15 chapter

Moisture and Total Solids Analysis

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15.1 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. Water molecules are small and ubiquitous in the environment in which foods are produced, stored, and used. Moisture exchange between foods and the environment can lead to under- or overestimation from moisture assays, and water can be difficult to completely remove from foods. The first sections of this chapter describe various methods for moisture content analysis – their principles, procedures, applications, cautions, advantages, and disadvantages. Water activity measurement also is described later in this chapter, since it parallels the measurement of total moisture as an important stability and quality factor. Determining both the water content and the water activity of a food provides a complete moisture analysis. With an understanding of the techniques described, one can apply appropriate moisture analyses to a wide variety of food products.

15.1.1 Importance of Moisture Assays

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, referred to as the **moisture or water content** of the food [1–4]. In this context, the words "water" and "moisture" are generally used interchangeably. 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, and there are legal limits as to how much water must or can be present in some foods. Some examples in which moisture content is important to the food processor are provided in Table 15.1.

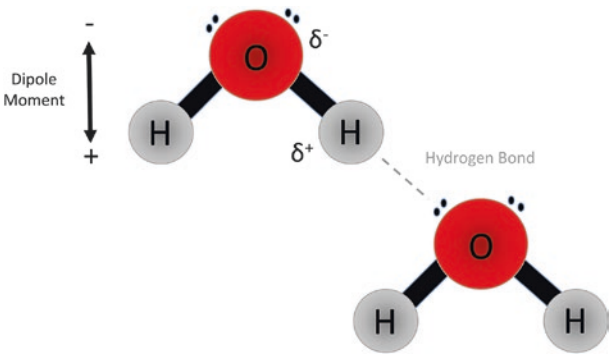
In addition to quantifying the amount of water in foods, it is also important to document the energy status of the water in the food by determining the **water activity** [5]. It is the water activity, more so than the moisture content, that influences microbial growth, physical properties, and chemical and enzymatic reactions in foods. Additionally, it is differences in water activity, not moisture content, that drive moisture migration between different food components (such as between a crust and a filling) or between a food and the environment. Water molecules move from regions of high water activity to regions of low water activity until equilibrium water activity is reached.

15.1 table Importance of moisture content in the food industry

Moisture content is important for	*Food examples*
Preservation and stability	Dehydrated vegetables, potatoes, and fruits Dried milks and infant formulas Powdered eggs, coffees, and teas Spices and herbs Crispy fried or baked chips and crackers Cotton candy
Quality factors	Jams and jellies, to inhibit sugar crystallization Sugar syrups Prepared cereals: conventional 4–8% H_2O, puffed 7–8% H_2O
Convenience in packaging or shipping (usually reduced moisture)	Concentrated milks and fruit juices Liquid cane sugar (67% solids) and liquid corn sweetener (80% solids) Dehydrated products (which clump or become sticky and difficult to package if moisture content is too high)
Meeting compositional standards and standards of identity	Cheddar cheese must be ≤39% H_2O Enriched flour must be ≤15% H_2O Pineapple juice must have soluble solids of ≥10.5°Brix (conditions specified) Glucose syrup must have ≥70% total solids % H_2O in processed meats is commonly specified
Accurate computation of nutritional value	
Expressing results of other analytical determinations on a uniform basis	

15.1.2 Water in Foods

The amount, physical state, and location of water in foods will affect the types of analyses best suited to a particular food product, the ease and rate of water removal, the time required for assay equilibration, and the sample handling.



15.1 figure Schematic of the water molecule and hydrogen bonding

15.2 table Properties of water

Property	*Value*
Molecular weight	18.0153 g
Melting point (1 atm)	0.000 °C
Boiling point (1 atm)	100.000 °C
Enthalpy of vaporization (1 atm)	40.647 kJ/mol
Glass transition temperature (1 atm)	136 K
Critical temperature	373.99 °C
Density (20 °C)	0.99821 g/cm^3
Vapor pressure (20 °C)	2.3388 kPa
Heat capacity (20 °C)	4.1818 J/gK
Thermal conductivity (20 °C)	0.5984 W/mK
Thermal diffusivity (20 °C)	1.4×10^{-7} m^2/s
Dielectric constant (20 °C)	80.20
Water activity (20 °C)	1.000
Water activity (−20 °C)	0.82

Adapted from [5, 6, 38]

15.1.2.1 *Structure of the Water Molecule*

Water molecules are comprised of two hydrogen atoms covalently bound to an oxygen atom in a distorted tetrahedral arrangement (Fig. 15.1) [6]. There is a slight partially negative charge on the oxygen atom and a slight partially positive charge on the hydrogen atoms. Thus, water molecules are small and highly polar, have two hydrogen bond donor sites (the hydrogen atoms), and have two hydrogen bond acceptor sites (the two nonbonding electron pairs on the oxygen atom). Hydrogen bonds are relatively weak attractive interactions with short life spans (picoseconds), readily breaking and reforming as water molecules are constantly moving, particularly when temperature or humidity fluctuations occur [7, 8]. This poses challenges for accurately quantifying the amount or activity of water in a sample since the target can be moving from or escaping to the environment during sample handling.

15.1.2.2 *Physical States and Properties of Water*

The temperature, pressure, and extent of intermolecular hydrogen bonding between water molecules result in water existing in different physical states: as a solid (ice crystals), liquid (water), and gas (water vapor, humidity). Determining the total amount of water as well as the water activity in foods may involve analyzing water in all three of these states. Many direct moisture assays are based on the weight lost from evaporating all water from a sample, often by application of heat to overcome the enthalpy of vaporization. Other properties of water may be exploited for the indirect determination of moisture contents, including the dielectric constant, density, and freezing point. These and other properties of water are summarized in Table 15.2.

15.1.2.3 *Water Interactions with Food Ingredients*

Although water does not covalently bond with food ingredients, it can be difficult to remove from foods. Having some understanding of the water intermolecular interactions with food ingredients and the locations of water in foods can improve the application of moisture assays. The hydrogen bond donor and acceptor sites on water molecules interact with food ingredients via hydrogen bonds, dipole-dipole interactions, ionic attractions, and van der Waals forces [6]. Water molecules tend to cluster around ions and charged groups, as well as other hydrogen bond donor and acceptor groups such as hydroxyl groups (-OH), carbonyl oxygens (=O), and amine groups (-NH_2), adopting a more ordered structure in these hydration shell clusters than is found in bulk water [9]. To remove water from food, enough energy must be applied to overcome these intermolecular interactions. There are also different mechanisms by which water interacts with solids, including surface interactions (adsorption), condensed water (capillary condensation and deliquescence), and internalized water (absorption and crystal hydrate formation) [10]. Water also can be physically entrapped in food matrices, such as in gel structures or dense dehydrated or fried products, making it more difficult to remove than surface water. It is nearly impossible to remove all water from foods, particularly the internalized water for which heat and mass transfer rates and the properties of the food material can complicate complete moisture egress.

15.1.3 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. 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 transfer between the sample and the container environment will likely occur. It is critical to control temperature fluctuations since moisture will migrate in a sample to the colder part. To control this potential error, the entire sample should be removed from the container and reblended quickly, and a new test portion should then be sampled [11, 12].

To illustrate the need for optimum efficiency and speed in weighing samples for analysis, Bradley and Vanderwarn [13] 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 (RH). At 50% RH, it required only 5 s to lose 0.01% moisture. This time doubled at 70% RH 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. Samples with lower a_w than the environmental RH, such as many powders and crispy fried products, may pick up moisture during handling leading to overestimation of moisture contents. These examples demonstrate the necessity of absolute control during collection of samples through weighing, before drying or other analysis.

15.2 MOISTURE/WATER CONTENT

15.2.1 Overview

The moisture content of foods varies greatly, as shown in Table 15.3 [3]. Water is a major constituent of many food products. The approximate, expected moisture content of a food can affect the choice of the method of measurement. It also can guide the analyst in determining the practical level of accuracy required when measuring moisture content, relative to other food constituents. The method used for determining moisture content may measure more or less of the water present. This is the reason for official methods with stated procedures [14–16]. 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; and 969.19, distillation [14]. Usually, the first method listed by AOAC International is preferred over others in any section.

