16 chapter

Ash Analysis

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16.1 INTRODUCTION

Ashing is an important first step in proximate or specific mineral analysis. **Ash** refers to the inorganic (mineral) residue remaining after the combustion or complete acid-facilitated oxidation of organic matter in food. A basic knowledge of the characteristics of various ashing procedures and types of equipment is essential to ensure reliable results. Two major types of ashing are used: dry ashing and wet ashing. Dry ashing is primarily used for proximate composition and for some types of specific mineral analyses. Dry ashing requires very high (500–600 °C) temperatures, which may be achieved by conventional or microwave heating. Wet ashing (acid-facilitated oxidation) is used as a preparation for the analysis of minerals that might be volatilized and lost during dry ashing. Wet ashing uses lower temperatures than dry ashing and relies on strong acids and chemical oxidizers to rid samples of organic material. Microwave systems can also be used for both dry and wet ashing. In either case, microwave systems tend to speed the ashing process, although sample throughput may be a limiting factor. Most dry samples (i.e., whole grain, cereals, dried vegetables) need no preparation, while fresh vegetables and other high-moisture foods are generally dried prior to ashing. High-fat products such as meats may need to be dried and fat extracted before ashing to prevent smoke generation during heating steps. Ashing is a gravimetric analysis, in which the final (ashed) weight is compared to the original weight of the sample. The ash content of foods can then be expressed on either a wet weight (as is) or dry weight basis. For general and food-specific information on measuring ash content, see references [1–14].

16.1.1 Definitions

Dry ashing refers to the use of a muffle furnace capable of maintaining temperatures of 500–600 °C. Water and volatiles are vaporized, and organic substances are burned in the presence of oxygen to form CO_2 and oxides of N_2. In this way, organic materials are removed from the sample. The remaining minerals are converted to oxides, sulfates, phosphates, chlorides, and silicates. Elements such as Fe, Se, Pb, and Hg may partially volatilize with this procedure, so other methods must be used if ashing is a preliminary step for the analysis of these minerals.

Wet ashing is a procedure used to chemically oxidize and remove organic substances using strong acids, oxidizing agents, or combinations thereof. The acids and oxidizing agents used must also be capable of solubilizing the remaining minerals. Hydrochloric, sulfuric, nitric, and perchloric acids are commonly used. Perchloric acid requires a specially designed hood, since it may leave explosive peroxides behind. Since wet ashing is conducted either at lower temperatures than dry ashing or in sealed vessels, minerals are not lost due to volatility. For this reason, wet ashing is preferable to dry ashing as a preparation for specific elemental analysis.

16.1.2 Importance of Ash in Food Analysis

Ash content represents the total mineral content in foods. Determining the ash content may be important for several reasons. It is a part of proximate analysis for nutritional evaluation. Ashing is also the first step in preparing a food sample for specific elemental analysis, whether for essential nutrients or for highly toxic heavy metals. Because certain foods are high in particular minerals, ash content can be important from nutritional, toxicological, and food quality standpoints. For example, dairy and beef are known to be rich sources of calcium and iron. Ash content is commonly part of the ingredient specification for flours and whole grains. Rice grown in arsenic-containing soil effectively concentrates it. High levels of transition metals in lipid-rich foods may speed rancidity and limit shelf life. The mineral content of plant foods tends to be more variable than that of animal products.

16.1.3 Ash Contents in Foods

The ash content of food can range from 0 to 12%, but rarely exceeds 5% for fresh foods.

The average ash content for various food groups is given in Table 16.1.

16.2 METHODS

Principles, materials, instrumentation, general procedures, and applications are described below for various ash determination methods. Refer to methods cited for detailed instructions of the procedures.

16.2.1 Sample Preparation

Ashing does not require large sample sizes. A 2–10 g sample is generally sufficient for ash determination. Some newer instruments allow for sample sizes as low as 250 mg. For this reason, it is necessary to ensure that a homogenous, representative sample is obtained. Milling, grinding, and other methods used to homogenize samples will not significantly alter ash values for proximate analysis. In contrast, if ashing is used as a preparatory step for specific mineral analyses, mineral contamination from the environment or from grinding equipment is of potential concern and may require the use of sample blanks to account for this. (This is particularly true of metals such as iron, since most grinders and mincers are of steel construction.) Repeated use of

