U.S. Department of Health & Human Services

FDA U.S. Food and Drug Administration

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 https://www.fda.gov/food/laboratory-methods-food/elemental-analysis-manual-eam-food-and-related-products

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Elemental Analysis Manual

for Food and Related Products

2.2 Food Homogenization

Version 3.0 (September, 2021)

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GLOSSARY and ACRONYMS

Obtaining representative analytical portions is critical for decision makers to evaluate/interpret analysis results. Since the properties of food matrices varies so greatly, analyst experience and judgment are of paramount importance. While foods such as clear fruit juices are inherently homogeneous without need for further treatment, most foods will require homogenization so that representative analytical portions can be obtained. Heterogeneity is particularly problematic when analytical portions are small relative to the overall size of the analytical sample but. EAM methods assume the analyst has made the analytical sample homogeneous with respect to the size of the analytical portion taken for analysis.

The effectiveness of a homogenization procedure can be evaluated by analyzing replicate analytical portions ($n \geq 3$). If precision is significantly higher than that typically observed for the method, procedural issues could be at play (instrument instability, simple blunders, etc.) but more than likely the increase reflects the effects of heterogeneity. If heterogeneity is observed, the decision must be made whether it is too large, and therefore whether additional replicates will be analyzed (to obtain an average that will counter heterogeneity effects) of if further homogenization is needed. Replicate analysis of unhomogenized sample material can also be useful - if results are satisfactorily precise, then homogenization is not necessary.

Homogenized foods will vary greatly in texture and viscosity and may separate into liquid and solid components, especially if stored for a time before analysis. Re-homogenization may be needed and is a good practice immediately before taking the analytical test portion from the sample. The physical characteristics of the homogenate will determine how best to select a representative analytical portion. In some cases, an analytical portion will need to be removed directly during active mixing, such as by using a pipette or a spatula.

A selection of useful laboratory equipment and homogenization procedures are described in the following sections to assist the analyst in obtaining homogenous, representative analytical portions.

2.2.1. LABORATORY EQUIPMENT

Simple mixing (*e.g.,* stirring or shaking by hand in a plastic bag or bottle) or manual size reduction coupled with mixing (*e.g.,* chopping by hand, grinding using a mortar and pestle) may, in some cases, be adequate and common kitchen utensils will often be used. However, commercially available and purpose built mechanical devises that are specifically designed for laboratory use will usually be needed to produce a more uniform, consistent homogenate. These also can be expected to have advantages in materials, options and safety features. The following are common types of equipment used for homogenization:

- **Blender** - A blender is a kitchen appliance used to blend ingredients or puree food. The term typically refers to a stationary, upright electrical device, which is to be distinguished from a hand-powered or electric mixer that may be used for similar purposes. A typical blender is built around a vessel for the ingredients to be blended. At the top of the vessel is a cap to prevent ingredients from escaping when the blender is switched on. At the bottom is a blade assembly, typically removable for cleaning purposes. The bottom seal is most likely watertight. The vessel rests upon a base containing a motor (for turning the blade assembly) with controls on its surface. Most modern blenders offer several possible speeds.

- **Food Processor** - A food processor is a kitchen appliance used to facilitate various repetitive tasks in the process of preparation of food. Today, the term usually refers to an electric-motor-driven appliance, although there are some manual devices also referred to as "food processors". Food processors are similar to blenders in many ways. The primary difference is that food processors use swappable blades and disks (attachments) instead of a fixed blade. In addition, their bowls are wider and shorter, a more appropriate shape for the solid or semi-solid foods usually worked in a food processor. Usually little or no liquid is required in the operation of the food processor unlike a blender, which requires some amount of liquid to move the particles around its blade.

- **Laboratory Homogenizer** - Laboratory homogenizers are high-speed, high-shear mixers that reduce samples to uniformed-sized particles through maceration, cutting, and blending. They are used to process liquids, slurries, or granular substances. Product specifications for laboratory homogenizers include media viscosity, capacity, feed rate, motor speed, motor power, pressure range, and operating temperature. There are three basic types of laboratory homogenizers: fluidized bed, rotor-stator, and ultrasonic. Fluidized-bed homogenizers are durable vessels that fluidize the complete product bed. Rotor-stator homogenizers are single-shaft mixers with an impeller that rotates in close proximity to a stationary housing. Ultrasonic or vibrational homogenizers apply ultrasonic waves in a mixed medium to produce a steep gradient of acoustical pressure and, therefore, fluid movement and a very-fine level of mixing action. Homogenizers that produce high shear and ultrasonic waves can disrupt cellular structure of some foods leading to a better homogenization of the material.

