Elemental Analysis Manual

for Food and Related Products

The following is a section of the Elemental Analysis Manual for Food and Related Products.

For additional information and to view other sections of the manual, visit the Elemental Analysis Manual for Food and Related Products web page at http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006954.htm.

U.S. Department of Health & Human Services

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2.3 Digestion and Separation

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This section provides general information on digesting sample material for preparation of an analytical solution and references for procedures to further separate or extract analytes from the matrix. Full details for an analysis are provided in the analytical methods.

2.3.1 MICROWAVE DIGESTION (general applications)

Microwave digestions are performed using commercial equipment specifically made for performing acid digestions.

Analytical Portion for Closed Vessels

Pressure built up by digestion products governs maximum analytical portion mass. Typical analytical portions range from 0.5 to 2 g. Mass must be lower for high-fat foods while larger masses can be used with high-water content foods. Use 1 g reagent water for method blanks (MBKs).

Often the maximum analytical portion mass that can be safely digested may be determined using a sample's known energy (*i.e.*, caloric) content as an indicator of pressure produced during digestion. For some products (*e.g.*, juice concentrates) the energy content may not be known. Maximum energy release permitted for 600 and 800 psi digestion vessels (90 mL capacity) was empirically determined as 3 and 6 kcal, respectively. This maximum vessel energy prevents a digestion from reaching the vessel's maximum operating pressure before digestion is complete. For safe operation, the energy of an analytical portion must not exceed these values.

A food's energy content (usually provided as kcal/100 g) is available from many sources[1,2]. A food's energy content may also be estimated from the calories and serving size provided on a food's label.

Example: For a 50g sports nutrition bar, the nutrition label lists a serving size as the entire bar with a mass of 50 g and an energy value of 210 Calories (Consumer label "Calories" are kilocalories). 2.3 Equation 1 shows calculation of the food energy-to-mass ratio (kcal/g):

$$kcal/g = \frac{210\ kcal}{50\ g} = 4.2\ kcal/g \qquad \text{2.3 Equation 1}$$

The maximum analytical portion mass (max mass) for an 800 psi digestion vessel (6 kcal energy maximum) is calculated using 2.3 Equation 2, which shows that up to 1.4 g of the sports nutrition bar could be digested in this microwave vessel.

$$Max\ mass\ (g) = \frac{Vessel\ Max\ Energy\ (kcal)}{Food\ Energy\ Ratio\ (kcal/g)} = \frac{6\ kcal}{4.2\ kcal/g} = 1.4 g \qquad \text{2.3 Equation 2}$$

For safety, the analytical portion mass for samples of unknown composition should be limited to 0.5g. If maximum pressure attained for this unknown is less than the vessel limit then a greater mass may be analyzed. An analytical portion should not exceed 5 g even if calculation indicates that a larger portion could be taken. Limiting the mass will prevent excess dilution of the nitric acid used for digestion and ensure a complete digestion. Also, in many cases the maximum analytical portion mass will not be necessary or should not be used because of the risk of causing matrix effects (s.a. can occur with a high level of salt) during the determinative step.

2.3 Table 1 provides examples of typical analytical portion masses for selected foods when using 800 psi digestion vessels. Although these calculations will reveal the maximum mass that can be safely decomposed in a vessel, generally, about 1 g is recommended for an analytical portion.

2.3 Table 1. Typical Analytical Portion Mass for Selected Foods

Food	Portion (g)	Food	Portion (g)
American cheese	1.2	Lettuce, Iceberg	5
Beef liver	2.4	Nuts, mixed	0.8
Peaches, canned	5	Peanut butter	0.8
Spaghetti, canned	5	Raisin bran cereal	1.6
Dill pickles	4.0[a]	Tomato catsup	3.0[a]
Spinach	5	Yellow mustard	3.0[a]
Fruit cocktail, canned	5	White bread	1.8

[a] Recommended portion is less than the calorie limit of the digestion vessel due to interference produced by the high-salt content.

Digestion Procedure

If possible, place analytical balance in clean hood (class 100) or on clean bench to minimize contamination while weighing analytical portions. Transfer analytical portion with a pipette, spatula or by pouring and into a tared, clean digestion vessel liner. Avoid placing analytical portions on walls of digestion vessels. Determine mass of analytical portion to an accuracy of 0.001 g.

Move vessel liner to an exhausting clean hood. (Note: suitable for acid use. Plastic construction such as polypropylene is recommended.) For dry samples, dry CRM materials and reactive samples, adding 1 g of reagent water can help control exothermic reactions during the digestion. Pipette 8.0 mL or weigh 11.3 g of high purity nitric acid (sp gr 1.41 g/mL) into vessel liner, washing down any material on walls. Weighing acid using a top loading balance and Teflon® FEP wash bottle is suggested. Use double distilled grade for lowest method blank values. The trade name for double distilled grade will vary by manufacturer. Acid should be added drop wise for the first few mL until it can be established that the sample will not react violently. Some foods, especially those high in sugar, will react with nitric acid within several minutes. If foaming or reaction with the acid is observed, let the vessels sit uncovered in a class 100 exhausting clean hood for 20 minutes or until reaction subsides.

If a clean hood is unavailable, cap vessels but loosen the pressure relief nut (with the safety membrane) to allow pressure to escape. If, however, it appears that excessive foaming would result in the sample-acid mixture expanding out of the vessel then cap the vessel and tighten to appropriate torque to prevent loss of sample or acid.