16.1 table Ash content of selected foods

Food item	*Percent ash (wet weight basis)*
Cereals, bread, and pasta	
Rice, brown, long grain, raw	1.5
Corn meal, whole grain, yellow	1.1
Hominy, canned, white	0.9
White rice, long grain, regular, raw, enriched	0.6
Wheat flour, whole grain	1.6
Macaroni, dry, enriched	0.9
Rye bread	2.5
Dairy products	
Milk, reduced fat, fluid, 2 %	0.7
Evaporated milk, canned, with added vitamin A	1.6
Butter, with salt	2.1
Cream, fluid, half and half	0.7
Margarine, hard, regular, soybean	2.0
Yogurt, plain, low fat	1.1
Fruits and vegetables	
Apples, raw, with skin	0.2
Bananas, raw	0.8
Cherries, sweet, raw	0.5
Raisins	1.9
Potatoes, raw, skin	1.6
Tomatoes, red, ripe, raw	0.5
Meat, poultry, and fish	
Eggs, whole, raw, fresh	0.9
Fish fillet, battered or breaded and fried	2.5
Pork, fresh, leg (ham), whole, raw	0.9
Hamburger, regular, single patty, plain	1.9
Chicken, broilers or fryers, breast meat only, raw	1.0
Beef, chuck, arm pot roast, raw	1.1

From 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

glassware can also be a source of contaminants. This can be addressed by soaking crucibles and glassware in an acid bath to solubilize mineral contaminants and rinsing repeatedly with distilled-deionized water. The crucibles themselves can also be "pre-ashed" in a muffle furnace to remove organic contaminants.

16.2.1.1 *Plant Materials*

Plant materials are generally oven-dried prior to grinding and ashing. The primary goal for this oven-drying step is to remove moisture. Since samples are often used for multiple determinations (e.g., protein, fiber, lipid), it may be necessary to keep oven temperatures at or below 100 °C to prevent the destruction or alteration of non-mineral analytes. Fresh stem and leaf tissue should be dried in two stages (i.e., first at a lower temperature of 55 °C, then at a higher temperature) to prevent artifact lignin and other undesired products. Plant material with 15 % or less moisture may be ashed without prior drying. If the goal of the analysis is ash or specific mineral analysis alone, low-temperature drying can be accomplished via the use of a muffle furnace equipped with temperature gradient settings. Samples can thus be dried at low temperature and then ashed in the same crucible.

16.2.1.2 *Fat and Sugar Products*

Animal products, syrups, and spices require special consideration prior to ashing because of high fat, moisture, or high sugar content that may result in loss of sample due to spattering, swelling, or foaming. Depending on the application, crucible covers or the use of sealed crucibles may be used to contain samples. Meats, sugars, and syrups should be evaporated to dryness on a steam bath or with an infrared (IR) lamp prior to ashing. For products that tend to form a crust on heating, one or two drops of olive oil (which contains no ash) may be added to allow steam to escape as the crust forms.

Smoking and burning may occur upon ashing for some products (e.g., cheese, seafood, spices). Allow this process to finish slowly by keeping the muffle door slightly open until combustion stops, then close the door fully, and proceed with the normal ashing protocol. A sample may be ashed after drying and fat extraction. In most cases, mineral loss is minimal during drying and fat extraction. Fat-extracted samples should not be heated until flammable extraction solvents (hexane, ether, etc.) have been evaporated completely to avoid the potential risk of solvent ignition or explosion.

16.2.2 Dry Ashing

16.2.2.1 *Principles and Instrumentation*

Dry ashing is incineration at high temperature (525 °C or higher). Incineration is accomplished with a muffle furnace. Several models of muffle furnaces are available, ranging from large capacity units requiring either 208- or 240-V electric supplies to small benchtop units utilizing 110-V outlets.

The muffle furnace may have to be placed in a heat-proof room along with drying ovens. It is important to make sure large furnaces of that type are equipped with a double-pole, single-throw switch. Heating coils are generally exposed, and care must be taken when taking samples in and out with metal tongs.

Crucible selection is critical in ashing because the type depends upon the specific use. Primary considerations relate to chemical stability and resistance to high temperatures. **Quartz crucibles** are resistant to acids and halogens, but not alkali, at high temperatures. **Vycor® brand crucibles** are stable to 900 °C, but **Pyrex® Gooch crucibles** are limited to 500 °C. Ashing at a lower temperature of 500–525 °C may result in slightly higher ash values because of less decomposition of carbonates and loss of volatile salts. **Porcelain crucibles** resemble quartz crucibles in their properties but will crack with rapid temperature changes. Since they are relatively inexpensive, porcelain crucibles are often the crucible of choice. **Steel crucibles** are resistant to both acids and alkalis and are inexpensive, but they are composed of iron, chromium, and nickel, which are possible sources of contamination. **Platinum crucibles** are very inert and are probably the best overall, but they are currently far too expensive for routine use for large numbers of samples. **Quartz fiber crucibles** are disposable, unbreakable, and can withstand temperatures up to 1,000 °C. They are porous, allowing air to circulate around the sample and speed combustion. This reduces ashing times significantly and makes them ideal for solids and viscous liquids. Quartz fiber also cools in seconds, virtually eliminating the risk of burns.