15.3 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 the US Department of Agriculture, Agricultural Research Service (2016) USDA National Nutrient Database for Standard Reference. Release 28. Nutrient Data Laboratory Home Page, http://ndb.nal.usda.gov [3]

The different types of moisture content assays can be generally categorized into direct and indirect methods. **Direct methods** for moisture content are often done by removing water, although the method for

moisture removal may vary. Drying, distillation, and extraction are commonly used for moisture removal followed by weighing, volumetry, or titration to determine moisture content. **Indirect methods** are based on properties of the food that are related to the presence of water, such as capacitance, specific gravity, density, refractive index, freezing point, and electromagnetic absorption.

15.2.2 Oven Drying Methods

In **oven drying methods**, which are direct 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 ~1 h to over 24 h.

15.2.2.1 *General Information*

15.2.2.1.1 Removal of Moisture

Water evaporates more quickly at higher temperatures. 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. According to Raoult's law, 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 drying process as solute concentrations increase.

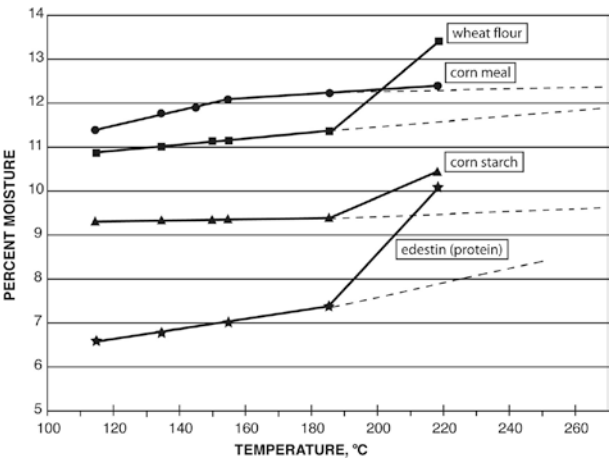
Moisture removal is sometimes best achieved in a two-stage process. Liquid products (e.g., juices, milk) are commonly pre-dried over a **steam bath** before drying in an oven. Products such as bread and field-dried grain are often air-dried and then ground and oven dried, with the moisture content calculated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

15.2.2.1.2 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 content 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 elevated temperatures and release water to form dehydrated hydrocarbon compounds. The moisture generated in this decomposition leads to overestimation of the moisture content and 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. The loss of **volatile constituents**, such as acetic, propionic, and butyric acids, and alcohols, esters, and aldehydes among flavor compounds, can also lead to errors. 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 [17] 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. 15.2). Distinct breaks were shown that indicated the temperature at which decomposition became measurable.



15.2 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 [17]. Copyright 1920, American Chemical Society)

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.

15.2.2.1.3 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 have the added benefit of horizontal air movement 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 [13].

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.

15.2.2.1.4 Types of Pans for Oven Drying Methods

Pans used for moisture content determinations are varied in shape and may or may not have a cover. The AOAC International [14] 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 [13].

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.

15.2.2.1.5 Handling and Preparation of Pans

The preparation and handling of pans before use require consideration. Use only **tongs** to handle any pan or wear gloves. Even the oils in 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, and therefore longer drying times are not needed [13]. Store dried moisture pans in a functioning **desiccator**. The glass fiber covers should be dried for 1 h before use.

15.2.2.1.6 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 pre-weighed 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 content determinations, especially for sticky fruits.

The inert matrices such as sand and diatomaceous earth function to disperse the food constituents and minimize 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 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 and reweigh. The difference between weighing must be less than 0.5 mg for any suitable matrix [18].

15.2.2.1.7 *Calculations*

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

$$\%\ \text{Moisture(wwb)} = \frac{\text{wt of wet sample} - \text{wt of dry sample}}{\text{wt of wet sample}} \times 100 = \frac{\text{wt } H_2O \text{ in sample}}{\text{wt of wet sample}} \times 100 \quad (15.1)$$

$$\%\ \text{Moisture(dwb)} = \frac{\text{wt } H_2O \text{ in sample}}{\text{wt of dry sample}} \times 100 \quad (15.2)$$

$$\%\ \text{Total solids(wt/wt)} = \frac{\text{wt of dry sample}}{\text{wt of wet sample}} \times 100 = 100 - \%\,\text{Moisture (wwb)} \quad (15.3)$$

15.2.2.2 *Forced Draft Oven*

When using a forced draft oven, the sample is rapidly weighed into a pre-dried 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 15.4), 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 pre-drying 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.

15.2.2.3 *Vacuum Oven*

By drying under reduced pressure (25–100 mm Hg), the rate of evaporation is faster, and one is able to obtain a more complete removal of water without decomposition within a 3–6 h drying time. Vacuum

15.4 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
Fruit, dried		70	6
Coffee, roasted		70	16

From Wehr and Frank [15] p. 492, with permission
[a]*X* = samples must be partially dried on steam bath before being placed in oven

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 water saturation (such as DrieRite™). 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:

1. **Temperature** used depends on the product, such as 95–102 °C for some foods and lower temperatures (60–70 °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.

15.2.2.4 *Microwave Analyzer*

Determination of moisture contents in food products has traditionally been done using a standard oven, which, though accurate, can take many hours to dry a sample. **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 content 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, and 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 under-processed; 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 (4–8 min), 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 [19].

15.2.2.5 *Infrared Drying*

Infrared drying involves penetration of heat into the sample being dried, as compared to 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. 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 moist 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.

15.2.2.6 *Rapid Moisture Analyzer Technology*

Many rapid moisture/solids analyzers based on thermogravimetric principles are used by the food industry. In addition to those based on infrared and microwave drying as described previously, compact instruments that depend on high heat created by different types of heaters are available. Two main categories of heating elements include halogen heaters (e.g., Halogen Moisture Analyzers, Mettler Toledo, Columbus, OH) and ceramic heaters (e.g., Computrac®, Arizona Instrument LLC, Chandler, AZ). These analyzers detect moisture levels from 50 ppm to 100% using sample weights of 150 mg to 54 g. Smaller samples tend to dry more quickly, but it is important to use enough sample to be representative of the product. The test sample is placed on an aluminum pan or filter paper, and the heat control program (with a heating range of 25 °C to >200 °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. The samples are not removed from the oven which minimizes weighing errors, and accurate results are obtained within minutes. These analyzers are utilized for both production and laboratory assays with results comparable to reference methods.

15.2.2.7 *Thermogravimetric Analyzer*

In a thermogravimetric analyzer (TGA), the mass of a sample is continuously measured as it is heated at a controlled rate in a controlled atmosphere. The sample (often 10–50 mg) is loaded into a pan that is then placed into the TGA instrument that contains a furnace and a precision balance. Foods can lose mass during heating through release of adsorbed compounds (such as water), chemical reactions, and decomposition. To determine moisture content, the sample chamber is purged with an inert gas (e.g., nitrogen) so the sample only reacts with temperature during heating and mass changes due to oxidation are minimized. The furnaces in TGA instruments cover a wider temperature range than many other moisture content determination techniques and may range from subambient temperatures to >1500 °C. This enables not only moisture assessment (moisture content, temperature of dehydration, stoichiometry of a hydrate, and dehydration kinetics) but also at higher temperatures measurement of pyrolysis, decomposition, and weight % ash. More detailed information on TGA is provided in the chapter on thermal analysis (Chap. 30). To use TGA, a survey scan is often run on a sample from approximately 100 °C below to 100 °C above the transition of interest at a heating rate of 20 °C per minute. For water content determination of many foods, scans from ambient to 200 °C are generally sufficient, and water content is determined by the mass lost up to ~100 °C. A derivative plot of the rate of mass loss will generate a peak showing the onset, midpoint, and endpoint temperatures for water loss. The precision balance and *in situ* mass monitoring result in precise and accurate moisture content determinations; however, decomposition or volatile loss at temperatures overlapping water evaporation can lead to errors in moisture content determination.

15.2.3 Distillation Procedures

15.2.3.1 *Overview*

Distillation is used as another direct measure of moisture content. 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 content 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.

