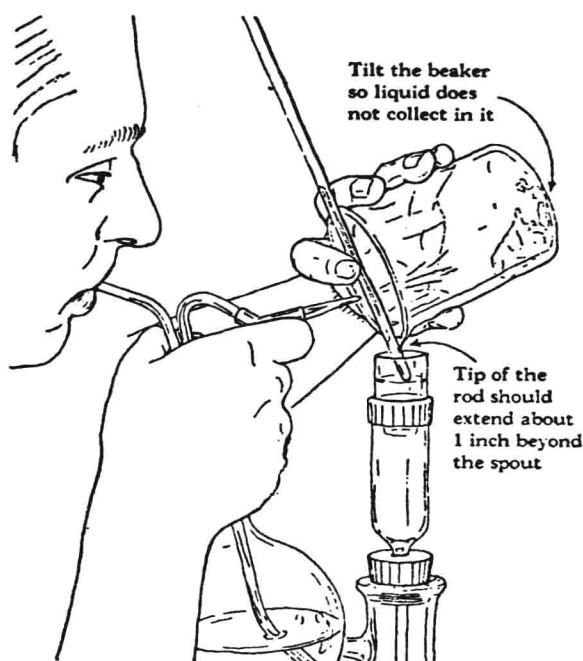


Figure 3.1

The usual method of weighing is by difference. The sample and container are first weighed and then are reweighed after removal of the sample. This is the most convenient procedure and generally does not require quantitative transfer techniques. If, however, the sample is to be transferred to a narrow-neck container such as a volumetric flask, then quantitative rinsing must be done as in Figure 3.2.

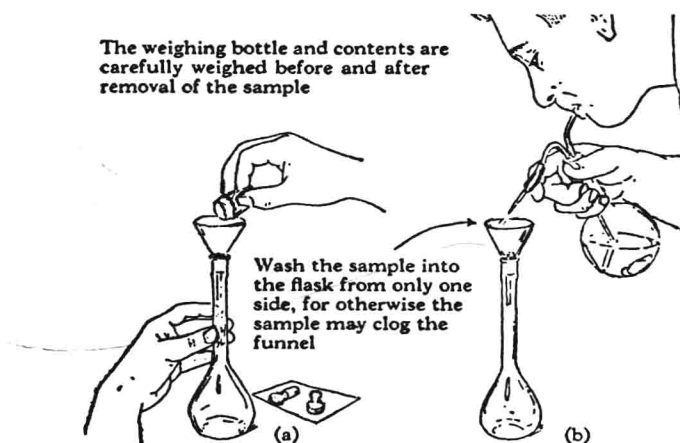


Figure 3.2

### 3.3 LIQUID AND SEMI-SOLID TRANSFERS

Most liquid transfers are done using volumetric or other pipettes. Pipettes are designed either to deliver a set volume or to contain a volume. Volumetric pipettes are designed to deliver and should never be blown out. The proper technique for volumetric pipettes is illustrated in Figure 3.3. Remember to never pipette by mouth, always use a safety bulb or vacuum tube. Note that drainage time for a grade A pipette is marked on its' side.

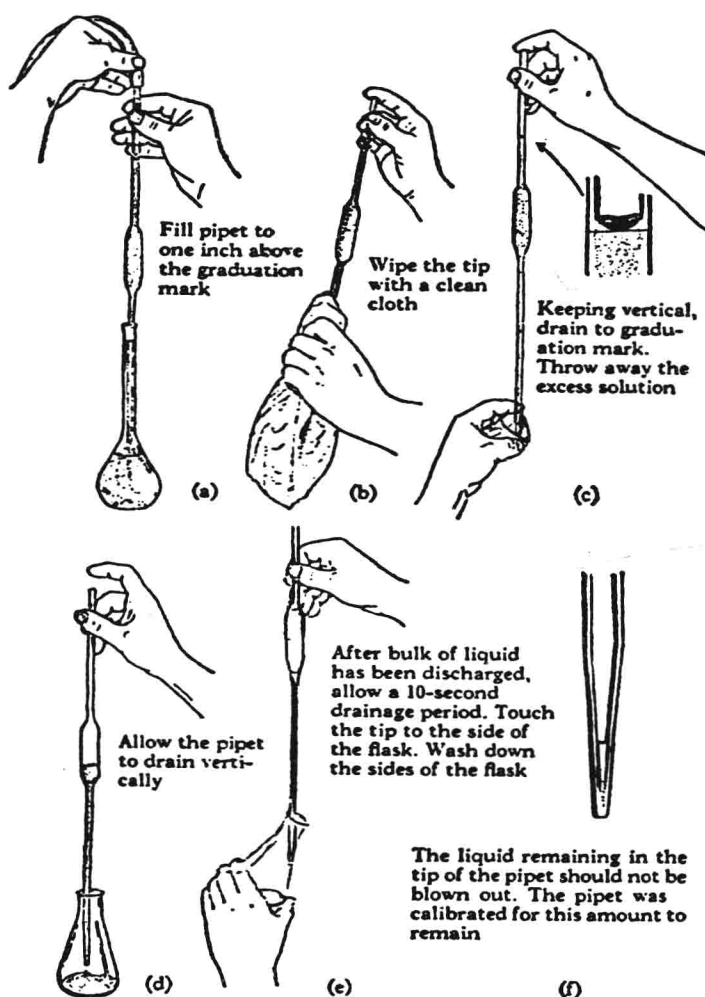


Figure 3.3

Pipettes designed 'to contain' must have the last liquid blown out. These pipettes are not as accurate as volumetric but can be used where only moderate volume accuracy is required. Often they are calibrated in multiple units such as a 10 ml pipette divided into 1 ml increments.