All crucibles should be marked for identification. Marks on crucibles with a felt-tip marking pen will disappear during ashing in a muffle furnace. Laboratory inks scribed with a steel pin are available commercially. Crucibles also may be etched with a diamond point and marked with a 0.5 *M* solution of $FeCl_3$, in 20 % HCl. An iron nail dissolved in concentrated HCl forms a thick brown pigment that is a satisfactory marker. The crucibles should be fired and cleaned prior to use.

The *advantages* of conventional dry ashing are that it is relatively a safe and inexpensive method, that less sample is required than for other methods, that it requires no acids or other added reagents or blank subtraction, that, depending on the size of the oven, many samples may be processed at once, that sample recovery is high, and that little attention is needed once ignition begins [2]. Usually a large number of crucibles can be handled at once, and the resultant ash can be used additionally in other analyses for most individual elements, acid-insoluble ash, and water-soluble and insoluble ash. The *disadvantages* are the length of time required (12–18 h or overnight) and expensive equipment relative to wet ashing. There will be some loss of the volatile elements and interactions between mineral components and crucibles. Volatile elements at risk of being lost include As, B, Cd, Cr, Cu, Fe, Pb, Hg, Ni, P, V, and Zn.

16.2.2.2 *Procedures*

AOAC International has several dry ashing procedures (e.g., AOAC Methods 900.02 A or B, 920.117, 923.03) for certain individual foodstuffs. The general procedure includes the following steps:

1. Weigh a 5- to 10-g sample into a tared crucible (making sure to account for the weight of crucible covers if they are used). Pre-dry if the sample is very moist.
2. Place crucibles in a cool muffle furnace. Use tongs, gloves, and protective eyewear if the muffle furnace is warm.
3. Heat samples for 12–18 h (or overnight) at about 550 °C.
4. Turn off muffle furnace and wait to open it until the temperature has dropped to at least 250 °C, preferably lower. Open the furnace door slowly to avoid losing the powdery ash that may be disturbed by air movement.
5. Using safety tongs, quickly transfer crucibles to a desiccator with a porcelain plate and desiccant. Cover crucibles, close desiccator, and allow crucibles to cool prior to weighing.

Note: Warm crucibles will heat air within the desiccator. With hot samples, a cover may bump to allow air to escape. A vacuum may form on cooling. At the end of the cooling period, the desiccator cover should be removed gradually by sliding to one side to prevent a sudden inrush of air. Covers with a ground-glass sleeve or fitted for a rubber stopper allow for slow release of a vacuum.

The ash content is calculated as follows:

$$\%\,\text{Ash (dry basis)} = \frac{(\text{WAA} - \text{TWOC})}{(\text{OSW} \times \text{DMC})} \times 100 \qquad (16.1)$$

where:

WAA = weight after ashing
TWOC = tare weight of crucible
OSW = original sample weight
DMC = % solids/100

Using the dry matter coefficient allows for the direct conversion of wet weight percent ash values to dry weight ash values. For example, if corn meal is 87 % dry matter, the dry matter coefficient would be 0.87. If it is necessary to calculate percent ash on an as-received or wet weight basis (includes moisture), delete the dry matter coefficient from the denominator. If moisture content was determined in the same crucible prior to ashing, the denominator becomes (dry sample wt – tared crucible wt).

16.2.2.3 *Special Applications*

Some of the AOAC procedures recommend steps in addition to those listed previously. If carbon is still present following the initial incineration, several drops of water or nitric acid should be added; then the sample should be re-ashed. If the carbon persists, such as with high-sugar samples, follow this procedure:

1. Suspend the ash in water.
2. Filter through ashless filter paper because this residue tends to form a glaze.
3. Dry the filtrate.
4. Place paper and dried filtrate in muffle furnace and re-ash.

Other suggestions that may be helpful and accelerate incineration:

1. High-fat samples should be extracted either by using the crude fat determination procedure or by burning off prior to closing the muffle furnace.
2. Glycerin, alcohol, and hydrogen will accelerate ashing.
3. Samples such as jellies will spatter and can be mixed with cotton wool to avoid this.
4. Salt-rich foods may require a separate ashing of water-insoluble components and salt-rich water extract. Use a crucible cover to prevent spattering.
5. An alcoholic solution of magnesium acetate can be added to accelerate ashing of cereals. An appropriate blank determination is necessary.