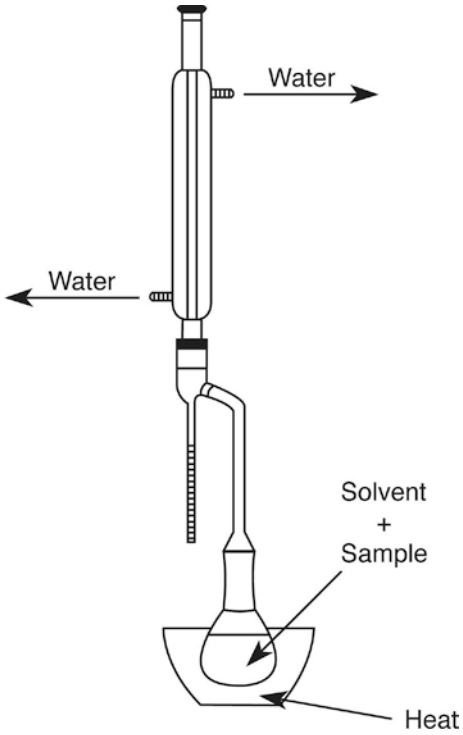
15.2.3.2 *Reflux Distillation with Immiscible Solvent*

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 the 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. 15.3) 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 requires about 1 h and involves using a brush to dislodge adhering water drops from the glassware, thereby minimizing error.

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

1. Formation of water-solvent 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 even with this, a burette brush is needed to dislodge water droplets.
3. Decomposition of the sample with production of water. If this is a measurable problem, discontinue method use and find an alternative procedure.



15.3 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

15.2.4 Chemical Method: Karl Fischer Titration

The **Karl Fischer titration**, a direct measure of moisture content, 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 content 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 and accurate and uses no heat. This method is based on the fundamental reaction described by Bunsen in 1853 [20] involving the reduction of iodine by SO_2 in the presence of water:

$$2H_2O + SO_2 + I_2 \rightarrow H_2SO_4 + 2HI \qquad (15.4)$$

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

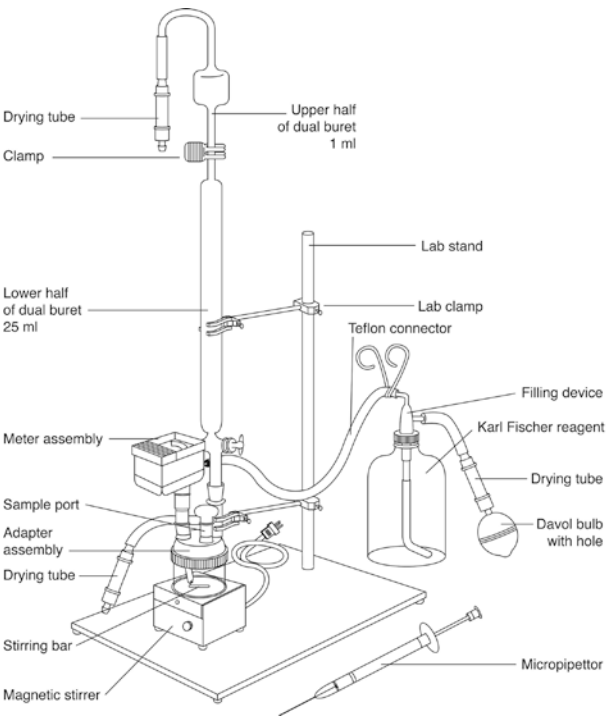
$$C_5H_5N \bullet I_2 + C_5H_5N \bullet SO_2 + C_5H_5N + H_2O \rightarrow 2C_5H_5N \bullet HI + C_5H_5N \bullet SO_3 \qquad (15.5)$$

$$C_5H_5N \bullet SO_3 + CH_3OH \rightarrow C_5H_5N(H)SO_4 \bullet CH_3 \qquad (15.6)$$

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. 15.4 is manual titration unit; Fig. 15.5 is an 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 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 water. The amount of iodine required to titrate the water is determined by the current needed to generate the iodine. Just like for volumetric titration, automated coulometric titration units are available commercially.



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

15.5 figure Automated Karl Fischer volumetric titration unit (Courtesy of Mettler Toledo, Columbus, OH)

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 noxious 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 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 water 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:

$$\mathrm{KFReq}\left(\mathrm{mg\,H_2O/mL}\right) = \frac{36\ \mathrm{g\,H_2O/mol\ Na_2C_4H_4O_6 \cdot 2H_2O} \times S \times 1000}{230.08\ \mathrm{g/mol} \times A} \quad (15.7)$$

where:

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

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

$$\%\,\mathrm{H_2O} = \frac{\mathrm{KFReq} \times Ks}{S} \times 100 \quad (15.8)$$

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.

15.2.5 Physical Methods

Most physical methods are indirect measures of moisture content and do not separate the water from the sample for analysis. These techniques can be rapid and nondestructive, which has led to their widespread use in food production and quality control; however, they must be calibrated against data collected by a direct method to quantify the amount of water in samples.

15.2.5.1 *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 direct 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 content 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 property 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.

15.2.5.2 *Hydrometry*

Hydrometry is the science of measuring **specific gravity** or **density**, which can be done using several differ-

ent 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.

15.2.5.2.1 Hydrometer

One 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. 15.6). 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 °C 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.

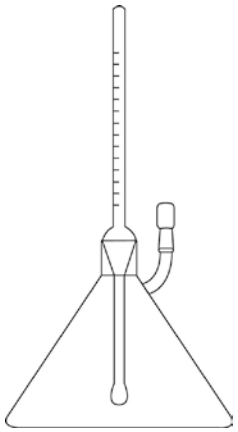
15.6 figure Hydrometers (Courtesy of Cole-Parmer Instrument Company, Vernon Hills, IL)

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 1.015–1.040 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.

15.2.5.2.2 Pycnometer

Another 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. 15.7). 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 any spillage from filling and is reweighed. The density of the sample is calculated as follows:



15.7 figure Pycnometer

$$\frac{\begin{array}{l}\text{weight of sample} - \text{filled pycnometer}\\ -\text{weight of empty pycnometer}\end{array}}{\begin{array}{l}\text{weight of water} - \text{filled pycnometer}\\ -\text{weight of empty pycnometer}\end{array}} = \text{density of sample} \qquad (15.9)$$

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).

15.2.5.3 *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 932.14C, 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).

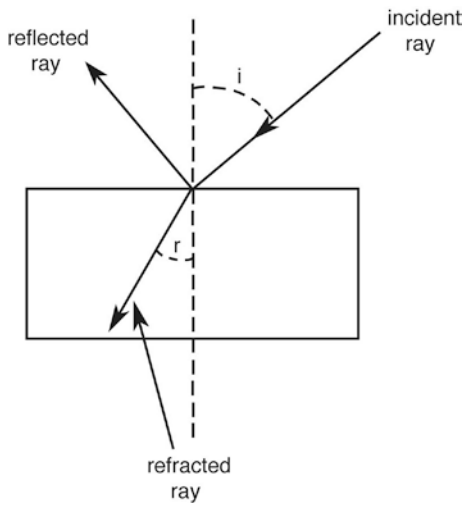
The **refractive index** (RI) of an oil, syrup, or other liquid is a dimensionless constant that can be used to describe the nature of the food. While some refractometers are designed only to provide results as refractive indices, others, particularly handheld, quick-to-use units, are equipped with scales 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 handheld 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 [21].

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. 15.8). The (RI) (η) is a ratio of the sines of the angles:

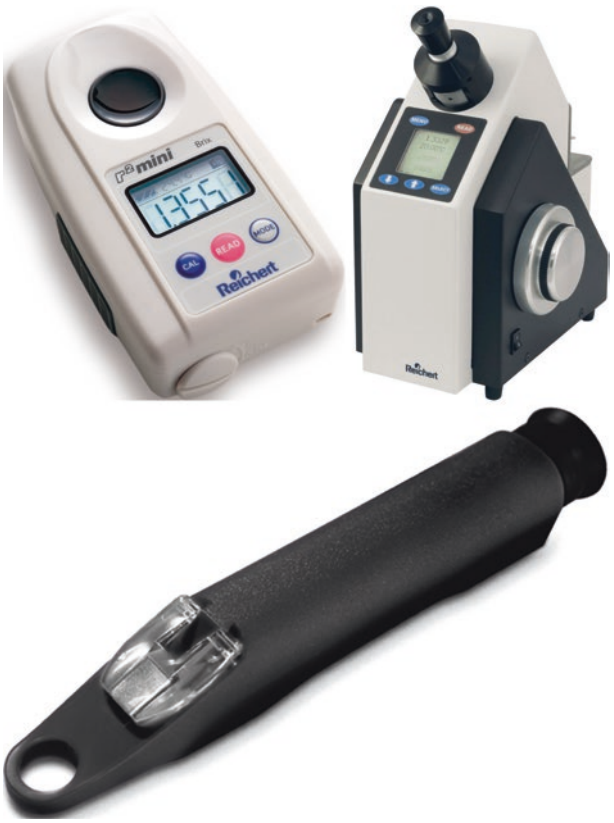
$$\eta = \frac{\text{sine incident ray angle}}{\text{sine refracted ray angle}} \qquad (15.10)$$

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. Benchtop or handheld units use **Amici prisms** to obtain the **D line of the sodium spectrum** or 589 nm from white light. Whenever refractive indices of standard fluids are given, these are prefaced with $\eta_D^{20} = \text{a value from } 1.3000 \text{ to } 1.7000$. 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.

