6

chapter

Moisture and Total Solids Analysis

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6.1 INTRODUCTION

Moisture assays can be one of the most important analyses performed on a food product and yet one of the most difficult from which to obtain accurate and precise data. This chapter describes various methods for moisture analysis – their principles, procedures, applications, cautions, advantages, and disadvantages. Water activity measurement also is described, since it parallels the measurement of total moisture as an important stability and quality factor. With an understanding of techniques described, one can apply appropriate moisture analyses to a wide variety of food products.

6.1.1 Importance of Moisture Assay

One of the most fundamental and important analytical procedures that can be performed on a food product is an assay for the amount of moisture (1–3). The dry matter that remains after moisture removal is commonly referred to as **total solids**. This analytical value is of great economic importance to a food manufacturer because water is an inexpensive filler. The following listing gives some examples in which moisture content is important to the food processor.

1. Moisture is a quality factor in the preservation of some products and affects stability in
   (a) Dehydrated vegetables and fruits
   (b) Dried milks
   (c) Powdered eggs
   (d) Dehydrated potatoes
   (e) Spices and herbs
2. Moisture is used as a quality factor for
   (a) Jams and jellies to prevent sugar crystallization
   (b) Sugar syrups
   (c) Prepared cereals – conventional, 4–8%; puffed, 7–8%
3. Reduced moisture is used for convenience in packaging or shipping of
   (a) Concentrated milks
   (b) Liquid cane sugar (67% solids) and liquid corn sweetener (80% solids)
   (c) Dehydrated products (these are difficult to package if too high in moisture)
   (d) Concentrated fruit juices
4. Moisture (or solids) content is often specified in compositional standards (i.e., Standards of Identity)
   (a) Cheddar cheese must be ≤39% moisture.
   (b) Enriched flour must be ≤15% moisture.
   (c) Pineapple juice must have soluble solids of ≥10.5°Brix (conditions specified).
   (d) Glucose syrup must have ≥70% total solids.
   (e) The percentage of added water in processed meats is commonly specified.
5. Computations of the nutritional value of foods require that you know the moisture content.
6. Moisture data are used to express results of other analytical determinations on a uniform basis [i.e., dry weight basis (dwb), rather than wet weight basis (wwb)].

6.1.2 Moisture Content of Foods

The moisture content of foods varies greatly as shown in Table 6-1 (4). Water is a major constituent of most food products. The approximate, expected moisture content of a food can affect the choice of the method of measurement. It can also guide the analyst in determining the practical level of accuracy required when measuring moisture content, relative to other food constituents.

6.1.3 Forms of Water in Foods

The ease of water removal from foods depends on how it exists in the food product. The three states of water in food products are:

1. **Free water:** This water retains its physical properties and thus acts as the dispersing agent for colloids and the solvent for salts.
2. **Adsorbed water:** This water is held tightly or is occluded in cell walls or protoplasm and is held tightly to proteins.
3. **Water of hydration:** This water is bound chemically, for example, lactose monohydrate; also some salts such as $Na_2SO_4 \cdot 10H_2O$.

Depending on the form of the water present in a food, the method used for determining moisture may measure more or less of the moisture present. This is the reason for official methods with stated procedures (5–7). However, several official methods may exist for a particular product. For example, the AOAC International methods for cheese include: Method 926.08, vacuum oven; 948.12, forced draft oven; 977.11, microwave oven; 969.19, distillation (5). Usually, the first method listed by AOAC International is preferred over others in any section.

6.1.4 Sample Collection and Handling

General procedures for sampling, sample handling and storage, and sample preparation are given in Chap. 5. These procedures are perhaps the greatest potential source of error in any analysis. Precautions must be taken to minimize inadvertent **moisture losses or gains** that occur during these steps.

6-1 table **Moisture Content of Selected Foods**

Food Item	*Approximate Percent Moisture (Wet Weight Basis)*
Cereals, bread, and pasta	
Wheat flour, whole-grain	10.3
White bread, enriched (wheat flour)	13.4
Corn flakes cereal	3.5
Crackers saltines	4.0
Macaroni, dry, enriched	9.9
Dairy products	
Milk, reduced fat, fluid, 2%	89.3
Yogurt, plain, low fat	85.1
Cottage cheese, low fat or 2% milk fat	80.7
Cheddar cheese	36.8
Ice cream, vanilla	61.0
Fats and oils	
Margarine, regular, hard, corn, hydrogenated	15.7
Butter, with salt	15.9
Oil-soybean, salad, or cooking	0
Fruits and vegetables	
Watermelon, raw	91.5
Oranges, raw, California navels	86.3
Apples, raw, with skin	85.6
Grapes, American type, raw	81.3
Raisins	15.3
Cucumbers, with peel, raw	95.2
Potatoes, microwaved, cooked in skin, flesh and skin	72.4
Snap beans, green, raw	90.3
Meat, poultry, and fish	
Beef, ground, raw, 95% lean	73.3
Chicken, broilers and fryers, light meat, meat and skin, raw	68.6
Finfish, flatfish (flounder and sole species), raw	79.1
Egg, whole, raw, fresh	75.8
Nuts	
Walnuts, black, dried	4.6
Peanuts, all types, dry roasted with salt	1.6
Peanut butter, smooth style, with salt	1.8
Sweeteners	
Sugar, granulated	0
Sugar, brown	1.3
Honey, strained or extracted	17.1

From US Department of Agriculture, Agricultural Research Service (2009) USDA National Nutrient Database for Standard Reference. Release 22. Nutrient Data Laboratory Home Page, http://www.ars.usda.gov/ba/bhnrc/ndl

Obviously, any exposure of a sample to the open atmosphere should be as short as possible. Any heating of a sample by friction during grinding should be minimized. Headspace in the sample storage container should be minimal because moisture is lost from the sample to equilibrate the container environment against the sample. It is critical to control temperature fluctuations since moisture will migrate in a sample to the colder part. To control this potential error, remove the entire sample from the container, reblend quickly, and then remove a test portion (8,9).

To illustrate the need for optimum efficiency and speed in weighing samples for analysis, Bradley and Vanderwarn (10) showed, using shredded Cheddar cheese (2–3 g in a 5.5-cm aluminum foil pan), that moisture loss within an analytical balance was a straight line function. The rate of loss was related to the relative humidity. At 50% relative humidity, it required only 5 s to lose 0.01% moisture. This time doubled at 70% humidity or 0.01% moisture loss in 10 s. While one might expect a curvilinear loss, the moisture loss was actually linear over a 5-min study interval. These data demonstrate the necessity of absolute control during collection of samples through weighing, before drying.

6.2 OVEN DRYING METHODS

In **oven drying methods**, the sample is heated under specified conditions, and the loss of weight is used to calculate the moisture content of the sample. The amount of moisture determined is highly dependent on the type of oven used, conditions within the oven, and the time and temperature of drying. Various oven methods are approved by AOAC International for determining the amount of moisture in many food products. The methods are simple, and many ovens allow for simultaneous analysis of large numbers of samples. The time required may be from a few minutes to over 24 h.

6.2.1 General Information

6.2.1.1 Removal of Moisture

Any oven method used to evaporate moisture has as its foundation the fact that the boiling point of water is 100°C; however, this considers only pure water at sea level. Free water is the easiest of the three forms of water to remove. However, if 1 molecular weight (1 mol) of a solute is dissolved in 1.0 L of water, the boiling point would be raised by 0.512°C. This boiling point elevation continues throughout the moisture removal process as more and more concentration occurs.

Moisture removal is sometimes best achieved in a two-stage process. Liquid products (e.g., juices, milk) are commonly predried over a **steam bath** before drying in an oven. Products such as bread and field-dried grain are often air dried, then ground and oven dried, with the moisture content calculated from moisture loss at both air and oven drying steps. Particle size, particle size distribution, sample sizes, and surface area during drying influence the rate and efficiency of moisture removal.