When a liquid transfer is made from an item such as a beaker, then the technique using a glass rod illustrated in Figure 3.4 should be used.

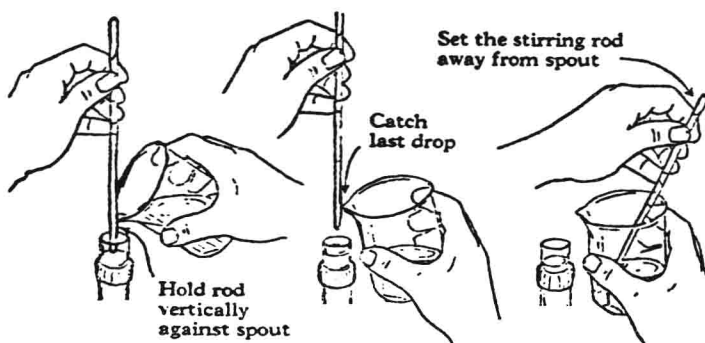


Figure 3.4



Filtration is commonly used to separate liquids from solids. Fluted filter paper is often available, but if not available, the technique to prepare a fluted filter illustrated in Figure 3.5 should be used. The filter paper is first folded in half and again in quarters, and opened up as shown in (a). The edge 2,1 is then folded on to 2,4 and edge 2,3 on to 2,4, producing, when the paper is opened, new folds at 2,5 and 2,6. The folding is continued, 2,1 to 2,6 and 2,3 to 2,5, thus producing folds at 2,7 and 2,8 respectively (b); further 2,3 to 2,6 giving 2,9, and 2,1 to 2,5 giving 2,10 (c). The final operation consists in making a fold in each of the eight segments - between 2,3 and 2,9, between 2,9 and 2,6, etc. - in a direction opposite to the first series of folds, i.e., the folds are made outwards instead of inwards as at first. The result is a fan arrangement (d), and upon opening, the fluted filter paper (e) is obtained.

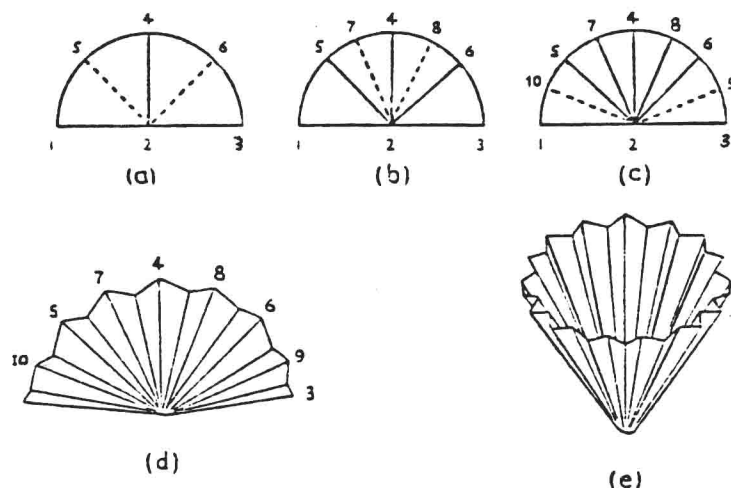


Figure 3.5

### 3.4 USE OF VOLUMETRIC WARE

The proper use of volumetric pipettes was discussed in Section 3.3. Burettes and their use will be discussed in Section 4.5.

The volumetric flask is indispensable for accurate analytical work, but only if properly used and cleaned. When filling a volumetric flask, avoid incorporation of air and resultant foaming. Fill until the bottom of the meniscus rests on the calibration line. For most accurate work, remember that the volumetric flask was calibrated at 20°C, so that if it is filled at a different temperature, a temperature correction must be made. This is discussed in Section 3.5.

All volumetric glassware, such as flasks, pipettes, etc., must be kept scrupulously clean. Washing with detergent solutions is often not enough, and in fact can aggravate the problem by leaving detergent residues if not rinsed properly. One of the best cleaning methods is to treat the volumetric ware with dichromate cleaning solution, soak for some time, rinse with distilled water then alcohol. Dichromate cleaning solution can be made by carefully mixing 800 ml concentrated sulphuric acid with 500 ml of a saturated aqueous solution of potassium dichromate. The cleaning solution can be reused until it develops a greenish tinge. At that point it should be discarded.

Another, less rigorous, cleaning solution is 15% trisodium phosphate in water. A warm solution of this (about 70°C) has been shown to be very effective.