Benchtop instruments are more accurate compared to handheld units mainly because of tempera-



15.8 figure Reflection and refraction concepts of refractometry



15.9 figure

Rhino Brix handheld refractometer, R² mini digital handheld refractometer, and Mark III Abbe refractometer (Courtesy of Reichert Analytical Instruments, Depew, NY)

ture control (Fig. 15.9). These former units have provisions for water circulation through the head where the prism and sample meet. Digital and **Abbe refractometers** are common for laboratory use. Care must be taken when cleaning the prism surface following use. The contact surface should be wiped clean with lens paper and rinsed with distilled water and then ethanol. The prism chamber should be closed and the instrument covered 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.

15.2.5.4 *Infrared Analysis*

Infrared spectroscopy (see Chap. 8) has attained a primary position in monitoring the composition of food products before, during, and following processing [22]. It has a wide range of food applications and has proven successful in the laboratory and online. Similar to the use of ultraviolet (UV) or visible (Vis) light in UV-Vis spectroscopy, in infrared spectroscopy a sample is exposed to IR radiation (near IR 700–2400 nm or mid IR 2500–25,000 nm), specific wavelengths are absorbed, and an IR spectrum is measured by calculating the intensity of the IR radiation before and after passing through the sample. The absorbance peaks are related to the type and amount of functional groups present. However, 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 of the water molecule and can be used to determine the moisture content of a food. NIR has been applied to moisture content analysis of a wide variety of food commodities and is an official method for moisture content determination in dried vegetables (AOAC Method 967.19).

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. 8 of this text. 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:

$$a = \sum (TS - F - P - L)/n \qquad (15.11)$$

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

$$TS = a + F + P + L \qquad (15.12)$$

The a value is thus a standard value mathematically derived. Newer instruments have the algorithm in their computer software to ascertain this value automatically. Although not directly measured, the mois-

ture content can be calculated by subtracting the total solids content from 100.

15.2.5.5 *Microwave Absorption*

The absorption of microwaves, which are electromagnetic waves with wavelength of 0.001–1 m and frequency of 0.3–300 GHz, can be used to determine the moisture contents of a wide variety of food products. The approach is based on **dielectric constant** or **permittivity value** differences between water and dry materials. The permittivity of most dry materials is much lower than that of water, and small changes in the amount of water in a sample lead to measurable changes in permittivity. As microwave energy is passed through a sample (<40 mm thick) placed between a microwave transmitter and receiver, the absorption at 2.450 GHz (the most widely used frequency in microwaves) is linearly related to moisture content [23]. After calibration against laboratory standards, moisture contents can be determined in ~2 s using the microwave absorption method. Power absorption and attenuation may be affected by the dimensions, surface area, temperature, and dielectric properties of some food ingredients; however, the technique is rapid, nondestructive, and useful for some heterogeneous, powdered, multilayered, and frozen foods [4, 24].

15.2.5.6 *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. 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 and −0.541 °C, with the average very close to −0.521 °C. 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.

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. The Food and Drug Administration will reject all milk with freezing points above −0.507 °C. 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 thermistor used can sense temperature change to 0.001 °C. 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. Time required for the automated instruments is 1–2 min per prechilled sample.

15.2.6 Comparison of Moisture Content Determination Methods

15.2.6.1 *Principles*

Characteristics of the various moisture content analysis methods described in Sects. 15.2.2, 15.2.3, 15.2.4, and 15.2.5 are summarized in Table 15.5. Direct moisture content determination methods often remove water from a sample and determine moisture contents by mass, volumetry, or titration. 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 (and consequently also the total solids content). Non-water 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.

Indirect moisture content determination methods analyze some property of the food that is related to the presence of water. 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. Microwave absorption is based on the dielectric properties of water. Freezing point is a physical property of milk that is changed by a change in solute concentration.

15.2.6.2 *Nature of Sample*

While many 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 water or other compounds, and these reactions 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 vola-

15.5 table Comparison of moisture analysis methods

Method	*Principle*	*What is actually measured?*	*How is water removed/reacted/identified/etc.?*	*Cautions/things to control*	*Advantages*	*Disadvantages*	*Typical applications*
Forced draft oven	Sample is heated in oven to evaporate water. Weight loss equals moisture content	Weight change	Heat evaporates water when it boils at 100 °C	Control time, temperature; control sample particle size. Must pre-dry some samples to avoid splattering	Easy to handle many samples at one time	Takes long time to get results. High temperature can cause loss of volatiles, lipid oxidation, Maillard browning, sucrose hydrolysis, so not suitable for some types of foods	Official method for many types of samples. Not suitable of rapid quality control results. Not suitable for samples subject to loss of volatiles, lipid oxidation, Maillard browning, or sucrose hydrolysis
Vacuum oven	Sample is heated in oven under reduced pressure, so water evaporates at a lower temperature. Weight loss equals moisture content	Weight change	Heat sample under reduced pressure to evaporate water at ~70 °C	Control time and temperature. Pull and release vacuum slowly	Easy to handle many samples at one time. Lower temperature for evaporating water reduced problems with high-sugar products	Takes long time to get results (though usually less time than with forced draft oven). More expensive than forced draft oven	An official method for many types of products. Not suitable for rapid quality control. Not suitable for powdered products, since they can blow around when vacuum is pulled and released
Microwave drying oven	Sample is heated with microwave energy to evaporate water. Weight loss equals moisture content	Weight change	Heat from microwave energy causes water evaporation	Control power and time to prevent sample decomposition. Spread sample evenly. Check calibration of analytical balance	Rapid	More expensive than other drying methods listed. Can only run one sample at a time	Suitable for rapid quality control, especially for liquid products, since use of pads avoids splattering
Infrared drying oven	Infrared lamp supplies heat that penetrates sample to evaporate water. Weight loss equals moisture content	Weight change	Heat from infrared lamp evaporates water	Control time and temperature. Spread sample evenly	Rapid	Expensive. Can only run one sample at a time	Suitable for rapid quality control, but not for high moisture products (would get splattering)
Rapid moisture analyzer	Sample is heated with heating elements to evaporate water. Weight loss equals moisture content	Weight change	Heat evaporates water when it boils at 100 °C	Control time and temperature. Spread sample evenly. Regular calibration of analytical balance	Rapid	Expensive. Can only run one sample at a time	Suitable for rapid quality control, but not for high moisture products (would get splattering)

(continued)

Reflux distillation (with toluene)	When sample is heated to toluene (an immiscible liquid), the toluene and water are co-distilled. Collected moisture distills off, is condensed and collected, and volume of water is measured	Volume of water from sample collected after distillation and condensation	Co-distill water from sample with toluene. Collect water and measure	Any emulsion formed must break to read volume of water. Need very clean glassware with no water. Use caution with solvents (fire hazards; toxic)	Causes less thermal decomposition of some foods than oven drying. Solvent protects sample from losing volatiles and minimized oxidation. Water is measured directly	Can only run one sample at a time. Solvent is likely flammable and toxic. Reading volume of water in receiving tube may be less accurate than by gravimetric method	AOAC method for spices
Karl Fischer	In titration of sample with Karl Fischer reagent, water in sample reacts with sulfur dioxide to cause reduction of iodine. Endpoint of titration is detected when excess iodine cannot react with water. Volume of titrant is used to calculate % moisture	Volume of Karl Fischer Reagent titrated	Water in sample reacts with iodine and sulfur dioxide to cause reduction of iodine	Control particle size of sample and humidity of room. Prevent any water in glassware. Must standardize KRF. Choose another method if interferences from certain food constituents (e.g., ascorbic acid, carbonyl compounds, unsaturated fatty acids)	No heat, so no thermal decomposition; rapid. Higher accuracy than many other methods for low-moisture foods	Can only run one sample at a time. Expensive, if using automated unit	Method of choice for many low-moisture foods (e.g., dried fruits and vegetables, candies, chocolate, roasted coffee, oils and fats, and many low-moisture foods that are high in sugar or protein). Good method to try if method with heating and/or vacuum gives erratic results
Hydrometer	Archimedes' principle. Compare relative density (specific gravity) of sample to that of water at same temperature	Volume displaced by hydrometer. Read specific gravity directly from hydrometer. Measuring solids content	Based on solids content of the solution, to determine specific gravity compared to pure water	Control temperature. Need clean hydrometer	Rapid. Easy. Enexpensive	Limited applications. Measures only solids content	Commonly used as rapid method to measure solids content of beverages, salt brines, and sugar solutions. Best applied to solutions with only one solute in a medium of water