6.2.1.2 Decomposition of Other Food Constituents

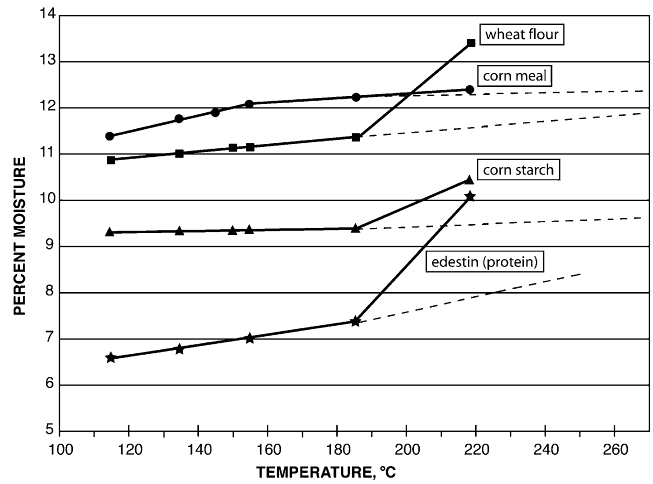
Moisture loss from a sample during analysis is a function of time and temperature. Decomposition enters the picture when time is extended too much or temperature is too high. Thus, most methods for food moisture analysis involve a compromise between time and a particular temperature at which limited decomposition might be a factor. One major problem exists in that the physical process must separate all the moisture without decomposing any of the constituents that could release water. For example, carbohydrates decompose at 100°C according to the following reaction:

$$C_6H_{12}O_6 \rightarrow 6C + 6H_2O \quad [1]$$

The moisture generated in carbohydrate decomposition is not the moisture that we want to measure. Certain other chemical reactions (e.g., sucrose hydrolysis) can result in utilization of moisture, which would reduce the moisture for measurement. A less serious problem, but one that would be a consistent error, is the loss of **volatile constituents**, such as acetic, propionic, and butyric acids; and alcohols, esters, and aldehydes among flavor compounds. While weight changes in oven drying methods are assumed to be due to moisture loss, weight gains also can occur due to oxidation of unsaturated fatty acids and certain other compounds.

Nelson and Hulett (11) determined that moisture was retained in biological products to at least 365°C, which is coincidentally near the critical temperature for water. Their data indicate that among the decomposition products at elevated temperatures were CO, CO_2, CH_4, and H_2O. These were not given off at any one particular temperature but at all temperatures and at different rates at the respective temperature in question.

By plotting moisture liberated against temperature, curves were obtained that show the amount of moisture liberated at each temperature (Fig. 6-1). Distinct breaks were shown that indicated the temperature at which decomposition became measurable. None of these curves showed any break before 184°C. Generally, proteins decompose at temperatures somewhat lower than required for starches and celluloses. Extrapolation of the flat portion of each curve to 250°C gave a true moisture content based on the assumption that there was no adsorbed water present at the temperature in question.



6-1 figure Moisture content of several foods held at various temperatures in an oven. The hyphenated line extrapolates data to 275°C, the true moisture content. [Reprinted with permission from (11) Nelson OA and Hulett GA. 1920. The moisture content of cereals. *J. Industrial Eng. Chem.* 12:40–45. Copyright 1920, American Chemical Society.]

6.2.1.3 Temperature Control

Drying methods utilize specified drying temperatures and times, which must be carefully controlled. Moreover, there may be considerable variability of temperature, depending on the type of oven used for moisture analysis. One should determine the extent of variation within an oven before relying on data collected from its use.

Consider the temperature variation in three types of ovens: **convection** (**atmospheric**), **forced draft**, and **vacuum**. The greatest temperature variation exists in a convection oven. This is because hot air slowly circulates without the aid of a fan. Air movement is obstructed further by pans placed in the oven. When the oven door is closed, the rate of temperature recovery is generally slow. This is dependent also upon the load placed in the oven and upon the ambient temperature. A 10°C temperature differential across a convection oven is not unusual. This must be considered in view of anticipated analytical accuracy and precision. A convection oven should not be used when precise and accurate measurements are needed.

Forced draft ovens have the least temperature differential across the interior of all ovens, usually not greater than 1°C. Air is circulated by a fan that forces air movement throughout the oven cavity. Forced draft ovens with air distribution manifolds appear to have added benefit where air movement is horizontal across shelving. Thus, no matter whether the oven shelves are filled completely with moisture pans or only half filled, the result would be the same for a particular sample. This has been demonstrated using a Lab-Line oven (Melrose Park, IL) in which three stacking configurations for the pans were used (10). In one configuration, the oven shelves were filled with as many pans holding 2–3 g of Cheddar cheese as the forced draft oven could hold. In the two others, one-half of the full load of pans with cheese was used with the pans (1) in orderly vertical rows with the width of one pan between rows, or (2) staggered such that pans on every other shelf were in vertical alignment. The results after drying showed no difference in the mean value or the standard deviation.

Two features of some **vacuum ovens** contribute to a wider temperature spread across the oven. One feature is a glass panel in the door. Although from an educational point of view, it may be fascinating to observe some samples in the drying mode; the glass is a heat sink. The second feature is the way by which air is bled into the oven. If the air inlet and discharge are on opposite sides, conduct of air is virtually straight across the oven. Some newer models have air inlet and discharge manifolds mounted top and bottom. Air movement in this style of vacuum oven is upward from the front and then backward to the discharge in a broad sweep. The effect is to minimize cold spots as well as to exhaust moisture in the interior air.

6.2.1.4 Types of Pans for Oven Drying Methods

Pans used for moisture determinations are varied in shape and may or may not have a cover. The AOAC International (5) moisture pan is about 5.5 cm in diameter with an insert cover. Other pans have covers that slip over the outside edge of the pan. These pans, while reusable, are expensive, in terms of labor costs to clean appropriately to allow reuse.

Pan covers are necessary to control loss of sample by spattering during the heating process. If the cover is metal, it must be slipped to one side during drying to allow for moisture evaporation. However, this slipping of the cover also creates an area where spattering will result in product loss. Examine the interior of most moisture ovens and you will detect odor and deposits of burned-on residue, which, although undetected at the time of occurrence, produce erroneous results and large standard deviations (10).

Consider the use of **disposable pans** whenever possible; then purchase **glass fiber discs** for covers. At 5.5 cm in diameter, these covers fit perfectly inside disposable aluminum foil pans and prevent spattering while allowing the surface to breathe. Paper filter discs foul with fat and thus do not breathe effectively. Drying studies done on cheese using various pans and covers have shown that fat does spatter from pans with slipped covers, and fiberglass is the most satisfactory cover.

6.2.1.5 Handling and Preparation of Pans

The preparation and handling of pans before use requires consideration. Use only **tongs** to handle any pan. Even fingerprints have weight. All pans must be oven treated to prepare them for use. This is a factor of major importance unless disproved by the technologist doing moisture determinations with a particular type of pan. Disposable aluminum pans must be vacuum oven dried for 3 h before use. At 3 and 15 h in either a vacuum or forced draft oven at 100°C, pans varied in their weight within the error of the balance or 0.0001 g (10). Store dried moisture pans in a functioning **desiccator**. The glass fiber covers should be dried for 1 h before use.

6.2.1.6 Control of Surface Crust Formation (Sand Pan Technique)

Some food materials tend to form a semipermeable crust or lump together during drying, which will contribute to erratic and erroneous results. To control this problem, analysts use the **sand pan technique**. Clean, dry sand and a short glass stirring rod are preweighed into a moisture pan. Subsequently, after weighing in a sample, the sand and sample are admixed with the stirring rod left in the pan. The remainder of the procedure follows a standardized method if available; otherwise the sample is dried to constant weight. The purpose of the sand is twofold: to prevent **surface crust** from forming and to disperse the sample so evaporation of moisture is less impeded. The amount of sand used is a function of sample size. Consider 20–30 g sand/3 g sample to obtain desired distribution in the pan. Similar to the procedure, applications, and advantages of using sand, other heat-stable inert materials such as diatomaceous earth can be used in moisture determinations, especially for sticky fruits.

The inert matrices such as sand and **diatomaceous earth** function to disperse the food constituents and minimize the retention of moisture in the food products. However, the analyst must ascertain that the inert matrix used does not give erroneous results for the assay because of decomposition or entrapped moisture loss. Test the sand or other inert matrix for weight loss before using in any method. Add approximately 25 g of sand into a moisture pan and heat at 100°C for 2 h and weigh to 0.1 mg. Add 5 ml of water and mix with the matrix using a glass rod. Heat dish, matrix, cover, and glass rod for at least 4 h at 100°C, reweigh. The difference between weighing must be less than 0.5 mg for any suitable matrix (12).