16.2.3 Wet Ashing

16.2.3.1 *Principle, Materials, and Applications*

Wet ashing is sometimes called **wet oxidation** or **wet digestion**. Its primary use is preparation for specific mineral analysis. Often, analytical testing laboratories use only wet ashing to prepare samples for certain mineral analyses (e.g., Fe, Cu, Zn, P), because losses would occur by volatilization during dry ashing. There are several *advantages* to using the wet ashing procedure. Minerals will usually stay in solution, and there is little or no loss from volatilization because of the lower temperature. The oxidation time is short and requires a hood, hot plate, and long tongs, plus safety equipment. The *disadvantages* of wet ashing are that it takes virtually constant operator attention, corrosive reagents are necessary, and only small numbers of samples can be handled at any one time. If the wet digestion utilizes perchloric acid, all work needs to be carried out in an expensive special fume hood called a **perchloric acid hood**, since working under a normal fume hood could lead to the deposit of explosive peroxides in the ventilation system.

Unfortunately, using a single acid for wet ashing does not give complete and rapid oxidation of organic material, so a mixture of acids often is used. Combinations of the following acid solutions are used most often: (1) **nitric acid**, (2) **sulfuric acid-hydrogen peroxide**, and (3) **perchloric acid**. Different combinations are recommended for different types of samples. The nitric-perchloric combination is generally faster than the sulfuric-nitric procedure.

While wet digestion with perchloric acid is an AOAC procedure (e.g., AOAC Method 975.03), many analytical laboratories avoid the use of perchloric acid in wet ashing, and instead use a combination of nitric acid with either sulfuric acid, hydrogen peroxide, or hydrochloric acid. This is because perchloric acid can form explosive peroxide by-products; it is ***extremely*** dangerous to work with. The reason that perchloric acid is still used is that it allows for better extraction of certain minerals from resistant matrices, such as bone. When working with perchloric acid, special fume hoods free of plastic or glycerol-based caulking compounds and with wash-down capabilities must be used. Precautions for use of perchloric acid are found in the AOAC methods under "Safe Handling of Special Chemical Hazards." Cautions must be taken when fatty foods are wet ashed using perchloric acid. While perchloric acid does not interfere with atomic absorption spectroscopy, it does interfere with the traditional colorimetric assay for iron.

16.2.3.2 *Procedures*

The following is a wet ash procedure using concentrated nitric and sulfuric acids (*to be performed in a fume hood*) (John Budin, Silliker Laboratories, Chicago, IL, personal communication):

1. Accurately weigh a dried, ground 1-g sample in a 125-mL Erlenmeyer flask (previously acid washed and dried).
2. Prepare a blank of 3 ml of H_2SO_4 and 5 ml of HNO_3, to be treated like the samples. (Blank is to be run with every set of samples.)
3. Add 3 ml of H_2SO_4 followed by 5 ml of HNO_3 to the sample in the flask.
4. Heat the sample on a hot plate at ca. 200 °C (boiling). Brown-yellow fumes will be observed.
5. Once the brown-yellow fumes cease and white fumes from decomposing H_2SO_4 are observed, the sample will become darker. Remove the flask from the hot plate. Do not allow the flask to cool to room temperature.
6. *Slowly* add 3–5 ml of HNO_3.
7. Put the flask back on the hot plate and allow the HNO_3 to boil off. Proceed to the next step when all the HNO_3 is removed and the color is clear to straw yellow. If the solution is still dark in color, add another 3–5 ml of HNO_3 and boil. Repeat the process until the solution is clear to straw yellow.
8. While on the hot plate, reduce the volume appropriately to allow for ease of final transfer.

Allow the sample to cool to room temperature, and then quantitatively transfer the sample to an appropriately sized volumetric flask.

9. Dilute the sample to volume with ultrapure water, and mix well. Dilute further, as appropriate, for the specific type of mineral being analyzed.

A combination of dry and wet ash procedure is given in AOAC Method 985.35 “Minerals in Infant Formula, Enteral Products, and Pet Foods.”