(continued)

15.5 table (continued)

Method	*Principle*	*What is actually measured?*	*How is water removed/reacted/identified/etc.?*	*Cautions/things to control*	*Advantages*	*Disadvantages*	*Typical applications*
Refractometer	Based on bending of light (i.e., refraction. Measure refractive index) as it hits surface of product. Refractive index can be used to determine concentration of compound of interest if nature of compound, temperature of sample, and wavelength of light are constant	Refractive index. Measuring solids content. Commonly calibrated in degree Brix (g of sucrose/100 g sample)	Based on solids content of solution, to determine refractive index	Control temperature. Need clean contact surface	Rapid. Easy. inexpensive	Limited applications; measures only solids content	Commonly used as rapid method to measure solids content of beverages and milk, and soluble solids of fruits and fruit products and of tomato products
Infrared analyzer	Measure absorption of infrared radiation at wavelength characteristic of the -OH stretch of the water molecule. Concentration of water is determined by energy that is reflected or transmitted, which is inversely proportional to energy absorbed	Amount of NIR light reflected from sample	Molecular vibration of functional groups of water determines absorption, which is inversely related to reflected light what is measured	Must calibrate instrument for each type of product and each analyte being analyzed. Control sample particle size. Prevent scratches on glass container for sample; remember that values obtained are only a prediction	Rapid. Easy. Can be used to estimate content of various food constituents	Expensive. Can only run one sample at a time. Value obtained are only estimates/predictions. Must calibrate the instrument for each analyte for each type of sample	Has wide range of food applications, in the laboratory, at-line, and on-line. NIR is heavily used in the grain/cereal industry for moisture, protein, and fat. NIR is heavily used in the dairy industry for total solids, fat, protein, and lactose in milk

15.6 table Common food industry uses of moisture content and water activity assays

Technique		*Where used: Production, quality control*	*Product development*	*Basic research*
Water content measurement				
Direct methods	Forced draft oven drying	X	X	X
	Vacuum oven drying	X	X	X
	Microwave analyzer	X	X	
	Infrared drying	X	X	
	Rapid moisture analyzer technology	X	X	
	Thermogravimetric analyzer		X	X
	Lyophilization		X	X
	Chemical desiccation		X	X
	Karl Fischer titration	X	X	X
Indirect methods	Dielectric capacitance	X		
	Hydrometer	X		
	Pycnometer	X		
	Refractometer	X	X	X
	NIR spectroscopy (absorbance or reflectance)	X	X	X
	Freezing point, cryoscope		X	X
	Microwave absorption	X	X	X
	Conductivity	X	X	
Water activity measurement	Dewpoint analyzer	X	X	X
	Electric (capacitance or electrolyte) hygrometer	X	X	X
	Freezing point depression			X
	Tunable diode laser sensor		X	X

tilization 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.

15.2.6.3 *Intended Purposes*

Moisture content 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 rapid moisture analyzer techniques 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, refractive index, and microwave absorption 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. A survey of food industry usage of the moisture content assays is summarized in Table 15.6, wherein the most commonly used techniques across a variety of companies for production, product development, and basic research applications are identified.

15.3 WATER ACTIVITY

15.3.1 Overview

Water content alone is not a reliable indicator of food stability, since foods with the same water content differ in their perishability [6]. It is the water activity (a_w) of foods that has been correlated to microbial growth, physical properties, and chemical and enzymatic reactions.

The water activity (a_w) of foods varies greatly, as shown in Table 15.7. For referring to water activity, the a is lower case and the w is a subscript because an activity coefficient of water is being used to describe its energy state. Generally, foods with higher moisture contents also have higher a_ws, although the relationship between moisture content and a_w is not linear. **Water activity** is a thermodynamic property of water in foods defined as the ratio of the fugacity (or escaping tendency) of water in the food to the fugacity of pure water at the same temperature and pressure [4, 6, 25]. Because fugacity cannot be directly measured, a_w is more commonly determined

15.7 Table Water activity (25 °C) of foods and saturated solutions of food ingredients and salts

Foods		*Saturated solutions*			
		Food ingredients		*a_w control salts*	
Type	a_w	Type	a_w	Type	a_w
Potato chips	0.07	*Single ingredients*		LiCl	0.11
Hard candy	0.12	Malic acid	0.58	CH_3CO_2K	0.23
Crisp crackers	0.13–0.20	Fructose	0.62	$MgCl_2$	0.33
Sugar-free hard candy	0.25	Sorbitol	0.67	K_2CO_3	0.43
Crisp cookie	0.25	Glucose	0.74	$Mg(NO_3)_2$	0.53
Chewy cookie	0.55	Citric acid	0.78	$CoCl_2$	0.65
Honey	0.56	Xylitol	0.79	NaCl	0.75
Beef jerky	0.61	Sucrose	0.85	KCl	0.84
Gummy candy	0.66	Lactose	0.97	K_2SO_4	0.97
High fructose corn syrup	0.75	Maltose	0.97		
Condensed milk, strawberry preserves	0.84	*Ingredient blends*			
Soy sauce	0.87	NaCl +fructose	0.45		
Salted butter	0.90	Fructose + citric acid	0.50		
Bread	0.94	NaCl+sucrose	0.65		
Reduced sugar ketchup, unsalted butter	0.97	NaCl+glucose	0.71		
Juices, milk, fruits, vegetables	0.98–0.99				

All measurements were done using an AquaLab 4TE instrument (Decagon Devices, Pullman, WA)

as the ratio of the vapor pressure of water in a food (p) to the vapor pressure of water (p_o) at the same temperature and barometric pressure, as shown in Eq. 15.13.