6.2.1.7 Calculations

Moisture and total solids contents of foods can be calculated as follows using oven drying procedures:

$$\%\text{Moisture (wt/wt)} = \frac{\text{wt } H_2O \text{ in sample}}{\text{wt of wet sample}} \times 100 \quad [2]$$

$$\%\text{Moisture (wt/wt)} = \frac{\text{wt of wet sample} - \text{wt of dry sample}}{\text{wt of wet sample}} \times 100 \quad [3]$$

$$\%\text{Total solids (wt/wt)} = \frac{\text{wt of dry sample}}{\text{wt of wet sample}} \times 100 \quad [4]$$

6.2.2 Forced Draft Oven

When using a forced draft oven, the sample is rapidly weighed into a predried moisture pan covered and placed in the oven for an arbitrarily selected time if no standardized method exists. Drying time periods for this method are 0.75–24 h (Table 6-2), depending on the food sample and its pretreatment; some liquid samples are dried initially on a steam bath at 100˚C to minimize spattering. In these cases, drying times are shortened to 0.75–3 h. A forced draft oven is used with or without a steam table predrying treatment to determine the solids content of fluid milks (AOAC Method 990.19, 990.20).

An alternative to selecting a time period for drying is to weigh and reweigh the dried sample and pan until two successive weighings taken 30 min apart agree within a specified limit, for example, 0.1–0.2 mg for a 5-g sample. The user of this second method must be aware of sample transformation, such as browning which suggests moisture loss of the wrong form. Lipid oxidation and a resulting sample weight gain can occur at high temperatures in a forced draft oven. Samples high in carbohydrates should not be dried in a forced draft oven but rather in a vacuum oven at a temperature no higher than 70°C.

6.2.3 Vacuum Oven

By drying under reduced pressure (25–100 mm Hg), one is able to obtain a more complete removal of water and volatiles without decomposition within a 3–6-h drying time. Vacuum ovens need a dry air purge in addition to temperature and vacuum controls to operate within method definition. In older methods, a vacuum flask is used, partially filled with concentrated sulfuric acid as the desiccant. One or two air bubbles per second are passed through the acid. Recent changes now stipulate an air trap that is filled with calcium sulfate containing an indicator to show moisture saturation. Between the trap and the vacuum oven is an appropriately sized rotameter to measure air flow (100–120 ml/min) into the oven.

The following are important points in the use of a vacuum drying oven:

6-2 table **Forced Draft Oven Temperature and Times for Selected Foods**

Product	Dry on Steam Bath	Oven Temperature (°C ± 2)	Time in Oven (h)
Buttermilk, liquid	X[a]	100	3
Cheese, natural type only		100	16.5 ± 0.5
Chocolate and cocoa		100	3
Cottage cheese		100	3
Cream, liquid and frozen	X	100	3
Egg albumin, liquid	X	130	0.75
Egg albumin, dried	X	100	0.75
Ice cream and frozen desserts	X	100	3.5
Milk	X	100	3
Whole, low fat, and skim		100	3
Condensed skim		100	3
Nuts: almonds, peanuts, walnuts		130	3

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[a] *X* = samples must be partially dried on steam bath before being placed in oven.

1. **Temperature** used depends on the product, such as 70°C for fruits and other high-sugar products. Even with reduced temperature, there can be some decomposition.
2. If the product to be assayed has a high concentration of **volatiles**, you should consider the use of a correction factor to compensate for the loss.
3. Analysts should remember that in a **vacuum**, heat is not conducted well. Thus pans must be placed directly on the metal shelves to conduct heat.
4. **Evaporation** is an endothermic process; thus, a pronounced cooling is observed. Because of the cooling effect of evaporation, when several samples are placed in an oven of this type, you will note that the temperature will drop. Do not attempt to compensate for the cooling effect by increasing the temperature, otherwise samples during the last stages of drying will be overheated.
5. The **drying time** is a function of the total moisture present, nature of the food, surface area per unit weight of sample, whether sand is used as a dispersant, and the relative concentration of sugars and other substances capable of retaining moisture or decomposing. The drying interval is determined experimentally to give reproducible results.

6.2.4 Microwave Analyzer

Determination of moisture in food products has traditionally been done using a standard oven, which, though accurate, can take many hours to dry a sample. Other methods have been developed over the years including infrared and various types of instruments that utilize halogen lamps or ceramic heating elements. They were often used for "spot checking" because of their speed, but they lacked the accuracy of the standard oven method. The introduction of microwave moisture/solids analyzers in the late 1970s gave laboratories the accuracy they needed and the speed they wanted. **Microwave moisture analysis**, often called **microwave drying**, was the first precise and rapid technique that allowed some segments of the food industry to make in-process adjustment of the moisture content in food products before final packaging. For example, processed cheese could be analyzed and the composition adjusted before the blend was dumped from the cooker. The ability to adjust the composition of a product in-process helps food manufacturers reduce production costs, meet regulatory requirements, and ensure product consistency. Such control could effectively pay for the microwave analyzer within a few months.

A particular microwave moisture/solids analyzer (CEM Corporation, Matthews, NC), or equivalent, is specified in the AOAC International procedures for total solids analysis of processed tomato products (AOAC Method 985.26) and moisture analysis of meat and poultry products (AOAC Method 985.14).

The general procedure for use of a microwave moisture/solids analyzer has been to set the microprocessor controller to a percentage of full power to control the microwave output. Power settings are dependent upon the type of sample and the recommendations of the manufacturer of the microwave moisture analyzer. Next, the internal balance is tared with two sample pads on the balance. As rapidly as possible, a sample is placed between the two pads, then pads are centered on the pedestal, and weighed against the tare weight. Time for the drying operation is set by the operator and "start" is activated. The microprocessor controls the drying procedure, with percentage moisture indicated in the controller window. Some newer models of microwave moisture analyzers have a temperature control feature to precisely control the drying process, removing the need to guess appropriate time and power settings for specific applications. These new models also have a smaller cavity that allows the microwave energy to be focused directly on the sample.

There are some considerations when using a microwave analyzer for moisture determination: (1) the sample must be of a uniform, appropriate size to provide for complete drying under the conditions specified; (2) the sample must be centrally located and evenly distributed, so some portions are not burned and other areas are underprocessed; and (3) the amount of time used to place an appropriate sample weight between the pads must be minimized to prevent moisture loss or gain before weight determination. Sample pads also should be considered. There are several different types, including fiberglass and quartz fiber pads. For optimum results, the pads should not absorb microwave energy, as this can cause the sample to burn, nor should they fray easily, as this causes them to lose weight and can affect the analysis. In addition, they should absorb liquids well.

Another style of microwave oven that includes a vacuum system is used in some food plants. This vacuum microwave oven will accommodate one sample in triplicate or three different samples at one time. In 10 min, the results are reported to be similar to 5 h in a vacuum oven at 100°C. The vacuum microwave oven is not nearly as widely used as conventional microwave analyzers, but can be beneficial in some applications.

Microwave drying provides a fast, accurate method to analyze many foods for moisture content. The method is sufficiently accurate for routine assay.

The distinct advantage of rapid analysis far outweighs its limitation of testing only single samples (13).

6.2.5 Infrared Drying

Infrared drying involves penetration of heat into the sample being dried, as compared with heat conductivity and convection with conventional ovens. Such heat penetration to evaporate moisture from the sample can significantly shorten the required drying time to 10–25 min. The infrared lamp used to supply heat to the sample results in a filament temperature of 2000–2500 K (degrees Kelvin). Factors that must be controlled include distance of the infrared source from the dried material and thickness of the sample. The analyst must be careful that the sample does not burn or case harden while drying. Infrared drying ovens may be equipped with forced ventilation to remove moisture air and an analytical balance to read moisture content directly. No infrared drying moisture analysis techniques are approved by AOAC International currently. However, because of the speed of analysis, this technique is suited for qualitative in-process use.

