



ADPI Analytical Method #008

Total Ash and Alkalinity of Ash

#008a: Total Ash

#008b: Alkalinity of Ash

1.0 Purpose

This Analytical Method defines the standard operating procedure for determination of total ash and alkalinity of ash in milk and dairy products, by the traditional combustion and titrimetric means. Ash determination via spectroscopic means is not addressed in this method.

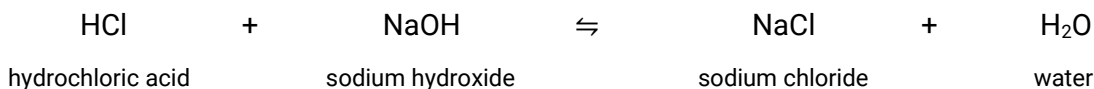
2.0 Scope

This SOP is applicable to determination of total ash and alkalinity of ash in fluid milk and in other dairy products.

3.0 Definitions

- 3.1 **Ash** is the inorganic residue of a sample which remains after ignition (combustion) of the sample at high temperature until the residue exhibits a constant weight. **Total ash** in dairy samples consists primarily of minerals, plus any trace inorganic impurities such as metals which, if present, also would not be eliminated by ignition.
- 3.2 **Titration** is the process of controlled, incremental volumetric addition of a reagent (the **titrant**) to a sample preparation, allowing that titrant to react with components of the sample until a predefined chemical endpoint is achieved.
- 3.3 Titration is usually accomplished either manually using a volumetric apparatus called a **burette** (or sometimes “buret”) where the titrant is added manually, and the added quantity is read visually from a finely graduated scale incorporated into the burette; or automatically via a specialized lab instrument called an **autotitrator** where the addition process and the determination of the added titrant quantity are automated. Manual titration is most often accompanied by use of an indicator substance, while autotitration is most often performed to a point which can be measured by a probe such as a pH electrode or via a potentiometric electrode such as a dual pin platinum probe.

- 3.4 An **indicator** is a substance added to a titration which enables the visualization of the end of the chemical reaction in question. Typical indicator substances change from one color to another, or from a colorless form to a colored one. In the case of this method, phenolphthalein is the indicator substance, and it changes from colorless at acidic pH levels to bright pink at an alkaline pH of 8.3 and higher.
- 3.5 The **endpoint** of a titration, sometimes called the equivalence point or the inflection point, is reached when the quantity of titrant added to the sample preparation becomes equivalent to the corresponding reactant in the sample. In this case, the endpoint is that moment when the alkaline titrant has reacted equivalently with the acidic components of the sample and the pH of the reacted solution rises to pH 8.3, where the phenolphthalein indicator changes color from colorless (acidic pH) to its first persistent, characteristic pink color.
- 3.6 **Stoichiometry** is the known quantitative relationship between reagents in a specific chemical reaction. In this method, the key stoichiometric relationship is the quantitative reaction between alkaline constituents of the sample (presumed to be specifically sodium hydroxide) and the acid titrant of known concentration (specifically hydrochloric acid). Each sodium hydroxide equivalent molecule is neutralized by one molecule of hydrochloric acid as the titrant is added, forming sodium chloride and water in the process. When all alkaline substances have reacted, any further hydrogen ions which are added then begin to accumulate in excess in the solution, causing an immediate drop in pH which corresponds to the endpoint of the chemical reaction.



4.0 Principle

As the term 'ash' might suggest, the determination of inorganic, non-volatile constituents of a sample has long relied on the simple combustion of the sample under controlled conditions until the weight of the residue remains constant. In some methods for ash determination, the decomposition of the sample to yield its mineral residues is aided by the use of one or more concentrated mineral acids, most notably sulfuric acid, hydrochloric acid, nitric acid, or some combination thereof (e.g., aqua regia). These 'wet' ashing methods (including that specified in *Standard Methods for the Examination of Dairy Products*) are substantially more dangerous to perform and are most often reserved for subsequent determination of the precise content of specific individual minerals or trace metal residues in the sample, while this basic 'dry' ashing method is adequate for determination of total ash content in dairy materials.