$$a_w = \frac{p}{p_o} \tag{15.13}$$

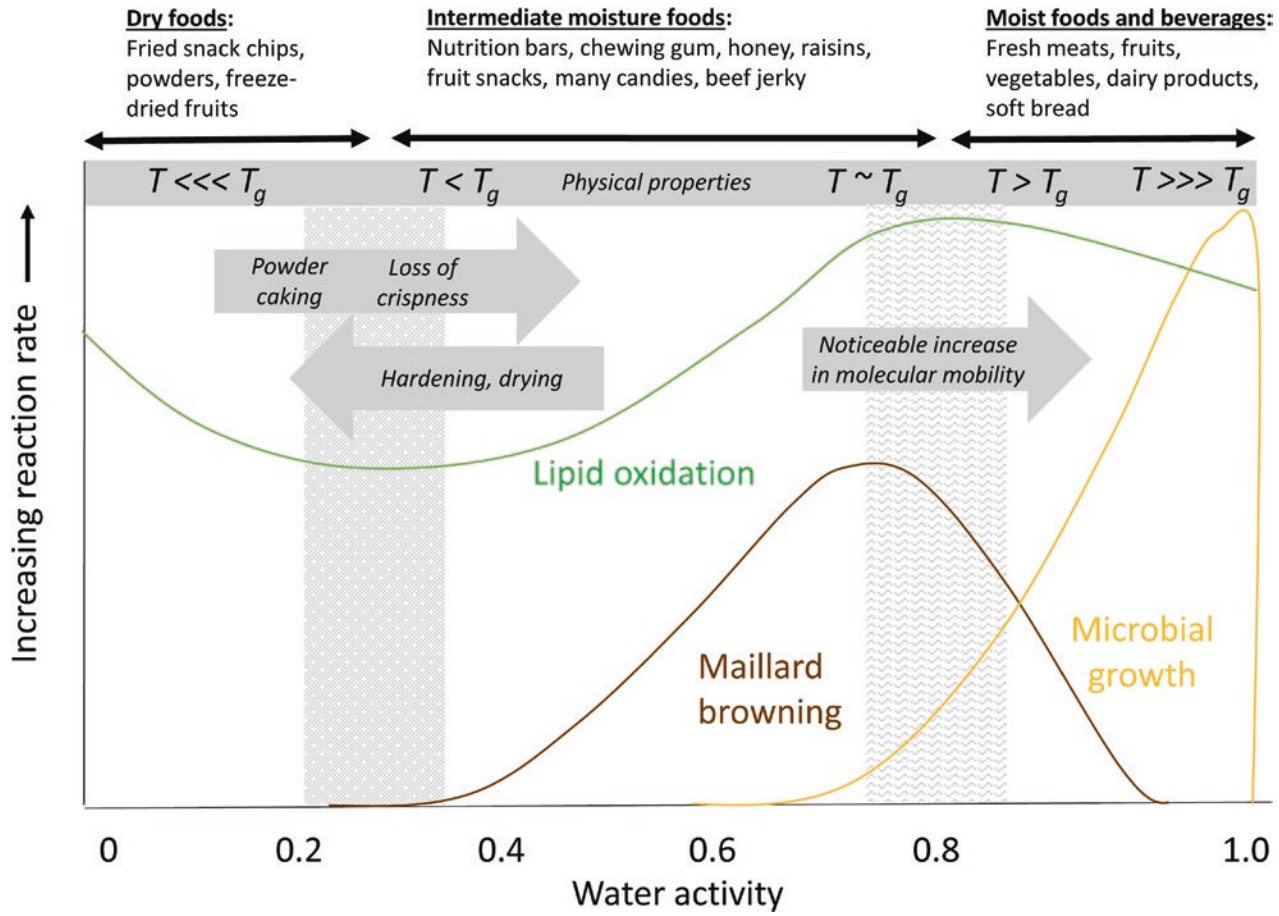
This concept is similar to how the relative humidity (RH) of the atmosphere is determined. The relationship between the a_w and equilibrium relative humidity (ERH) can be expressed as $a_w = \mathrm{ERH}/100$. Water activity is a dimensionless number between 0 (absolute no water) and 1 (pure water).

There are fewer official methods for determining the a_w of foods than there are for determining the moisture content [4, 14]. AOAC Method 978.18 describes techniques to determine the a_w of canned vegetables: in the regulation of acidified foods, an a_w of 0.85 is used as the cutoff for pathogen growth. In 21CFR Part 114 [26], low-acid foods are defined as foods with an equilibrium pH of >4.6 and $a_w > 0.85$, and acidified foods are defined as low-acid foods to which acid has been added to create a finished equilibrium pH of ≤4.6 and that have an $a_w > 0.85$. Foods with $a_w < 0.85$ are not covered by 21CFR Part 108, 113, or 114 [26]; however, it is important to recognize that lowering the a_w is not a kill step.

15.3.2 Importance of Water Activity

The a_w of foods affects important quality and safety factors, and therefore a_w measurements are commonly used in food development and production. The a_w influences all aspects of microbial growth, and each microorganism has a threshold a_w below which it will not grow, although microorganisms may not be dead below this threshold a_w [27]. Understanding which spoilage or pathogenic microorganisms are a concern for a specific food and their a_w limits for growth can provide a foundation for formulation efforts to reduce the a_w of the food below that level. Because of the direct correlation between a_w and microbial growth, there are many food safety regulations that incorporate a_w guidelines (21CFR 110, 113, and 114 [26]; ANSI/NSF Standard 75 [28]; ISO 21807:2004(E) [29]; AOAC Method 978.18 [14]), and a_w can be a critical control point in HACCP plans.

Food stability maps have been created to display the relationships between water activity, microbial growth, chemical and biochemical reaction rates, and physical properties [30]. An example of a general map for an amorphous food is provided in Fig. 15.10, although the reaction rates and properties will vary across different a_w ranges for different foods. In addition to controlling microbial growth, maintaining the desired a_w of a product is a critical aspect in maintaining its texture and quality throughout shelf-life, and there will be critical a_ws beyond which unwanted physical and/or chemical changes will occur in most products. If a crispy fried product (potato chip) is exposed to an environment with a higher RH than its a_w, moisture will migrate into the potato chip, increasing its a_w and leading to a softening of its texture once the critical a_w is exceeded. Likewise, if a food product contains multiple components with different a_ws (cheese and cracker, pizza sauce on crust, pastry with filling, etc.), water migrates from



15.10 figure Food stability map (Adapted from Labuza et al. [30] and overlaid with data from Table 15.7)

the region of high *aw* to the region of low a_w until a_w equilibration is attained, which can result in a hardening of one phase and a softening of the other. If these changes are unwanted, then reformulation of the phases to the same a_w (if possible) or physical separation of the phases in barrier packaging could extend shelf-life. Increasing the a_w above a critical value can lead to clumping and caking of powders, recrystallization of sugars or salts in some products, a glassy-to-rubbery transition in amorphous solids, or even deliquescence of water-soluble deliquescent crystalline compounds.

15.3.3 Water Activity Measurement

15.3.3.1 *Principles*

Since $a_w = p/p_o$, determining the a_w often involves the direct measurement of vapor pressures, although there are also indirect measurements for a_w. For the majority of a_w measurement techniques, a representative food sample is sealed in a container and allowed to reach equilibrium (i.e., the water will migrate out of the sample into the container headspace, or vice versa, until the a_w of the food and the RH of the headspace are equal) (Fig. 15.11). Enough sample must be used to assure that moisture migration during equilibration will not dry the sample but not so much sample that the sensor is compromised. A general recommendation is to fill half of the container with sample. More

15.11 figure Dewpoint water activity meter (Courtesy of Decagon Devices, Pullman, WA)

sample might be warranted for dense, dry products, while less sample could be used for wet products or liquids (AOAC Method 978.18 recommends canned vegetables fill >1/20 of the sample container volume).

The time required to attain headspace equilibrium can vary between samples and between assay types. Slow water-emitting samples (dense, dry, dehydrated, high fat, high viscosity) may require several hours for precise a_w measurement, while samples containing more water or those with water as the continuous phase may equilibrate much faster. For establishing the equilibrium criterion for a sample, the a_w could be monitored over time, such as at 15, 30, 60, 120 min (AOAC Method 978.18), extending out in 60 min intervals until consecutive readings vary by <0.01 a_w (or other designated equilibrium criteria for a particular product). While some samples may require 24 h for equilibration, many foods (barring the slow water emitters) may reach equilibrium within 5 min for dewpoint analyses or 30–90 min for electric hygrometers [31].

Temperature is an important factor in equilibration and a_w – higher temperatures often lead to higher a_w readings for many foods if the moisture content is kept constant (water molecules move faster as temperature is increased). This is why temperature is reported along with a_w values. Additionally, inequalities in temperature between samples and containers or equipment can lead to unwanted water condensation and/or errors in determining the a_w. For example, a 1 °C temperature difference between sample and dew point leads to an a_w error of 0.06 in dewpoint instruments [4, 32]. The majority of reported a_w values were measured at 25 °C (as stipulated in AOAC Method 978.18) or 20 °C, although the temperatures of interest for a particular product may vary (particularly for refrigerated or frozen products).

It is important to use a calibrated sensor for a_w measurements. Calibration is often done using salt solution standards of known a_w (Table 15.7). It is important to comply with instrument manufacturer guidelines for cleaning, calibrating, and maintaining a_w instruments. Calibration may involve at least three measurement points (ISO 21807) or ≥5 salts (AOAC Method 978.18) that encompass the a_w range of interest for the sample and sensor to generate a standard curve and/or determine an offset adjustment. Once the instrument is calibrated and temperature and headspace equilibrium are reached, the headspace is analyzed to determine the a_w. There are a variety of sensors and methods to directly or indirectly determine the equilibrium water vapor pressure in the headspace, although only the most common used by the food industry (Tables 15.6 and 15.8) will be further described. Examples of a_w methods include dewpoint measurement, electric hygrometer sensors (including capacitance and electrolyte sensors), direct measurement of manometric pressure, change in length of a hair/thread, increase in sorbent mass in an isopiestic method, tunable diode laser sensor, thermocouple psychrometry, and freezing point determination [5, 33].