6.2.6 Rapid Moisture Analyzer Technology

Many rapid moisture/solids analyzers are available to the food industry. In addition to those based on infrared and microwave drying as described previously, compact instruments that depend on high heat are available, such as analyzers that detect moisture levels from 50 ppm to 100% using sample weights of 150 mg to 40 g (e.g., Computrac®, Arizona Instrument LLC, Chandler, AZ). Using a digital balance, the test sample is placed on an aluminum pan or filter paper and the heat control program (with a heating range of 25–275°C) elevates the test sample to a constant temperature. As the moisture is driven from the sample, the instrument automatically weighs and calculates the percentage moisture or solids. This technology is utilized to cover a wide range of applications within the food industry and offers quick and accurate results within minutes. These analyzers are utilized for both production and laboratory use with results comparable to reference methods.

6.3 DISTILLATION PROCEDURES

6.3.1 Overview

Distillation techniques involve codistilling the moisture in a food sample with a high boiling point solvent that is immiscible in water, collecting the mixture that distills off, and then measuring the volume of water. Two distillation procedures are in use today: **direct** and **reflux distillations**, with a variety of solvents. For example, in direct distillation with immiscible solvents of higher boiling point than water, the sample is heated in mineral oil or liquid with a flash point well above the boiling point for water. Other immiscible liquids with boiling point only slightly above water can be used (e.g., toluene, xylene, and benzene). However, reflux distillation with the immiscible solvent toluene is the most widely used method.

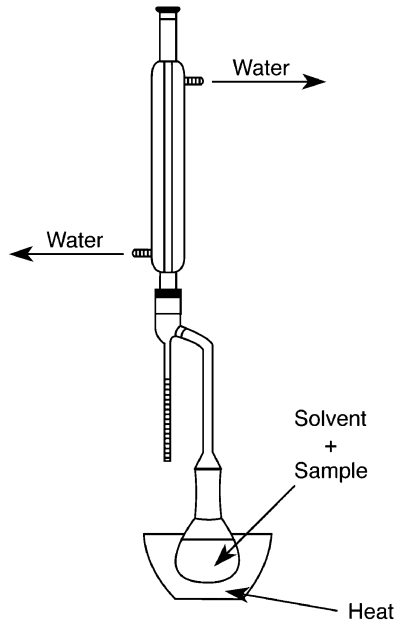
Distillation techniques were originally developed as rapid methods for quality control work, but they are not adaptable to routine testing. The distillation method is an AOAC-approved technique for moisture analysis of spices (AOAC Method 986.21), cheese (AOAC Method 969.19), and animal feeds (AOAC Method 925.04). It also can give good accuracy and precision for nuts, oils, soaps, and waxes.

Distillation methods cause less thermal decomposition of some foods than oven drying at high temperatures. Adverse chemical reactions are not eliminated but can be minimized by using a solvent with a lower boiling point. This, however, will increase distillation times. Water is measured directly in the distillation procedure (rather than by weight loss), but reading the volume of water in a receiving tube may be less accurate than using a weight measurement.

6.3.2 Reflux Distillation with Immiscible Solvent

Reflux distillation uses either a solvent less dense than water (e.g., toluene, with a boiling point of 110.6°C; or xylene, with a boiling range of 137–140°C) or a solvent more dense than water (e.g., tetrachlorethylene, with a boiling point of 121°C). The advantage of using this last solvent is that material to be dried floats; therefore it will not char or burn. In addition, there is no fire hazard with this solvent.

A **Bidwell–Sterling moisture trap** (Fig. 6-2) is commonly used as part of the apparatus for reflux distillation with a solvent less dense than water. The distillation procedure using such a trap is described in Fig. 6-3, with emphasis placed on dislodging adhering water drops, thereby minimizing error. When the toluene in the distillation just starts to boil, the analyst will observe a hazy cloud rising in the distillation flask. This is a vaporous emulsion of water in toluene. Condensation occurs as the vapors rise, heating the vessel, the Bidwell–Sterling trap, and the bottom of the condenser. It is also hazy at the cold surface of the condenser, where water droplets are visible. The emulsion inverts and becomes toluene dispersed in water. This turbidity clears very slowly on cooling.



6-2 figure Apparatus for reflux distillation of moisture from a food. Key to this setup is the Bidwell–Sterling moisture trap. This style can be used only where the solvent is less dense than water.

Three potential sources of error with distillation should be eliminated if observed:

1. Formation of emulsions that will not break. Usually this can be controlled by allowing the apparatus to cool after distillation is completed and before reading the amount of moisture in the trap.
2. Clinging of water droplets to dirty apparatus. Clean glassware is essential, but water seems to cling even with the best cleaning effort. A burette brush, with the handle end flattened so it will pass down the condenser, is needed to dislodge moisture droplets.
3. Decomposition of the sample with production of water. This is principally due to carbohydrate decomposition to generate water ($C_6H_{12}O_6 \rightarrow 6H_2O + 6C$). If this is a measurable problem, discontinue method use and find an alternative procedure.

REFLUX DISTILLATION

Place sample in distillation flask and cover completely with solvent.

⇓

Fill the receiving tube (e.g., Bidwell-Sterling Trap) with solvent, by pouring it through the top of the condenser.

⇓

Bring to a boil and distill slowly at first then at increased rate.

⇓

After the distillation has proceeded for approximately 1 hr, use an adapted buret brush to dislodge moisture droplets from the condenser and top part of the Bidwell-Sterling trap.

⇓

Slide the brush up the condenser to a point above the vapor condensing area.

⇓

Rinse the brush and wire with a small amount of toluene to dislodge adhering water drops.

⇓

If water has adhered to the walls of the calibrated tube, invert the brush and use the straight wire to dislodge this water so it collects in the bottom of the tube.

⇓

Return the wire to a point above the condensation point, and rinse with another small amount of toluene.

⇓

After no more water has distilled from the sample, repeat the brush and wire routine to dislodge adhering water droplets.

⇓

Rinse the brush and wire with toluene before removing from the condenser.

⇓

Allow the apparatus to cool to ambient temperatures before measuring the volume of water in the trap.

⇓

Volume of water x 2 (for a 50 g sample) = % moisture

6-3 figure Procedures for reflux distillation with toluene using a Bidwell–Sterling trap. Steps to dislodge adhering moisture drops are given.

6.4 CHEMICAL METHOD: KARL FISCHER TITRATION

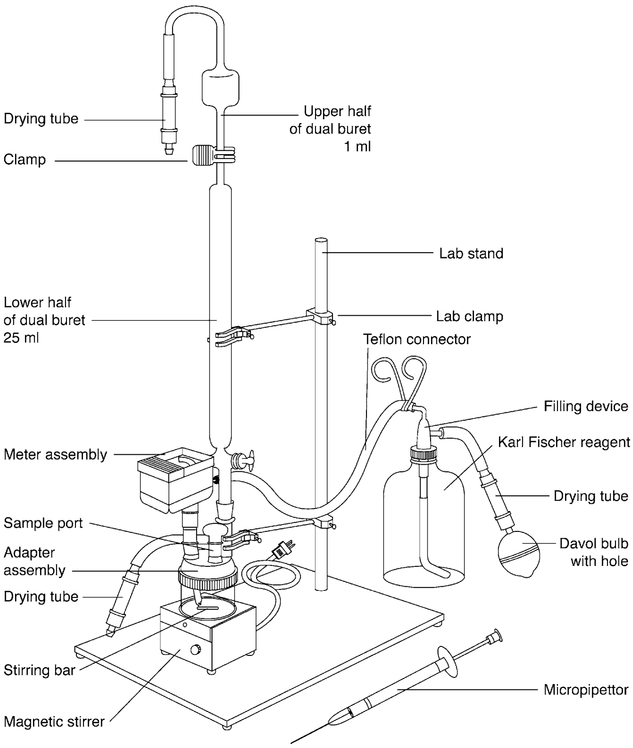
The **Karl Fischer titration** is particularly adaptable to food products that show erratic results when heated or submitted to a vacuum. This is the method of choice for determination of water in many low-moisture foods such as dried fruits and vegetables (AOAC Method 967.19 E-G), candies, chocolate (AOAC Method 977.10), roasted coffee, oils and fats (AOAC Method 984.20), or any low-moisture food high in sugar or protein. The method is quite rapid, is accurate, and uses no heat. This method is based on the fundamental reaction described by Bunsen in 1853 (14) involving the reduction of iodine by SO_2 in the presence of water:

$$2H_2O + SO_2 + I_2 \rightarrow H_2SO_4 + 2HI \quad [5]$$

This was modified to include methanol and pyridine in a four-component system to dissolve the iodine and SO_2:

$$C_5H_5N \cdot I_2 + C_5H_5N \cdot SO_2 + C_5H_5N + H_2O \rightarrow 2C_5H_5N \cdot HI + C_5H_5N \cdot SO_3 \quad [6]$$

$$C_5H_5N \cdot SO_3 + CH_3OH \rightarrow C_5H_5N(H)SO_4 \cdot CH_3 \quad [7]$$



6-4 figure Manual Karl Fischer titration unit. (Courtesy of Lab Industries, Inc., Berkeley, CA.)