The combination of the known concentration of the titrant, the measured volume of titrant addition, and the stoichiometry of the chemical reaction of the titration, allow for accurate determination of the quantity of the corresponding sample constituent that reacts with the chosen titrant. In the case of the alkalinity of ash method, which depends on simple acid/base titration, the titrant is 0.100 N hydrochloric acid solution, which reacts stoichiometrically with alkaline constituents of the sample preparation. For purposes of this method, those alkaline constituents are presumed to be sodium hydroxide, and the

results of the test are reported as the quantity of hydrochloric acid, on the basis of milliliters per 100 grams of sample.

Because the visualization of a colorimetric endpoint can be subjective (and may even be entirely unachievable on a consistent basis, whether due to limitations in acuity of the specific analyst and/or due to coloration of the sample being tested), the reliance on non-visual means (e.g., pH measurement with an instrument probe) for endpoint determination has become commonplace and may contribute to more reliable, consistent, repeatable results.

5.0 Reagents and Materials

Adhere to the following requirements carefully for consistent and accurate results.

Both methods require all of the following:

- 5.1 Crucible, 50 mL, porcelain, Coors No. 1, or platinum, or equivalent;
- 5.2 Concentrated hydrochloric acid, ACS reagent grade or better;
- 5.3 Concentrated nitric acid, ACS reagent grade or better;
- 5.4 Water, sediment-free, distilled or efficiently filtered;
- 5.5 Fume hood, suitable for handling fuming inorganic acids;
- 5.6 Aqua regia solution: In a fume hood, combine three parts concentrated hydrochloric acid with one part nitric acid, and then carefully dilute the resulting mixture 1:1 with water by slowly adding the acids solution to the water.
- 5.7 Laboratory oven, forced air type, or equivalent, capable of achieving a consistent temperature of 100°C;
- 5.8 Muffle furnace, capable of maintaining a consistent temperature of 540-550°C, appropriately ventilated;
- 5.9 Tongs, suitable for handling the crucibles into and out of the muffle furnace;
- 5.10 Dessicator, suitable for cooling and holding the prepared crucibles;
- 5.11 Laboratory balance, with capacity of approximately 500 grams and with sensitivity of ± 0.1 grams or better;
- 5.12 Spatula, or equivalent, suitable for handling solid samples;
- 5.13 Steam bath, or equivalent, suitable for preliminary drying of liquid samples prior to ignition (for preparing liquid samples only);
- 5.14 Bunsen burner;
- 5.15 Stirring rod, policeman, or equivalent, for breaking up lumps of ash in the crucible during sample processing;

Determination of the alkalinity of ash (method #008b) additionally requires the following:

- 5.16 Beaker, 400 mL, or equivalent, suitable for the titration steps;
- 5.17 Watch glass, of suitable size to cover the 400 mL beaker;
- 5.18 Graduated cylinder, 100 mL, or equivalent, suitable for adding water to the titration sample preparation;
- 5.19 Pipette, Class A, 50.0 mL, or equivalent, calibrated to deliver, suitable for adding the dilute hydrochloric acid to the titration sample preparation;
- 5.20 Hydrochloric acid (HCl) solution, 0.100 N, certified to a known and reported normality;
- 5.21 Hot plate, or equivalent, suitable for boiling the acidified sample preparation;
- 5.22 Pipette, Class A, 30.0 mL, or equivalent, calibrated to deliver, suitable for adding the concentrated calcium chloride solution;
- 5.23 Calcium chloride (CaCl₂), anhydrous, ACS reagent grade or better;
- 5.24 Calcium chloride solution, 40%: Combine 40 g of anhydrous calcium chloride and 60 g of water in a suitable container and mix to dissolve. Add a few drops of phenolphthalein indicator solution (below) and adjust the pH of the solution to neutrality with 0.100 N hydrochloric acid. (If necessary, filter the preparation to yield a clear solution. A Whatman #1 filter paper or similar would be suitable.)
- 5.25 Burette, Class A, 50 mL, calibrated to deliver, graduated in 0.1 mL increments (auto-zeroing type is acceptable), or equivalent autotitrator may be substituted;
- 5.26 Phenolphthalein indicator solution, ethanolic, 1% m/v;
- 5.27 Pipette, or equivalent apparatus, suitable for dropwise addition of the phenolphthalein indicator solution;
- 5.28 Autotitrator probe, pH, potentiometric, dual-pin, gold, or equivalent, capable of accurately and consistently sensing the pH 8.3 endpoint of the titration (accompanies the autotitrator, if substituted for a manual burette);
- 5.29 Sodium hydroxide (NaOH) solution, 0.100 N, certified to a known and reported normality.