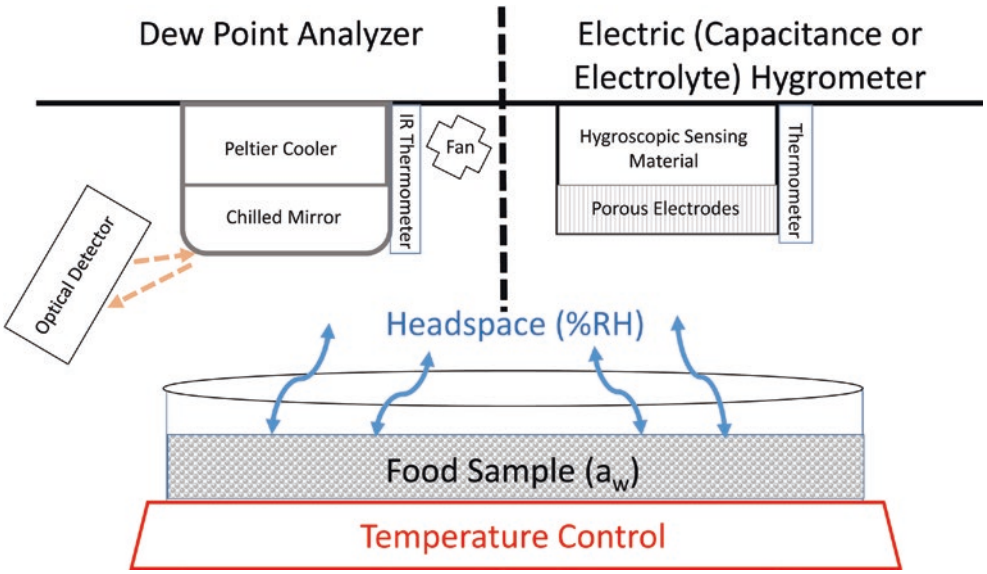
15.8 table Comparison of water activity measurement methods

Method	*Instrument*	*aw range*	*Accuracy*	*Repeatability*	*Resolution*	*Internal temperature control*	*Read time*	*Volatile interference*
Dewpoint	AquaLab4 (Decagon Devices)	0.030–1.000	0.003	0.001	0.0001	15–50 °C	2–5 min	Yes
Electric (resistive electrolytic based on electrolyte)	LabMaster-aw (Novasina)	0.03–1.000	0.003	0.001	0.001	0–50 °C	10–15 min	Yes[a]
Electric (capacitance)	HygroLab C1 (Rotronic)	0.0–1.000	0.008	0.001	0.001	No	4–6 min	Minimal[b]
Electric (capacitance)	Aw Therm (Rotronic)	0.005–1.000	0.005	0.001	0.001	10–60 °C	4–6 min	Minimal[b]
Tunable diode laser	TDL (Decagon Devices)	0.03–1.000	0.005	0.001	0.0001	15–50 °C	2–5 min	No

Adapted from Fontana and Campbell [4] and personal communications with Brady Carter (Decagon Devices), Markus Bernasconi (Novasina), and Harry Trainor and Rico Hasler (Rotronic)

[a]For the LabMaster-aw, filters can be used to block out low to medium levels of volatiles, or use the CM-3 sensor that is resilient to alcohol

[b]The sensors in the HygroLab C1 and Aw Therm instruments are affected by volatiles when subjected to high concentrations and long-term exposure. A single a_w measurement in 4–6 min is not enough exposure in most cases for volatiles to affect the analysis



15.12 figure

Schematic of water activity measurement chambers for the two most common techniques used by the food industry: dewpoint and capacitance/electrolyte sensors

15.3.3.2 *Chilled Mirror Dew Point*

In a chilled mirror dewpoint instrument, such as a Decagon Devices AquaLab (Fig. 15.12), a sample is equilibrated in a temperature-controlled sealed chamber that contains a fan to circulate air in the headspace, an infrared thermometer to measure sample temperature, a temperature-controlled mirror, and a sensor that detects condensation on the mirror [5]. The mirror temperature is controlled by a thermoelectric Peltier cooler, and a photodetector monitors the reflectance of light off of the mirror. The a_w of the sample is the same as the RH of the headspace at equilibrium ($a_w = \mathrm{ERH}/100$), and it is the headspace RH that is analyzed. The temperature of the mirror is cooled until condensation from the headspace first appears, which is determined by a change in the reflectance. When condensation occurs, the temperatures of the sample and the mirror are recorded. The temperature of the sample is used to determine the water vapor pressure (p_o), and the dewpoint temperature is used to determine the vapor pressure of water in the headspace (p). The a_w of the sample is determined by applying Eq. 15.13, $a_w = p/p_o$. Volatiles that may condense on the mirror, such as alcohols, acetic acid, or propylene glycol, interfere with accurate a_w measurements using dewpoint techniques.

15.3.3.3 *Electric Hygrometer*

In an electric hygrometer, which for our purposes will include capacitance and electrolyte sensors such as Novasina and Rotronic instruments, a sample container is equilibrated in a chamber with a potentiometer and a sensor (such as an electrolytic moisture sensor) that measures the headspace RH and temperature [31]. Some models have internal temperature control features, while others may require an incubator or water-jacketed sample chamber for temperature control. The sensors monitor the electrical conductivity of their immobilized salt solutions or a hygroscopic polymer (the sensor composition is proprietary) that sorb or desorb water in response to the headspace RH. Changes in electric conductivity are calibrated to reflect changes in the headspace RH, and a_w is calculated as $a_w = \mathrm{ERH}/100$. Volatile compounds may interfere with a_w measurements by electric hygrometers, and tunable diode laser techniques may be a better option for accurately determining the a_w of samples that contain alcohols, acetic acid, or propylene glycol.

15.4 MOISTURE SORPTION ISOTHERMS

15.4.1 Overview

A **moisture sorption isotherm** is a plot of the equilibrium relationship between a sample's moisture content and a_w at a constant temperature and pressure. The **hygrocapacity**, or water-holding capacity, of a sample is the amount of water it contains when equilibrated at a set a_w. Hygrocapacity depends on the affinity between water and the sample, temperature, RH, surface area, and water-solid interaction mechanism [34]. A key to establishing moisture sorption isotherms is to ensure that the samples have equilibrated at the set a_ws. Different samples exhibit different kinetics of moisture sorption and desorption. In the absence of equilibrium, a plot of moisture content versus a_w

would simply be called a **sorption profile** (not an isotherm).

Generally as a_w increases, moisture content also increases although the relationship is most certainly not linear. Different types of solids and different foods have different shapes of moisture sorption profiles, and these shapes have been organized into six major types (I–IV) [35]. Types II (sigmoid) and III (_/-shaped) are most common for foods. The inflection points and slopes of the different isotherms provide useful information about hygroscopicity, potential for reformulating or drying to alter a_w, how a sample might respond to different environments or formulations, and stability (microbial, physical, and chemical). Inflection points in sorption profiles occur during phase changes, such as a glassy-to-rubbery transition, hydrate formation, or deliquescence. Recrystallization events are often accompanied by expulsion of water from the matrix and a corresponding weight loss. Overlaying isotherms from different food types is a useful practice when considering co-formulation and multicomponent foods, keeping in mind that water does not move if the a_ws of the different components are equal. If differences in a_w are initially present between the different food components, water will migrate until a_w equilibrium is reached.

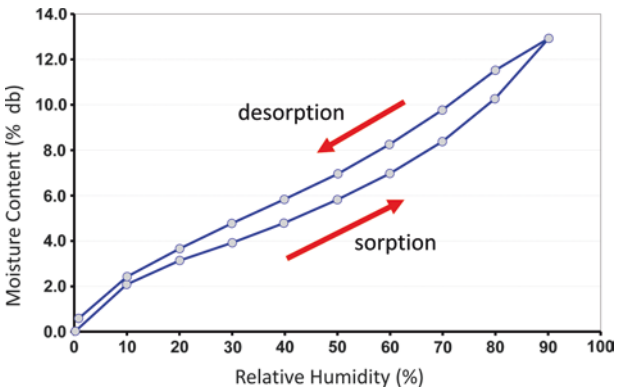
There are several approaches and instruments used to generate moisture sorption isotherms (or profiles), although there are no government regulations or AOAC official methods dictating techniques and parameters. A description of two common approaches for moisture sorption isotherm measurement is provided below.