6-5 figure Automated Karl Fischer volumetric titration unit. (Courtesy of Mettler-Toledo, Columbus, OH.)

These reactions show that for each mole of water, 1 mol of iodine, 1 mol of SO_2, 3 mol of pyridine, and 1 mol of methanol are used. For general work, a methanolic solution is used that contains these components in the ratio of 1 iodine:3 SO_2:10 pyridine, and at a concentration so that 3.5 mg of water = 1 ml of reagent. A procedure for standardizing this reagent is given below.

In a **volumetric titration** procedure (Fig. 6-4 is manual titration unit; Fig. 6-5 is example of automated titration unit), iodine and SO_2 in the appropriate form are added to the sample in a closed chamber protected from atmospheric moisture. The excess of I_2 that cannot react with the water can be determined **visually**. The endpoint color is dark red-brown. Some instrumental systems are improved by the inclusion of a potentiometer (i.e., **conductometric method**) to electronically determine the endpoint, which increases the sensitivity and accuracy. The volumetric titration can be done manually (Fig. 6-4) or with an automated unit (Fig. 6-5 is one example instrument). The automated volumetric titration units (used for 100 ppm water to very high concentrations) use a pump for mechanical addition of titrant and use the conductometric method for endpoint determination (i.e., detection of excess iodine is by applying a current and measuring the potential).

The volumetric titration procedure described above is appropriate for samples with a moisture content greater than ∼0.03%. A second type of titration, referred to as **coulometric titration**, is ideal for products with very low levels of moisture, from 0.03% down to parts per million (ppm) levels. In this method, iodine is electrolytically generated ($2I \rightarrow I_2 + 2e^-$) to titrate the moisture. The amount of iodine required to titrate the moisture is determined by the current needed to generate the iodine. Just like for volumetric titration, automated coulometric titration units are available commercially.

In a Karl Fischer volumetric titration, the **Karl Fischer reagent** (KFR) is added directly as the titrant if the moisture in the sample is accessible. However, if moisture in a solid sample is inaccessible to the reagent, the moisture is extracted from the food with an appropriate solvent (e.g., methanol). (Particle size affects efficiency of extraction directly.) Then the methanol extract is titrated with KFR.

The obnoxious odor of pyridine makes it an undesirable reagent. Therefore, researchers have experimented with other amines capable of dissolving iodine and sulfur dioxide. Some aliphatic amines and several other heterocyclic compounds were found suitable. On the basis of these new amines, **one-component reagents** (solvent and titrant components together) and **two-component reagents** (solvent and titrant components separate) have been prepared. The one-component reagent may be more convenient to use, but the two-component reagent has greater storage stability.

Before the amount of water found in a food sample can be determined, a **KFR water (moisture) equivalence** (KFReq) must be determined. The KFReq value represents the equivalent amount of moisture that reacts with 1 ml of KFR. Standardization must be checked before each use because the KFReq will change with time.

The KFReq can be established with **pure water**, a **water-in-methanol standard**, or **sodium tartrate dihydrate**. Pure water is a difficult standard to use because of inaccuracy in measuring the small amounts required. The water-in-methanol standard is premixed by the manufacturer and generally contains 1 mg of water/ml of solution. This standard can change over prolonged storage periods by absorbing atmospheric moisture. Sodium tartrate dihydrate ($Na_2C_4H_4O_6{\cdot}2H_2O$) is a primary standard for determining KFReq. This compound is very stable, contains 15.66% water under all conditions expected in the laboratory, and is the material of choice to use.

The KFReq is calculated as follows using sodium tartrate dihydrate:

$$\text{KFReq}\ (\text{mg}\ H_2O/\text{ml}) = \frac{36\,\text{g}\ H_2O/\text{mol}\ Na_2C_4H_4O_6 \cdot 2H_2O \times S \times 1000}{230.08\,\text{g/mol} \times A} \quad [8]$$

where:

KFReq = Karl Fischer reagent moisture equivalence
S = weight of sodium tartrate dihydrate (g)
A = ml of KFR required for titration of sodium tartrate dehydrate

Once the KFReq is known, the moisture content of the sample is determined as follows:

$$\%H_2O = \frac{\text{KFReq} \times \text{Ks}}{S} \times 100 \quad [9]$$

where:

KFReq = Karl Fischer reagent water (moisture) equivalence
Ks = ml of KFR used to titrate sample
S = weight of sample (mg)

The major difficulties and sources of error in the Karl Fischer titration methods are as follows:

1. **Incomplete moisture extraction**. For this reason, fineness of grind (i.e., particle size) is important in preparation of cereal grains and some foods.
2. **Atmospheric moisture**. External air must not be allowed to infiltrate the reaction chamber.
3. **Moisture adhering** to walls of unit. All glassware and utensils must be carefully dried.
4. **Interferences** from certain food constituents. **Ascorbic acid** is oxidized by KFR to dehydroascorbic acid to overestimate moisture content; **carbonyl compounds** react with methanol to form acetals and release water to overestimate moisture content (this reaction also may result in fading endpoints); **unsaturated fatty acids** will react with iodine, so moisture content will be overestimated.

6.5 PHYSICAL METHODS

6.5.1 Dielectric Method

The electrical properties of water are used in the **dielectric method** to determine the moisture content of certain foods, by measuring the change in **capacitance** or **resistance to an electric current** passed through a sample. These instruments require calibration against samples of known moisture content as determined by standard methods. Sample density or weight/volume relationships and sample temperature are important factors to control in making reliable and repeatable measurements by dielectric methods. These techniques can be very useful for process control measurement applications, where continuous measurement is required. These methods are limited to food systems that contain no more than 30–35% moisture.

The moisture determination in dielectric-type meters is based on the fact that the dielectric constant of water (80.37 at 20°C) is higher than that of most solvents. The **dielectric constant** is measured as an index of capacitance. As an example, the dielectric method is used widely for cereal grains. Its use is based on the fact that water has a dielectric constant of 80.37, whereas starches and proteins found in cereals have dielectric constants of 10. By determining this properly on samples in standard metal condensers, dial readings may be obtained and the percentage of moisture determined from a previously constructed standard curve for a particular cereal grain.

6.5.2 Hydrometry

Hydrometry is the science of measuring **specific gravity** or **density**, which can be done using several different principles and instruments. While hydrometry is considered archaic in some analytical circles, it is still widely used and, with proper technique, is highly accurate. Specific gravity measurements with various types of **hydrometers** or with a **pycnometer** are commonly used for routine testing of moisture (or solids) content of numerous food products. These include beverages, salt brines, and sugar solutions. Specific gravity measurements are best applied to the analysis of solutions consisting of only one solute in a medium of water.

6.5.2.1 Hydrometer

A second approach to measuring specific gravity is based on **Archimedes' principle**, which states that a solid suspended in a liquid will be buoyed by a force equal to the weight of the liquid displaced. The weight per unit volume of a liquid is determined by measuring the volume displaced by an object of standard weight. A hydrometer is a standard weight on the end of a spindle, and it displaces a weight of liquid equal to its own weight (Fig. 6-6). For example, in a liquid of low density, the hydrometer will sink to a greater depth, whereas in a liquid of high density, the hydrometer will not sink as far. Hydrometers are available in narrow and wide ranges of specific gravity. The spindle of the hydrometer is calibrated to read specific gravity directly at 15.5 or 20°C. A **hydrometer** is not as accurate as a pycnometer, but the speed with which you can do an analysis is a decisive factor. The accuracy of specific gravity measurements can be improved by using a hydrometer calibrated in the desired range of specific gravities.