6.0 Personal Safety Precautions

In all cases, the practitioner's company's internal policies and procedures regarding personal safety supersede the following ADPI recommendations:

- 6.1 Milk (dairy) is globally classified as an allergen and should be properly handled with personal safety needs in mind.
- 6.2 Read and understand all precautions for safe handling and disposal shown in the Safety Data Sheets (SDS) for all the reagents required by this method, including use of any prescribed Personal Protective Equipment (PPE).

- 6.3 Dairy ingredients are foods and as such are exempt from U.S. requirements regarding Safety Data Sheets (SDS), where ingredient-specific safe handling instructions would be provided. Despite this exemption, many dairy ingredients are manufactured and marketed in powder form, and powders should be recognized as potential physical irritants, such as to the eyes, nose, and if inhaled.
- 6.4 Some testing apparatus described above may be susceptible to breakage, therefore be aware of associated personal risks. Inspect apparatus before use and replace any items which are compromised.
- 6.5 Concentrated hydrochloric and nitric acids are highly corrosive and are fuming. Handle in an appropriate fume hood, and use appropriate hand and eye PPE at all times.
- 6.6 The muffle furnace, laboratory oven, steam bath and hot plate generate substantial heat. Follow all manufacturers' recommendations for location, operation, and use. Use appropriate PPE.
- 6.7 While platinum crucibles are highly durable, porcelain crucibles are fragile and may be even more susceptible to breakage if they are scratched or chipped from handling. Exercise caution with the hot crucibles in any case, and replace damaged porcelain crucibles based on careful visual inspection.
- 6.8 The ethanolic phenolphthalein indicator solution is extremely flammable. Utilize caution when handling.

7.0 General Considerations

General procedural considerations:

- 7.1 The extensive preparation and maintenance requirements for the ash crucibles are necessary for maximizing accuracy and precision of test results. In order to improve laboratory safety, it may be adequate for general purposes to omit the aqua regia treatment and simply prepare the crucibles by heating in the muffle furnace to constant weight.
- 7.2 Samples should be homogeneous, representative, and equilibrated to ambient temperature before handling, in a manner which will not compromise their suitability for testing.
- 7.3 Follow Good Laboratory Practices (GLPs) wherever applicable.

8.0 Crucible Preparation

As previously stated, careful crucible preparation and maintenance is essential for maximizing the accuracy and precision of total ash determination. Ideal preparation involves aqua regia, a hazardous reagent whose preparation and use may be undesirable in certain laboratory settings and/or for routine ash testing. In that case, consider at least to pre-treat crucibles prior to use by physically cleaning and then heat-treating starting with step 8.4 below.

Optimal crucible preparation consists of the following:

- 8.1 Submerge used crucibles in aqua regia in a beaker for several hours.

- 8.2 Remove the crucibles from the aqua regia and rinse thoroughly with water.
- 8.3 Dry the rinsed crucibles in a forced air oven or on a steam bath at 100°C for 1 hour.
- 8.4 Heat the dried crucibles in the muffle furnace or over a Bunsen burner until they reach a dull red glow.
- 8.5 Cool the heated crucibles to room temperature.
- 8.6 Store the prepared crucibles in a dessicator until use.