15.4.2 Isopiestic Desiccator Method

In desiccator methods, samples are equilibrated in RH-controlled desiccators at set temperatures, and, upon equilibrium, their moisture contents are determined by either a mass change from a known starting amount or any of the moisture content methods described above. The RH in the desiccators is most often controlled by saturated salt solutions (Table 15.7) with the exception of the 0%RH condition, which is created using calcium sulfate (DrieRite™) or phosphorous pentoxide (P_2O_5), the latter of which is more effective [36]. Enough saturated salt solution and headspace must be present to maintain a constant RH as moisture is exchanged with stored samples. General recommendations are to use ≥10 % of the container volume filled with salt solution, >10:1 ratio of salt solution surface area to sample surface area, and a 20:1 ratio of headspace volume to sample volume. Vacuum may or may not be applied to the desiccators. Six to nine different RH chambers are recommended to generate a moisture sorption isotherm for a product [37]. Temperature is controlled by placing the desiccators in temperature-controlled incubators, ideally with temperature variation <1 °C. Samples should be stored and analyzed in triplicate over time, weighing to ±0.0001 g until some equilibrium criterion is reached. For example, equilibrium could be defined as <0.01 % mass change across three consecutive days of weighings. The amount of time it takes for samples to reach equilibrium varies by sample type and RH condition, often from 10 to 21 days. Desiccator methods are time and labor intensive, there is potential for RH fluctuation as samples are removed from the desiccators for weighing, and challenges with chemical change or microbial growth in samples during extended equilibration may be encountered. There are a limited number of RHs generated by saturated salt solutions, and therefore equations (such as the GAB and BET models) must be used to calculate sorption profiles from the measured data points [37]. However, the relative simplicity of the technique and ability to store multiple samples are attractive features of using the desiccator method to generate moisture sorption isotherms.

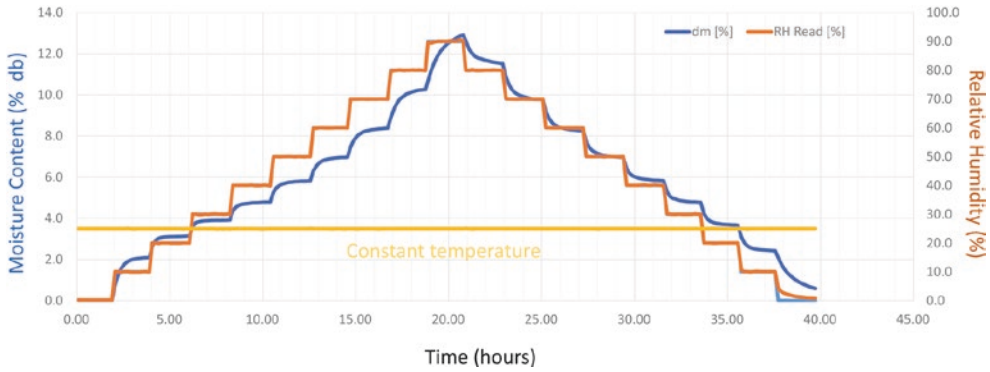
15.4.3 Automated Gravimetric Moisture Sorption Balance

In automated gravimetric moisture sorption balances, sometimes referred to as static dynamic vapor sorption (DVS) instruments, the mass of a sample is monitored as a function of time, while the sample is exposed to a series of tightly controlled RH step changes at constant temperature. An example of data collected using this technique is provided in Fig. 15.13. The initial moisture content of the sample must be known, or a sample can be dried in the instrument prior to analysis. The parameters used in generating automated moisture sorption isotherms (or profiles), including equilibrium criterion (% weight change in a set time period, e.g., 0.001 % or 0.01 %), step time (how long will a set RH be held before the next programmed RH step is initiated, in the absence of attaining the equilibrium criterion), and RH step size (1 % RH, 5 % RH,



15.13 figure

Moisture sorption isotherm (type II) of an amorphous powder



15.14 figure Moisture sorption data for an amorphous powder collected on a DVS instrument (SPS, ProUmid GmbH, Germany) and used to generate the moisture sorption isotherm shown in Fig. 15.13

10% RH, etc.), have a profound influence on the curves generated (Fig. 15.14).

When a DVS instrument moves to a programmed RH step, air with the programmed RH is moved into the sample chamber, and the mass of the sample is monitored as it sorbs (or desorbs) water. Usually the initial sorption is more rapid than later stages of sorption, as seen in Fig. 15.14. If true equilibrium is reached between the sample and the surrounding environmental RH, then no more moisture uptake would occur and the equilibrium criterion would be 0.00% mass change over any time scale. However, due to the labile nature of some foods and the need to collect data for foods over a period of days not months, an equilibrium criterion of 0.01% weight change over some time scale (1–5 h) is more common. It is important to recognize that the shorter the step time, the more likely the sample is not in equilibrium with the surrounding RH resulting in sorption profiles and not isotherms.

Careful control and reporting of test parameters are important for reproducibility of moisture sorption isotherms. Advantages of DVS methods compared to desiccator methods include precise RH control and mass determinations, a smaller amount of sample (5 mg–1.5 g), smaller RH step sizes, and possibly a reduced time to generate a moisture sorption isotherm for a single sample. Disadvantages of DVS methods include high instrumentation costs, the upper RH limit being 95% (or lower RH at higher temperatures), and a more limited sample size (many DVS instruments only analyze one sample at a time, although there is currently one that analyzes up to 23 samples simultaneously (SPS, ProUmid GmbH & Co., Germany)).

A modified approach to the DVS instrumentation is found in the AquaLab Vapor Sorption Analyzer (Decagon Devices, Pullman, WA) operated in DDI mode. In the DDI mode, a small amount of saturated (100% RH) or dry (0% RH) air is introduced into the sample chamber, the chamber is sealed, the RH is continuously monitored by a capacitance RH sensor until a constant RH is reached (on average 5–6 min), and at this point both the mass and the water activity of the sample (based on the chilled mirror dewpoint technique) are recorded. While it is not possible to program RH steps or timing between measurements into the DDI technique, the DDI sorption profiles are very high resolution with numerous data points collected and may be collected in less time than the static DVS moisture sorption isotherms.

15.4.4 Phase Diagrams Containing a_w, Moisture Content, and T_g Relationships

In addition to routine moisture analyses for quality control, the food industry is increasingly taking a fundamental materials science approach to designing foods and processes and controlling food quality. An important practice here is to establish not only moisture sorption isotherm relationships for foods but also to establish the relationship between moisture content and glass transition temperature (T_g) as well as the relationship between water activity and glass transition temperature (T_g). More detailed information about T_g measurements is provided in Chap. 31.

15.5 SUMMARY

The moisture content and water activity of foods are 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. Direct moisture content analysis methods involve a separation of moisture in the sample from the solids and then quantitation by weight or volume. Indirect methods do not involve such a separation but instead are based on some physical or chemical property of the

sample that varies with respect to its moisture content. 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).

15.6 STUDY QUESTIONS

1. Identify five factors that one would need to consider when choosing a moisture content analysis method for a specific food product.
2. Why is standardized methodology needed for moisture content 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 containing DrieRite™ or P_2O_5 desiccant (~0%RH) 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 near-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 is it necessary, and how it is determined. Give the technician your answer.
8. You are fortunate to have available in your laboratory the equipment for doing moisture content 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
9. 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)?

10. 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 your correct them?
11. 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.
12. Compare and contrast moisture content and water activity in terms of measurement approaches, effects on food texture and moisture migration with the environment, influence on microbial growth, and roles in vitamin stability, lipid oxidation, and the Maillard reaction.
13. Identify three factors that would lead to underestimation of the water activity of a food sample. Then identify three factors that would lead to overestimation of the water activity of a food sample.
14. You are put in charge of creating moisture sorption isotherms of several food products. Describe what method you will use and what approach you will take for assuring you have created moisture sorption isotherms and not moisture sorption profiles of:
    (a) Potato chips
    (b) An intermediate moisture nutrition bar containing oats, chocolate pieces, and a binder syrup
    (c) A gummy candy
    (d) Cheddar cheese

15.7 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 gal at 8.67% solids and the weight is 8.5 lb 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 disk 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 (326.7–302.8 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 Eqs. 15.2, 15.3, and 15.4 in Sect. 15.2.2.1.7. To use any of the equations, you must subtract the weight of the dried pan and glass disk. 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 g/3.5898 g) × 100 = 19.35 % solids and (2.8953 g/3.5898 g) × 100 = 80.65 % water.

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