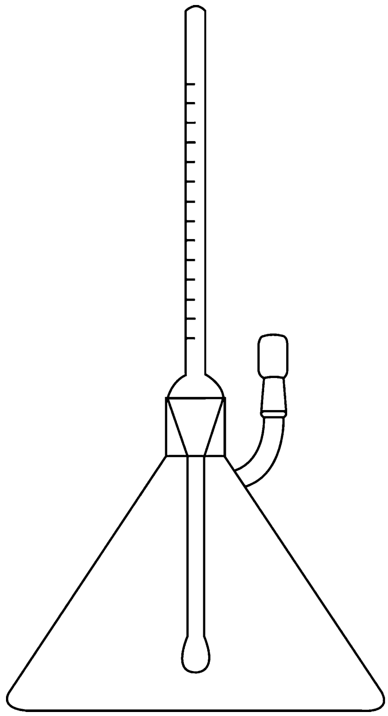
The rudimentary but surprisingly accurate hydrometer comes equipped with various modifications depending on the fluid to be measured:

1. The Quevenne and New York Board of Health **lactometer** is used to determine the density of milk. The Quevenne lactometer reads from 15 to 40 lactometer units and corresponds to 1.015 to 1.040 specific gravity. For every degree above 60°F, 0.1 lactometer unit is added to the reading, and 0.1 lactometer unit is subtracted for every degree below 60°F.
2. The **Baumé hydrometer** was used originally to determine the density of salt solutions (originally 10% salt), but it has come into much wider use. From the value obtained in the Baumé scale, you can convert to specific gravity of liquids heavier than water. For example, it is used to determine the specific gravity of milk being condensed in a vacuum pan.
3. The **Brix hydrometer** is a type of **saccharometer** used for sugar solutions such as fruit juices and syrups and one usually reads directly the percentage of sucrose at 20°C. **Balling saccharometers** are graduated to indicate percentage of sugar by weight at 60°F. The terms **Brix** and **Balling** are interpreted as the weight percentage of pure sucrose.
4. **Alcoholometers** are used to estimate the alcohol content of beverages. Such hydrometers are calibrated in 0.1 or 0.2° proof to determine the percentage of alcohol in distilled liquors (AOAC Method 957.03).
5. The **Twaddell hydrometer** is only for liquids heavier than water.

6-6 figure Hydrometers. (Courtesy of Cole-Parmer Instrument Company, Vernon Hills, IL.)

6.5.2.2 Pycnometer

One approach to measuring specific gravity is a comparison of the weights of equal volumes of a liquid and water in standardized glassware, a **pycnometer** (Fig. 6-7). This will yield density of the liquid compared to water. In some texts and reference books, 20/20 is given after the specific gravity number. This indicates that the temperature of both fluids was 20°C when the weights were measured. Using a clean, dry pycnometer at 20°C, the analyst weighs it empty, fills it to the full point with distilled water at 20°C, inserts the thermometer to seal the fill opening, and then touches off the last drops of water and puts on the cap for the overflow tube. The pycnometer is wiped dry in case of



6-7 figure Pycnometer.

any spillage from filling and is reweighed. The density of the sample is calculated as follows:

$$\frac{\text{weight of sample-filled pycnometer} \\ -\text{weight of empty pycnometer}}{\text{weight of water-filled pycnometer} \\ -\text{weight of empty pycnometer}} = \text{density of sample} \quad [10]$$

This method is used for determining alcohol content in alcoholic beverages (e.g., distilled liquor, AOAC Method 930.17), solids in sugar syrups (AOAC Method 932.14B), and solids in milk (AOAC Method 925.22).

6.5.3 Refractometry

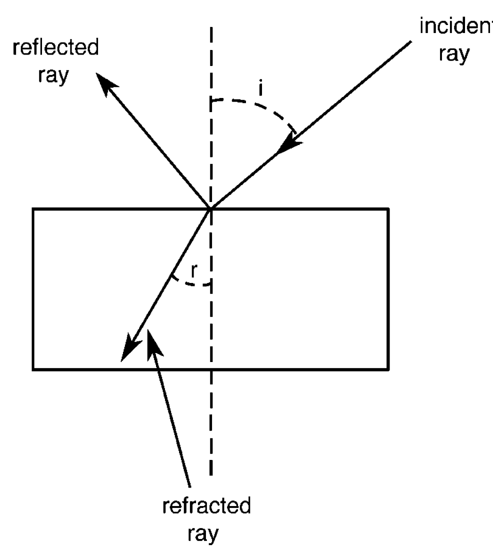
Moisture in liquid sugar products and condensed milks can be determined using a Baumé hydrometer (solids), a Brix hydrometer (sugar content), gravimetric means, or a **refractometer**. If it is performed correctly and no crystalline solids are evident, the refractometer procedure is rapid and surprisingly accurate (AOAC Method 9.32.14C, for solids in syrups). The refractometer has been valuable in determining the soluble solids in fruits and fruit products (AOAC Method 932.12; 976.20; 983.17).

The **refractive index** (RI) of an oil, syrup, or other liquid is a dimensionless constant that can be used to describe the nature of the food. While some refractometers are designed only to provide results as refractive indices, others, particularly hand-held, quick-to-use units, are equipped with scales calibrated to read the percentage of solids, percentage of sugars, and the like, depending on the products for which they are intended. Tables are provided with the instruments to convert values and adjust for temperature differences. Refractometers are used not just on the laboratory bench or as hand-held units. Refractometers can be installed in a liquid processing line to monitor the °Brix of products such as carbonated soft drinks, dissolved solids in orange juice, and the percentage of solids in milk (15).

When a beam of light is passed from one medium to another and the density of the two differs, then the beam of light is bent or refracted. Bending of the light beam is a function of the media and the sines of the angles of incidence and refraction at any given temperature and pressure and is thus a constant (Fig. 6-8). The (RI) (η) is a ratio of the sines of the angles:

$$\eta = \frac{\text{sine incident ray angle}}{\text{sine refracted ray angle}} \quad [11]$$

All chemical compounds have an index of refraction. Therefore, this measurement can be used for the qualitative identification of an unknown compound by comparing its RI with literature values. RI varies with **concentration** of the compound, **temperature**, and **wavelength of light**. Instruments are designed to give a reading by passing a light beam of a specific wavelength through a glass prism into a liquid, the sample. Bench-top or hand-held units use **Amici prisms** to obtain the **D line of the sodium spectrum** or



6-8 figure Reflection and refraction concepts of refractometry.

589 nm from white light. Whenever refractive indices of standard fluids are given, these are prefaced with $\eta_{\mathrm{D}}^{20} = a$ value from 1.3000 to 1.7000. The Greek letter η is the symbol for RI; the 20 refers to temperature in °C; and D is the wavelength of the light beam, the D line of the sodium spectrum.

Bench-top instruments are more accurate compared with hand-held units mainly because of temperature control (Fig. 6-9). These former units have provisions for water circulation through the head where the prism and sample meet. **Abbe refractometers** are the most popular for laboratory use. Care must be taken when cleaning the prism surface following use. Wipe the contact surface clean with lens paper and rinse with distilled water and then ethanol. Close the prism chamber and cover the instrument with a bag when not in use to protect the delicate prism surface from dust or other debris that might lead to scratches and inaccuracy.

The fact that the RI of a solution increases with concentration has been exploited in the analysis of total soluble solids of carbohydrate-based foods such as sugar syrups, fruit products, and tomato products. Because of this use, these refractometers are calibrated in °Brix (g of sucrose/100 g of sample), which is equivalent to percentage sucrose on a wt/wt basis. Refractive index measurements are used widely to approximate sugar concentration in foods, even though values are accurate only for pure sucrose solutions.