Add 1 mL high purity 30% H_2O_2. Seal vessels, tighten pressure relief nuts and run microwave digestion program as prescribed by the analytical method. After vessels have cooled to less than 50° C return them to an exhausting clean hood and vent excess pressure slowly. Quantitatively transfer and dilute digestion solution with reagent water as prescribed by the analytical method. This analytical solution should be transferred to a plastic bottle or a capped polypropylene centrifuge tube for storage.

A typical microwave digestion program is given in 2.3 Table 2.

2.3 Table 2. Microwave Digestion Program

Digestion	Peroxide Oxidation
Maximum Power (Watts)	1200
Control Pressure (psi)[a]	800
Ramp Time (min)	25
Hold Time (min)	15
Control Temperature (°C)	200

[a] Only use with non-venting vessels.

Note: Analysts should be cognizant that addition of concentrated nitric acid to some sample types may cause a strong exothermic reaction (especially those high in sugar). If the vessel is not immediately capped and sealed, a loss of nitric acid could occur through evaporation. Likewise, a portion of the sample can be lost through mechanical means when the sample reacts vigorously in an open vessel. For these samples, cap the vessels immediately and allow the sample to complete this exothermic reaction stage. When convenient, leave the vessel over night to go through this exothermic stage and cool. The cooled vessels should be vented before the heating program is initiated. For 'vent and reseal' type vessels (such as CEM Omni vessels), this may allow the use of slightly larger sample portions. For sealed vessels (such as CEM XP-1500 vessels) this step may prevent vessel blowout when dealing with samples having unknown behavior. Membranes are not a dependable defense against vessel blowout caused by sudden high pressure during the heating cycle.

Note: After diluting to volume, the analytical solutions should be clear and colorless to slightly yellow. Turbidity and/or a deep color usually indicate an incomplete digestion. Insoluble food ingredients may be present (e.g. titanium dioxide, silica). In these cases, determine if the microwave digestion system malfunctioned (such as lack of safety membrane, safety membrane cap not tight, wrong oven program, etc.). A copy of the computer generated graph of the digestion run's temperature and pressure versus time is helpful when diagnosing digestion problems. Correct problem and re-digest sample. If microwave digestion system did not malfunction then digest another analytical portion using a smaller mass (at least a factor of 2 less). Some foods (especially spinach) contain silica, which will not dissolve in this procedure. A very small amount of white silicate precipitate is to be expected and is not a problem. Centrifugation can separate these particles from the analytical solution. However, some matrices (i.e., certain dietary supplements) may yield a high proportion of undigested materials after the heating cycle. Additional treatments (e.g., hydrofluoric acid, smaller analytical portion) may be required for accurate results.

Microwave Digestion Vessel Cleaning

Digestion vessels are acid cleaned after each digestion. Vessels being used for the first time or after an incomplete digestion are cleaned with liquid laboratory-grade detergent and then subjected to the acid cleaning. Incomplete digestions are usually dark colored (yellow to brown), have a bad odor and may contain material that did not dissolve. The manufacturer of the microwave digestion equipment may provide additional information on cleaning vessels and

other components of the equipment. Be careful not to use anything that can scratch the vessel walls. The Teflon material is relatively soft and can scratch easily.

Detergent cleaning — Disassemble vessels and soak for at least 2 hours in a solution of liquid laboratory-grade detergent and hot water. Thermowells should be wiped down with a paper towel and detergent solution. Rinse thermowells and vessel components with warm tap water and rinse thoroughly with reagent water. Allow to dry in a clean area (preferably in a Class 100 clean area).

Acid cleaning — Add 10 mL nitric acid to each vessel and microwave them according to the Clean Program listed in 2.3 Table 3 or as suggested by the manufacturer. After vessels have cooled to less than 50 °C remove from oven and vent excess pressure slowly in a fume hood. Disassemble vessels, rinse off covers and liners with reagent water into a waste container. Thoroughly rinse covers and liners with copious quantities of reagent water. Dry in a clean area (preferably Class 100). Outside surfaces of vessels may be dried with laboratory tissues. If vessels are not used immediately after drying, store assembled in a Class 100 clean area or other appropriate contamination free environment.

2.3 Table 3. Microwave Digestion Clean Program

Stage:	**1**
Maximum Power (Watts)	1200
Control Pressure (psi)	{Not Used}
Ramp Time (min)	10
Hold Time (min)	3
Control Temperature (°C)	200

2.3.2 REFERENCES TO PROCEDURES IN VARIOUS METHODS

2.3.2.1 Leaching cadmium and lead from ceramicware---Method 4.6 (Section 4.6.5).

2.3.2.2 Mercury separation in seafood----------Method 4.8 (Section 4.8.5).

2.3.2.3 Arsenic speciation in rice----------Method 4.11 (Section 4.11.7).

REFERENCES

1. Souci, S. W., Fachmann, W., and Kraut, H. (1994) Food Composition and Nutrition Tables, 5th Ed., CRC Press, Boca Raton, FL.
2. U.S. Department of Agriculture, Agricultural Research Service (2013) USDA Nutrient Database for Standard Reference, Release 26 (**Note**: Release number changes when updated.) [accessed June 11, 2014]. Available via USDA website.