16.2.4 Microwave Ashing

Both **wet ashing** and **dry ashing** can be done using microwave instrumentation, rather than the conventional dry ashing in a muffle furnace and wet ashing in a flask or beaker on a hot plate. The CEM Corporation (Matthews, NC) has developed a series of instruments for dry and wet ashing, as well as other laboratory systems for microwave-assisted chemistry. While the ashing procedures by conventional means can take many hours, the use of microwave instrumentation can reduce sample preparation time to minutes, allowing laboratories to increase their sample throughput significantly (current CEM models have a throughput of 5–24 samples per hour). This advantage has led to widespread use of microwave ashing, especially for wet ashing, within both analytical and quality control laboratories in food companies.

16.2.4.1 *Microwave Wet Ashing*

Microwave wet ashing (acid digestion) may be performed safely in either an open- or closed-vessel microwave system. Choice of the system depends on the amount of sample and the temperatures required for digesting. Due to the ability of the closed vessels to withstand higher pressures (some vessels can handle up to 1,500 psi), acids may be heated past their boiling points. This ensures a more complete dissolution of hard-to-digest substances. It also allows the chemist to use nitric acid with samples that might normally require a harsher acid, such as sulfuric or perchloric. In closed vessels specifically designed for high-temperature/high-pressure reactions, nitric acid can reach a temperature of 240 °C. Thus, **nitric acid** is often the acid of choice, though hydrochloric, hydrofluoric, and sulfuric acids also are used, depending on the sample and the subsequent analysis being performed. **Closed-vessel microwave digestion systems** (Fig. 16.1) can process up to 40 samples at a time, with vessel liners available in Teflon®, TFM™ fluoropolymer, and quartz. These systems allow the input of time, temperature, and pressure parameters in a step-by-step format (ramping). In addition, some instruments enable the user to adjust the power and offer “change-on-the-fly” software, which allows the method to be changed while the reaction is running.

Typically, in a closed-vessel microwave system, samples are placed in vessels with the appropriate amount of acid. The vessels are sealed and set on a carousel where the temperature and pressure sensors are connected to a control vessel. The carousel then is placed in the microwave cavity and the sensors connected to the instrument. Time, temperature, pressure, and power parameters are chosen and the unit is started. Digestions normally take less than 30 min. Because of the pressure generated by raising the temperature of a reaction, vessels must be allowed to cool before being opened. The ability to process multiple samples simultaneously provides the chemist with greater throughput than traditional methods. (Note that some closed-vessel microwave digestion systems may also be used for acid concentration, solvent extraction, protein hydrolysis, and chemical synthesis with the proper accessories.)

Open-vessel digestion systems (Fig. 16.2) are used often for larger sample sizes (up to 10 g) and for samples that generate substantial amounts of gas as they are digested. Open-vessel systems can process up to six samples, each according to its own parameters in

16.1 figure Microwave closed-vessel digestion system (Courtesy of CEM Corporation, Matthews, NC)

16.2 figure Microwave open-vessel system (Courtesy of CEM Corporation, Matthews, NC) a sequential or simultaneous format. Teflon®, quartz, or Pyrex® vessels are used and condensers are added for refluxing. Acid (reagent) is automatically added according to the programmed parameters. Sulfuric, nitric, hydrochloric, and hydrofluoric acids, as well as hydrogen peroxide, can all be used in open-vessel systems. These instruments do not require the use of a fume hood, because a vapor containment system contains and neutralizes harmful fumes.

Generally, in an open-vessel microwave system, sample is placed in a vessel and the vessel set in a slot in the microwave system. Time, temperature, and reagent addition parameters are then chosen. The unit is started, the acid is added, and the vapor containment system neutralizes the fumes from the reaction. Samples are typically processed much faster and more reproducibly than on a conventional hot plate. (Note that some open-vessel systems may be used for evaporation and acid concentration as well.)

16.2.4.2 *Microwave Dry Ashing*

Compared to conventional dry ashing in a muffle furnace that often takes many hours, **microwave muffle furnaces** (Fig. 16.3) can ash samples in minutes, decreasing analysis time by as much as 97 %. Microwave muffle furnaces can reach temperatures of up to 1200 °C. These systems may be programmed with various methods to automatically warm up and cool down. In addition, they are equipped with exhaust systems that circulate the air in the cavity to help decrease ashing times. Some also have scrubber systems to neutralize any fumes. Any crucible that may be used in a conventional muffle furnace may be used in a microwave furnace, including those made of porcelain, platinum, quartz, and quartz fiber. Quartz fiber crucibles cool in seconds and are not breakable. Some systems can process up to 15 (25-ml) crucibles at a time.