6.5.4 Infrared Analysis

Infrared spectroscopy (see Chap. 23) has attained a primary position in monitoring the composition of food products before, during, and following processing (16). It has a wide range of food applications and has proven successful in the laboratory, at-line, and on-line. Infrared spectroscopy measures the absorption of radiation (near- or mid-infrared) by molecules in foods. Different frequencies of infrared radiation are absorbed by different functional groups characteristic of the molecules in food. Similar to the use of ultraviolet (UV) or visible (Vis) light in UV–Vis spectroscopy, a sample is irradiated with a wavelength of infrared light specific for the constituent to be measured. The concentration of that constituent is determined by measuring the energy that is reflected or transmitted by the sample, which is inversely proportional to the energy absorbed. Infrared spectrometers must be calibrated for each analyte to be measured and the analyte must be uniformly distributed in the sample.

For water, near-infrared (NIR) bands (1400–1450; 1920–1950 nm) are characteristic of the –OH stretch

6-9 figure Rhino Brix hand-held refractometer, R^2 mini digital hand-held refractometer, and Mark III Abbe refractometer. (Courtesy of Reichert Analytical Instrument, Depew, NY.)

of the water molecule and can be used to determine the moisture content of a food. NIR has been applied to moisture analysis of a wide variety of food commodities.

The use of mid-infrared milk analyzers to determine fat, protein, lactose, and total solids in milk (AOAC Method 972.16) is covered in Chap. 23. The midrange spectroscopic method does not yield moisture or solids results except by computer calculation because these instruments do not monitor at wavelengths where water absorbs. The instrument must be calibrated using a minimum of eight milk samples that were previously analyzed for fat (F), protein (P), lactose (L), and total solids (TS) by standard methods. Then, a mean difference value, a, is calculated for all samples used in calibration:

$$a = \Sigma(\mathrm{TS} - F - P - L)/n \qquad [12]$$

where:

a = solids not measurable by the F, P, and L methods
n = number of samples
F = fat percentage
P = protein percentage
L = lactose percentage
TS = total solids percentage

Total solids then can be determined from any infrared milk analyzer results by using the formula

$$\mathrm{TS} = a + F + P + L \qquad [13]$$

The a value is thus a standard value mathematically derived. Newer instruments have the algorithm in their computer software to ascertain this value automatically. Moreover, Fourier transform infrared spectroscopy (FTIR) is the latest development that allows greater flexibility in infrared assays.

6.5.5 Freezing Point

When water is added to a food product, many of the physical constants are altered. Some properties of solutions depend on the number of solute particles as ions or molecules present. These properties are vapor pressure, freezing point, boiling point, and osmotic pressure. Measurement of any of these properties can be used to determine the concentration of solutes in a solution. However, the most commonly practiced assay for milk is the change of the freezing point value. It has economic importance with regard to both raw and pasteurized milk. The **freezing point** of milk is its most constant physical property. The secretory process of the mammary gland is such that the osmotic pressure is kept in equilibrium with blood and milk. Thus, with any decrease in the synthesis of lactose, there is a compensating increase in the concentrations of Na^+ and Cl^-. While termed a physical constant, the freezing point varies within narrow limits, and the vast majority of samples from individual cows fall between −0.503°C and −0.541°C (−0.525 and −0.565°H, temperature in °H or **Hortvet**, the surname of the inventor of the first freezing point apparatus). The average is very close to −0.517°C (−0.540°H). Herd or bulk milk will exhibit a narrower range unless the supply was watered intentionally or accidentally or if the milk is from an area where severe drought has existed. All values today are given in °C by agreement. The following is used to convert °H to °C, or °C to °H (5, 6):

$$^\circ\mathrm{C} = 0.9623^\circ\mathrm{H} - 0.0024 \qquad [14]$$

$$^\circ\mathrm{H} = 1.03916^\circ\mathrm{C} + 0.0025 \qquad [15]$$

The principal utility of freezing point is to measure for **added water**. However, the freezing point of milk can be altered by mastitis infection in cows and souring of milk. In special cases, nutrition and environment of the cow, stage of lactation, and processing operations for the milk can affect the freezing point. If the solute remains constant in weight and composition, the change of the freezing point varies inversely with the amount of solvent present. Therefore, we can calculate the percent H_2O added:

$$\%\mathrm{H_2O\ added} = \frac{0.517 - T}{0.517} \times 100 \qquad [16]$$

where:

0.517 = freezing point in °C of all milk entering a plant
T = freezing point in °C of a sample

The AOAC Method 961.07 for water added to milk uses a **cryoscope** to test for freezing points, and assumes a freezing point for normal milk of −0.527°C (−0.550°H). The Food and Drug Administration will reject all milk with freezing points above −0.503°C (−0.525°H). Since the difference between the freezing points of milk and water is slight and since the freezing point can be used to calculate the amount of water added, it is essential that the method be as precise as possible. The thermister used can sense temperature change to 0.001°C (0.001°H). The general technique is to supercool the solution and then induce crystallization by a vibrating reed. The temperature will rise rapidly to the freezing point or eutectic temperature as the water freezes. In the case of pure water, the temperature remains constant until all the water is frozen. In the case of milk, the temperature is read when there is no further temperature rise.

6-10 figure A model 4D3 advanced instruments cryoscope for freezing point determination in milk. (Courtesy of Advanced Instruments, Inc., Norwood, MA.)

Instrumentation available is manufactured by Advanced Instruments (Fig. 6-10). Time required for the automated instruments is 1–2 min per sample using a prechilled sample.

6.6 WATER ACTIVITY

Water content alone is not a reliable indicator of food stability, since foods with the same water content differ in their perishability (17). This is at least partly due to differences in the way that water associates with other constituents in a food. Water tightly associated with other food constituents is less available for microbial growth and chemical reactions to cause decomposition. **Water activity** (a_w) is a better indication of food perishability than is water content. Water activity is defined as follows:

$$a_w = \frac{P}{P_o} \quad [17]$$

$$a_w = \frac{\text{ERH}}{100} \quad [18]$$

where:

a_w = water activity
P = partial pressure of water above the sample
P_o = vapor pressure of pure water at the same temperature (specified)
ERH = equilibrium relative humidity surrounding the product

There are various techniques to measure a_w. A commonly used approach relies on measuring the amount of moisture in the equilibrated headspace above a sample of the food product, which correlates directly with sample a_w. A sample for such analysis is placed in a small closed chamber at constant temperature, and a relative humidity sensor is used to measure the ERH of the sample atmosphere after equilibration. A simple and accurate variation of this approach is the chilled mirror technique in which the water vapor in the headspace condenses on the surface of a mirror that is cooled in a controlled manner. The dew point is determined by the temperature at which condensation takes place, and this determines the relative humidity in the headspace. Two other general approaches to measuring a_w are (1) using the sample freezing point depression and moisture content to calculate a_w, and (2) equilibrating a sample in a chamber held at constant relative humidity (by means of a saturated salt solution) and then using the water content of the sample to calculate a_w (17).

6.7 COMPARISON OF METHODS

6.7.1 Principles

Oven drying methods involve the removal of moisture from the sample and then a weight determination of the solids remaining to calculate the moisture content. Nonwater volatiles can be lost during drying, but their loss is generally a negligible percentage of the amount of water lost. Distillation procedures also involve a separation of the moisture from the solids, and the moisture is quantitated directly by volume. Karl Fischer titration is based on chemical reactions of the moisture present, reflected as the amount of titrant used.

The dielectric method is based on electrical properties of water. Hydrometric methods are based on the relationship between specific gravity and moisture content. The refractive index method is based on how water in a sample affects the refraction of light. Near-infrared analysis of water in foods is based on measuring the absorption at wavelengths characteristic of the molecular vibration in water. Freezing point is a physical property of milk that is changed by a change in solute concentration.

6.7.2 Nature of Sample

While most foods will tolerate oven drying at high temperatures, some foods contain volatiles that are lost at such temperatures. Some foods have constituents that undergo chemical reactions at high temperatures to generate or utilize moisture or other compounds, to affect the calculated moisture content. Vacuum oven drying at reduced temperatures may overcome such problems for some foods. However, a distillation technique is necessary for some food to minimize volatilization and decomposition. For foods very low in moisture or high in fats and sugars, Karl Fischer titration is often the method of choice. The use of a pycnometer, hydrometer, and refractometer requires liquid samples, ideally with limited constituents.