9.0 Method #008a: Total Ash

- 9.1 Accurately weigh a prepared crucible, recording the tare weight to the nearest 0.1 g.
- 9.2 Accurately weigh 2.0 g of dry dairy sample, or 10.0 g of other dairy material, into the tared crucible and record the weight of the sample to the nearest 0.1 g.
- 9.3 For dry samples, proceed to step 9.4. Otherwise, evaporate the sample to dryness in a forced air oven or on a steam bath at 100°C.
- 9.4 In a fume hood, carefully and slowly carbonize the sample in the crucible over a Bunsen burner. Carbonization should not generate smoke, nor should any part of the sample become white in color. Conditions should be controlled to achieve carbonization over a period of about 30 minutes.
- 9.5 Transfer the crucible with its carbonized contents into the muffle furnace at 540-550°C for a period of 1 hour.
- 9.6 Remove the crucible from the muffle furnace and cool to ambient temperature.
- 9.7 Add a few mL of water to the ash and break it up with a stirring rod or equivalent.
- 9.8 Evaporate to dryness in the forced air oven or on the steam bath again at 100°C.
- 9.9 Return the crucible to the muffle furnace for an additional hour.
- 9.10 Remove the crucible from the muffle furnace and cool to ambient temperature in a dessicator.
- 9.11 Accurately weigh the crucible and its contents, recording the total weight to the nearest 0.1 g.
- 9.12 Total ash may be calculated as shown in section 11.0 below. If alkalinity of ash is required, proceed to 10.0.

10.0 Method #008b: Alkalinity of Ash

Samples must first be prepared according to method #008a (total ash) before proceeding with this section.

- 10.1 Add a few mL of water to the ash and break it up with a stirring rod or equivalent.
- 10.2 Transfer the ash quantitatively to a 400 mL beaker, using 50-75 mL of water to ensure a complete transfer.
- 10.3 Add 50.0 mL of 0.100 N hydrochloric acid to the beaker.

- 10.4 Cover the beaker with a watch glass and gently boil the contents on a hot plate or steam bath for 5 minutes.
- 10.5 Allow the beaker to cool and rinse down the watch glass with additional water, collecting the rinses in the beaker.
- 10.6 Add 30.0 mL of 40% calcium chloride solution to the beaker, mix gently, cover with a watch glass, and allow to stand for 10 minutes.
- 10.7 Add about 10 drops of phenolphthalein indicator solution to the beaker and titrate the excess acid with 0.100 N sodium hydroxide titrant.
- 10.8 Alkalinity of ash may be calculated as shown in section 12.0 below.

11.0 Calculating Total Ash

$$\text{total ash, \% (w/w)} = \frac{(\text{weight of ash residue after ignition, g}) \times (100\%)}{(\text{weight of original sample, g})}$$

12.0 Calculating Alkalinity of Ash

The key stoichiometric equivalence for this titration is that 1.0 mL of 0.100 N sodium hydroxide corresponds to 1.0 mL of excess 0.100 N hydrochloric acid that was not neutralized by the alkalinity of the sample. The volume of titrant required to reach the endpoint is used to calculate the equivalent amount of alkalinity of the sample. Corrections are applied for the actual normality of the titrant as well as the dilute acid, and final results are reported in units of milliliters of 0.1 N hydrochloric acid per 100 g of dry sample.

The calculation is shown in simplified form, with factors for stoichiometric equivalence and conversion to a percentage basis worked into the formulas. Consult SMEDP for the long-form details if necessary.

Alkalinity of ash, mL per 100 g =

$$\frac{[(50.0 \text{ mL of acid}) \times (\text{actual normality of acid})] - [(mL \text{ of titrant}) \times (\text{actual normality of titrant})]}{(\text{weight of sample, g})} \times 1000$$

13.0 External References

- 13.1 *Standard Methods for the Examination of Dairy Products* ("SMEDP"), 17th edition, Ch. 15 – Chemical and Physical Methods, section 15.041 – Ash, Gravimetric;
- 13.2 *SMEDP*, section 15.042 – Alkalinity of Ash.

14.0 ADPI Document Linkages

Analytical Method #001: *Sampling Dry Powders*

15.0 Revision History

Version	Effective Date	Notes
1.0	???	First officially approved version of this Standard Operating Procedure.
2.0	09/11/2023	Migrated this analytical method to the new modernized Standard Operating Procedure format as established by the ADPI Vice President of Technical Services.