Typically, in microwave dry ashing, a desiccated crucible is weighed, sample is added, and the crucible is weighed again. The crucible then is placed in the microwave furnace, and the time and temperature parameters are set. A step-by-step (ramping) format may be used when programming the method. The system is started and the program is run to completion. The crucible then is carefully removed with tongs and reweighed. The sample then may be further analyzed, if necessary. Some tests call for acid to be added to a dry-ashed sample, which is then digested for further analysis.

16.3 figure Microwave muffle furnace (Courtesy of CEM Corporation, Matthews, NC)

A comparative study [14] showed that dry ashing various plants for 40 min using a microwave system (CEM Corporation, Matthews NC) was similar to the 4-h time in a conventional muffle furnace. Twenty minutes was shown to be adequate for the plant material used except for Cu determinations, which needed 40 min to obtain similar results. Other comparative examples include dried egg yolks, which can be ashed in 20 min in a microwave system, but require 4 h in a conventional muffle furnace. It takes 16 h to ash lactose in a conventional muffle furnace, but only 35 min in a microwave furnace. Though microwave furnaces may not hold as many samples as a conventional furnace, their speed actually allows significantly more samples to be processed in the same amount of time. Also, microwave furnaces do not require fume hood space.

16.2.5 Other Ash Measurements

The following are several special ash measurements and their applications:

1. **Soluble and insoluble ash** (e.g., AOAC Method 900.02). After dry ashing a sample, the ash is solubilized in boiling water and filtered, and the soluble and insoluble fractions are re-ashed. These measurements of soluble and insoluble ash are an index of the fruit content of preserves and jellies. A lower ash in the water-soluble fraction is an indication that extra fruit is added to fruit and sugar products.
2. **Ash insoluble in acid**. After dry ashing a sample, the ash is solubilized in 10 % HCl, boiled, and filtered, and the insoluble material is re-ashed. This is sometimes used as a measure of the surface contamination of fruits and vegetables and wheat and rice coatings. Those contaminants are generally silicates and remain insoluble in acid, except HBr.
3. **Alkalinity of ash** (e.g., AOAC Method 900.02, 940.26). To a dry-ashed sample, 10 mL 0.1*N* HCl and then boiling water are added. This sample is titrated with 0.1*N* NaOH using a methyl orange indicator. The volume of required NaOH in the titration determines the alkalinity of the ash. Ash of fruits and vegetable is alkaline, while ash of meats and some cereals is acid.
4. **Sulfated ash** (AOAC Method 900.02, 950.77). This method, applied mostly to sugars, syrups, and color additives, measures the amount of residual substance not volatilized when the sample is ignited in the presence of sulfuric acid. The sample is moistened with sulfuric acid and heated on a hot plate and then in a

muffle furnace. This entire procedure is repeated, with the final weight expressed as % sulfated ash.

16.3 COMPARISON OF METHODS

The four major ashing methods described in this chapter are summarized and compared in Table 16.2. Ash determination by dry ashing requires a muffle furnace, which is relatively more expensive than doing wet ashing on a hot plate. Wet ashing requires a hood (a special hood if perchloric acid is used), corrosive reagents, and constant operator attention. While wet oxidation causes little volatilization, dry ashing will result in the loss of volatile elements. If the intent is further elemental analyses, the specific elements being analyzed will dictate whether wet or dry ashing is used. Some micro- and most volatile elements will require special equipment and procedures. Refer to Chaps. 9 and 21 for specific preparation procedures for elemental analyses.

Both dry and wet ashing can be done using microwave systems that require relatively expensive instrumentation, but they greatly reduce the time for ashing and do not require use of a fume hood. New atomic absorption and mass spectrometry-based methods that allow for direct analysis of fresh sample may eventually replace both dry and wet ashing for proximate and mineral analysis. Given the novelty and expense of these new methods relative to traditional dry and wet ashing methods, it is unlikely that these newer methods will replace them in the short term.

16.4 SUMMARY

The two major types of ashing analysis, dry ashing and wet ashing (chemical oxidation), can be accomplished by conventional means or by the use of microwave systems. The procedure of choice depends upon the use of ash following its determination and its limitations based on cost, time, and sample numbers. Conventional dry ashing is based upon sample incineration at high temperatures in a muffle furnace. Except for certain volatile elements, which may be lost during dry ashing, the residue may be used for both proximate analysis and further specific mineral analyses. Wet ashing is often used as a preparation for specific elemental analysis by atomic absorption, inductively coupled plasma and/or mass spectrometry, since it simultaneously dissolves minerals and removes organic material via oxidation. Wet ashing conserves volatile elements but requires more operator time than dry ashing, is limited to a smaller number of samples, and requires the use of highly caustic solvents. Microwave ashing (dry or wet) is faster than conventional methods and requires little additional equipment or space, other than a dedicated fume hood. Ashing may be replaced at some point in the future by methods allowing for the direct determination of mineral identity and content from fresh samples. For the moment, however, ashing remains an essential component of proximate analysis and is a key preparation step for specific mineral analyses.