6.7.3 Intended Purposes

Moisture analysis data may be needed quickly for quality control purposes, in which high accuracy may not be necessary. Of the oven drying methods, microwave drying, infrared drying, and the moisture analyzer technique are fastest. Some forced draft oven procedures require less than 1 h drying, but most forced draft oven and vacuum oven procedures require a much longer time. The electrical, hydrometric, and refractive index methods are very rapid but often require correlation to less empirical methods. Oven drying procedures are official methods for a variety of food products. Reflux distillation is an AOAC method for chocolate, dried vegetables, dried milk, and oils and fats. Such official methods are used for regulatory and nutrition labeling purposes.

6.8 SUMMARY

The moisture content of foods is important to food processors and consumers for a variety of reasons. While moisture determination may seem simplistic, it is often one of the most difficult assays in obtaining accurate and precise results. The free water present in food is generally more easily quantitated as compared to the adsorbed moisture and the water of hydration. Some moisture analysis methods involve a separation of moisture in the sample from the solids and then quantitation by weight or volume. Other methods do not involve such a separation but instead are based on some physical or chemical property of the water in the sample. A major difficulty with many methods is attempting to remove or otherwise quantitate all water present. This often is complicated by decomposition or interference by other food constituents. For each moisture analysis method, there are factors that must be controlled or precautions that must be taken to ensure accurate and precise results. Careful sample collection and handling procedures are extremely important and cannot be overemphasized. The choice of moisture analysis method is often determined by the expected moisture content, nature of other food constituents (e.g., highly volatile, heat sensitive), equipment available, speed necessary, accuracy and precision required, and intended purpose (e.g., regulatory or in-plant quality control).

6.9 STUDY QUESTIONS

1. Identify five factors that one would need to consider when choosing a moisture analysis method for a specific food product.
2. Why is standardized methodology needed for moisture determinations?
3. What are the potential advantages of using a vacuum oven rather than a forced draft oven for moisture content determination?
4. In each case specified below, would you likely overestimate or underestimate the moisture content of a food product being tested? Explain your answer.
   (a) Forced draft oven:
      - Particle size too large
      - High concentration of volatile flavor compounds present
      - Lipid oxidation
      - Sample very hygroscopic
      - Alteration of carbohydrates (e.g., Maillard browning)
      - Sucrose hydrolysis
      - Surface crust formation
      - Splattering
      - Desiccator with dried sample not sealed properly

   (b) Toluene distillation:
      - Emulsion between water in sample and solvent not broken
      - Water clinging to condenser

   (c) Karl Fischer:
      - Very humid day when weighing original samples
      - Glassware not dry
      - Sample ground coarsely
      - Food high in vitamin C
      - Food high in unsaturated fatty acids
5. The procedure for an analysis for moisture in a liquid food product requires the addition of 1–2 ml of deionized water to the weighed sample in the moisture pan. Why should you add water to an analysis in which moisture is being determined?
6. A new instrument based on infrared principles has been received in your laboratory to be used in moisture analysis. Briefly describe the way you would ascertain if the new instrument would meet your satisfaction and company standards.
7. A technician you supervise is to determine the moisture content of a food product by the Karl Fischer method. Your technician wants to know what is this “Karl Fischer reagent water equivalence” that is used in the equation to calculate percentage of moisture in the sample, why it is necessary, and how it is determined. Give the technician your answer.

8. To explain and contrast the principles (not procedures) in determining the moisture content of food products by the following method, complete the table below. (Assume that sample selection and handling has been done appropriately.)

	What is actually measured?	*How is water removed/ reacted/ identified?*	*What assumptions are made in trusting the data obtained (or precautions taken to ensure accurate data)?*
Microwave oven			
NIR			
Karl Fischer			
Toluene distillation			

9. You are fortunate to have available in your laboratory the equipment for doing moisture analysis by essentially all methods – both official and rapid quality control methods. For each of the food products listed below (with the purpose specified as rapid quality control or official), indicate (a) the name of the method you would use, (b) the principle (not procedure) for the method, (c) a justification for use of that method (as compared to using a hot air drying oven), and (d) two cautions in use of the method to ensure accurate results.
   (a) Ice cream mix (liquid) – quality control
   (b) Milk chocolate – official
   (c) Spices – official
   (d) Syrup for canned peaches – quality control
   (e) Oat flour – quality control
10. You are a manufacturer of processed cheese. The maximum allowed moisture content for your product is 40%. Your current product has a mean moisture content of 38%, with a standard deviation of 0.7. It would be possible to increase your mean moisture content to 39.5% if you could reduce your standard deviation to 0.25. This would result in a saving of $3.4 million per year. You can accomplish this by rapidly analyzing the moisture content of the cheese blend prior to the cooking step of manufacture. The cheese blend is prepared in a batch process, and you have 10 min to adjust the moisture content of each batch.
    (a) Describe the rapid moisture analysis method you would use. Include your rationale for selecting the method.
    (b) How would you ensure the accuracy and precision of this method (you need to be sure your standard deviation is below 0.25)?
11. You work in a milk drying plant. As part of the production process, you need to rapidly analyze the moisture content of condensed milk.
    (a) What rapid secondary method would you use, and what primary method would you use to calibrate the secondary method? Additionally, how would you ensure the accuracy and precision of your secondary method?
    (b) Your results with the secondary method are consistently high (about 1%), based on the secondary method you chose. What are some potential problems and how would you correct them?
12. During a 12-h period, 1000 blocks (40 lbs each) from ten different vats (100 blocks per vat) of Cheddar cheese were produced. It was later realized that the cooking temperature was too low during cheesemaking. You are concerned that this might increase the moisture content of the cheese above the legal requirement. Describe the sampling plan and method of analysis you would use to determine the moisture content of the cheese. You want the results within 48 h so you can determine what to do with the cheese.

6.10 PRACTICE PROBLEMS

1. As an analyst, you are given a sample of condensed soup to analyze to determine if it is reduced to the correct concentration. By gravimetric means, you find that the concentration is 26.54% solids. The company standard reads 28.63%. If the starting volume were 1000 gallons at 8.67% solids and the weight is 8.5 pounds per gallon, how much more water must be removed?
2. Your laboratory just received several sample containers of peas to analyze for moisture content. There is a visible condensate on the inside of the container. What is your procedure to obtain a result?
3. You have the following gravimetric results: weight of dried pan and glass disc is 1.0376 g, weight of pan and liquid sample is 4.6274 g, and weight of the pan and dried sample is 1.7321 g. What was the moisture content of the sample and what is the percent solids?

Answers

1. The weight of the soup initially is superfluous information. By condensing the soup to 26.54% solids from 8.67% solids, the volume is reduced to 326.7 gal [(8.67%/26.54%) × 1000 gal]. You need to reduce the volume further to obtain 28.63% solids [(8.67%/28.63%) × 1000 gal] or 302.8 gal. The difference in the gallons obtained is 23.9 gal (326.7 gal − 302.8 gal), or the volume of water that must be removed from the partially condensed soup to comply with company standards.
2. This problem focuses on a real issue in the food processing industry – when do you analyze a sample and when don't you? It would appear that the peas have lost moisture that should be within the vegetable for correct results. You will need to grind the peas in a food mill or blender. If the peas are in a Mason jar or one that fits a blender head, no transfer is needed. Blend the peas to a creamy texture. If a container transfer was made, then

put the blended peas back into the original container. Mix with the residual moisture to a uniform blend. Collect a sample for moisture analysis. You should note on the report form containing the results of the analysis that the pea samples had free moisture on container walls when they arrived.

3. Note Equations [2]–[4]. To use any of the equations, you must subtract the weight of the dried pan and glass disc. Then you obtain 3.5898 g of original sample and 0.6945 g when dried. By subtracting these results, you have removed water (2.8953 g). Then $(0.6945\,\mathrm{g}/3.5898\,\mathrm{g}) \times 100 = 19.35\%$ solids and $(2.8953\,\mathrm{g}/3.5898\,\mathrm{g}) \times 100 = 80.65\%$ water.

6.11 REFERENCES

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