16.5 STUDY QUESTIONS

1. Identify four potential sources of error in the preparation of samples for ash analysis, and describe a way to overcome each.
2. You are determining the total ash content of a product using the conventional dry ashing method. Your boss asks you to switch to a conventional wet ashing method because he/she has heard it takes less time than dry ashing.
   (a) Do you agree or disagree with your boss concerning the time issue and why?
   (b) Not considering the time issues, why might you want to continue using dry ashing, *and* why might you change to wet ashing?
3. Your lab technician was to determine the ash content of buttermilk by conventional dry ashing. The technician weighed 5 g of liquid buttermilk into one weighed platinum crucible, immediately put the crucible into the muffle furnace using a pair of all stainless steel tongs, and ashed the sample for 48 h at 800 °C. The crucible was removed from the muffle furnace and set on a rack in the open until it was cool enough to reweigh. What errors were made in the preceding method (there were several)? What were the likely results of these errors? What instructions should you have given your technician before starting the procedure in order to avoid the errors you noted?
4. How would you recommend to your technician to overcome the following problems that could arise in conventional dry ashing of various foods?
   (a) You seem to be getting volatilization of phosphorus, when you want to later determine the phosphorus content.
   (b) You are getting incomplete combustion of a product high in sugar after a typical dry ashing procedure (i.e., the ash is dark colored, not white or pale gray).
   (c) The typical procedure takes too long for your purpose. You need to speed up the procedure, but you do not want to use the standard wet ashing procedure.

16.2 table Summary of common ashing methods

Ashing method	*Principle*	*Sources of error*	*Advantages*	*Disadvantages*	*Applications*
Dry ashing	Sample heated to very high (500–600 °C) temperature. All organic matter incinerated. Remaining inorganic material quantitated gravimetrically	Microelement contamination (from grinder or water used to clean crucibles). Sample loss during pre-ash drying step. Volatilization of some elements. Incomplete combustion	Can analyze many samples at once. Requires little technician time. Safe. No blanks needed	Slow (takes 12–18 h); some minerals volatilized. Minerals difficult to resolubilize	Total ash content for proximate analysis. May be used as preparation for specific mineral analysis
Wet ashing	Organic matter oxidized using acids and oxidizing agents, leaving inorganic matter	Microelement contamination. Must run blanks to correct for organic matter in acid and oxidizing agent. Sample loss may occur due to spattering	Shorter time (~2 h) than dry ashing. Minerals stay in solution. Little or no volatilization	Requires constant attention. Lower sample throughput than dry ashing. Can be dangerous. Use of strong acids and oxidizers. Sample loss due to spattering	Ashing of samples prior to mineral analysis for official analyses
Microwave ashing (dry)	Microwave energy heats sample to very high temperatures. Incinerates organic matter. Leaves inorganic matter to be quantitated gravimetrically	Microelement contamination (from grinder or water used to clean crucibles). Sample loss during pre-ash drying step. Volatilization of elements. Incomplete combustion	More rapid (~30 min) than regular dry ashing	Costly. Lower sample throughput than regular dry ashing. Some minerals volatilized. May be hard to resolubilize	Determine total ash content for determination of proximate composition or for quality control purposes
Microwave ashing (wet)	Microwave energy and acid (and sometimes oxidizing agent) are used to oxidize and incinerate organic matter, leaving inorganic matter	Microelement contamination. Must runs blanks to correct for any organic matter in acid and oxidizing agent	Takes less time (~30 min) than regular wet ashing. Minerals stay in solution. Little or no volatilization	Expensive. Can handle fewer samples per run than standard wet or dry ashing procedures	Rapid ashing of samples prior to mineral analysis by rapid or official methods

(d) You have reason to believe the compound you want to measure after dry ashing may be reacting with the porcelain crucibles being used.

(e) You want to determine the iron content of some foods but cannot seem to get the iron solubilized after the dry ashing procedure.

5. Identify advantages and disadvantages of using wet and dry microwave ashing compared with conventional wet and dry ashing.

16.6 PRACTICE PROBLEMS

1. A grain was found to contain 11.5 % moisture. A 5.2146-g sample was placed into a crucible (28.5053 g of tare). The ashed crucible weighed 28.5939 g. Calculate the percentage ash on (a) an as-received (wet weight) basis and (b) a dry matter basis. Make sure to adjust for significant figures in this and other practice problems.
2. A vegetable (23.5000 g) was found to have 0.0940 g acid-insoluble ash. What is the percentage acid-insoluble ash?
3. You wish to have at least 100 mg of ash from a cereal grain. Assuming 2.5 % of ash on average and no sample loss, what is the *minimum* weight of grain required for ashing?
4. You wish to have a coefficient of variation (CV) below 5 % with your ash analyses. The following ash data are obtained: 2.15 %, 2.12 %, and 2.07 %. Are these data acceptable, and what is the CV?
5. A sample of hamburger was analyzed. Here are the results: sample weight, 2.034 g; weight after drying, 1.0781 g; weight after ether extraction of the dried sample, 0.4679 g; and weight of ash, 0.0233 g. What is the percentage ash on (a) a wet weight basis, (b) a fat-free basis (including water), and (c) a fat-free basis (without water)?

Answers

1. (a) 1.70 %, (b) 1.92 %

Calculate ash from sample:

Crucible + ash	28.5939 g
Tared crucible	28.5053 g
Ash	0.0886 g

(a) Calculate for ash on a wet weight basis (a):

$$\frac{0.0886\,\text{g ash}}{5.2146\,\text{g sample}}\times 100\% = 1.70\%\text{ or }1.7\%$$

(b) Calculate for ash on a dry weight basis (b):

$$\frac{0.0886\,\text{g ash}}{5.2146\,\text{g sample}\times\left(\dfrac{100\%-11.5\%}{100\%}\,\text{dry matter coeff.}\right)}\times 100\%$$

$$= 1.92\%.$$

or

$$5.214\,\text{g sample}\times\frac{11.5\,\text{g water}}{100\,\text{g sample}} = 0.5997\,\text{g water}$$

$$5.214\,\text{g sample} - 0.5997\,\text{g water} = 4.6149\,\text{g sample dry wt}$$

$$\frac{0.0886\,\text{g ash}}{4.6149\,\text{g dry wt sample}}\times 100\% = 1.92\%$$

2. 0.4 %

Calculate % insoluble ash:

$$\frac{0.0940\,\text{g acid insoluble ash}}{23.5\,\text{g sample}}\times 100\% = 0.4\%$$

3. 4 g

100 mg = 0.1 g ash.
2.5 % = 2.5 g ash/100 g sample.

$$\frac{2.5\,\text{g ash}}{100\,\text{g sample}} = \frac{0.1\,\text{g ash}}{x}$$

$$2.5x = 10$$

$$x = 4\,\text{g sample}$$

4. Yes, 1.9 %

Calculate the mean:

$$\frac{2.15\% + 2.12\% + 2.07\%}{3} = 2.11\%$$

Calculation of mean and standard deviation was done using Excel:

1	2.15 %
2	2.12 %
3	2.07 %
	Average = 2.11 %
	Std deviation = 0.0404

Coefficient of variation (CV)

$$= \frac{\text{SD}}{\bar{\text{X}}}\times 100\%$$

$$\text{CV} = \frac{0.0404}{2.11}\times 100\% = 1.91\%$$

Is it within the 5 % level for CV? Yes.

5. (a) 1.1 %, (b) 1.64 %

Sample wet wt, 2.034 g
Sample dry wt, 1.0781 g
Wt after extraction, 0.4679 g
Wt of ash, 0.0233 g

(a) Calculate on a wet weight basis:

$$\frac{0.0233\text{ g ash}}{2.034\text{ g sample}} \times 100\% = 1.15\%$$

(b) Calculate on a fat-free, wet weight basis:

$$2.034\text{ g wet sample} - 1.0781\text{ g solids}$$
$$= 0.9559\text{ g water}\left(\text{this is }47\%\text{ moisture}\right)$$

$$1.0781\text{ g solids dry wt} - 0.4679\text{ g solids}$$
$$\text{after extraction} = 0.6102\text{ g fat}$$

$$2.034\text{ g wet sample} - 0.6102\text{ g fat}$$
$$= 1.4238\text{ g wet sample wt without fat}$$

$$\frac{0.0233\text{ g ash}}{\left(1.4238\text{ g wet sample wt without fat}\right)} \times 100\%$$
$$= 1.64\%\text{ ash, fat-free basis}$$

(c) Calculate on fat-free, dry weight basis:

$$\frac{0.0233\text{ g ash}}{\left(0.4679\text{ g drysample wt without fat}\right)} \times 100\%$$
$$= 4.98\%\text{ ash, fat-free dry basis}$$

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