- **Knife Mill** – a laboratory purpose design very similar to food processors, often designed with more matrix tolerant materials.

- **Rotor Mill** – Rotor mills have a multi-toothed blade rotating at high speed (3,000 - 28,000 rpm) in close proximity to an enclosing screen which combines impact, pressure, friction, cutting, shearing and sieving forces to reduce particle size.

- **Centrifugal Mill** – Dry, brittle samples are suitable for centrifugal grinding which uses impact and shearing effects between the rotor and the fixed ring sieve. Food passes onto the rotor and is finely ground between the rotor and the ring sieve.

- **Mortar Grinder** - Mortar grinders force the samples against two hard surfaces (i.e., mortar and pestle) to grind the sample by a combination of pressure and friction. A choice of materials in contact with the sample is available including agate, zirconium oxide, stainless steel, tungsten carbide and porcelain.

- **Impactor Mill** (including bead and ball mills and disc mills) – employ high energy impact and frictional forces created by oscillating, rotational and/or three-dimensional movement of vessels containing both the sample and one or more impactors.

- **Cryogenic Mill** - Cryogenic mills (typically of the impactor type) take advantage of the fact that sample materials become brittle at cold temperatures (dry ice at -78 or liquid nitrogen at -196 ºC) and virtually any food matrix can be homogenized quickly to a fine powder.

Contamination note:

The homogenization process always carries an inherent risk of contamination - such as from the components of the utensils and/or device or cross-contamination as samples are processed. Consideration should therefore be given to the choice of device and/or component (e.g., food processors may use bowls made from plastic, aluminum or stainless steel, and cutting heads made from stainless steel, titanium or ceramic materials, each of which may introduce contamination). Additionally, devises should be adequately cleaned between samples.

A homogenization procedure can be evaluated to determine whether it is adding contamination to samples by processing an analyte-free identical matrix sample (or a similar matrix certified reference material). When an analyte-free matrix sample is not available, a material of similar physical properties may be useful (e.g., water for highly liquid samples or microcrystalline cellulose for dry powdered samples).

2.2.2. HOMOGENIZATION PROCEDURES

Analysts are presented with many challenges for achieving homogeneous mixtures of foods and dietary supplements. Matrix composition and physical characteristics vary, and procedural options are limited by a laboratory's inventory of equipment. In addition, the amount of material received and the number of subsamples (in a laboratory sample), the number of replicates to be analyzed, the risks of contamination associated with different procedures and equipment, and the cleaning (of equipment

apparatus) between samples must be considered. Consequently, there is no single homogenization approach applicable to all food samples.

Food comes in an extremely wide variety of matrices and heterogeneity is expected. In contrast, dietary supplements and many kinds of candy are mass-produced in tablets, capsules, pieces, etc., and expected to be quite uniform. Regardless, since analytical portions are quite small (typically <1g), homogenization is needed to prevent composition biases. Using candy as an example, this is particularly evident because it can be multi-component (candy bars), assortments (assorted chocolates or multi-flavored life savers), or with powders, coatings, and fillings. These are all observations that explain why diversity necessitates a high degree of analyst discretion - on how to choose material (for homogenization) and how to process it.

Homogeneity is achieved by homogenizing multiple portions or units (i.e., tablets/capsules for dietary supplements or pieces of candy). For small-mass units such as tablets, this would likely include ≥ 20 units. The exact number and total mass will depend on the characteristics of the laboratory sample (product packaging, number of subsamples, etc.) and homogenizing equipment used.

Homogenization insight in the context of this manual - applicable primarily to food but also by extension to dietary supplements - is as diverse as is the range of matrices encountered in a food analysis laboratory. Most samples can be processed directly and at room temperature using relatively simple procedures (section 2.2.2.1) but some will be much easier to homogenize using strategies that alter the physical characteristics of difficult to process foods - heating (section 2.2.2.2), frozen sample processing (section 2.2.2.3), and adding liquid (section 2.2.2.4).

Sample homogenization is characterized by numerous factoids with procedures, observations, experiences, suggestions, cautions, etc., many of which are given in the discussions below.

2.2.2.1 General Guidelines on Food Homogenization

- Matrices believed to be inherently uniform (e.g., clear beverages, dry granulated powders, and syrupy or powdery candy) can often be processed by simple mixing using devices such as mechanical platform shakers, wrist-action shakers or rotary inversion mixers.
- Semi-solid, soft to medium hard, and fibrous matrices (e.g., bread, pasta, meat, fruit, vegetables, and some kinds of candy) may respond well with the cutting action of a blender, food processor, knife mill, rotor mill, or a laboratory homogenizer.
- Soft- to medium-hard fibrous matrices (e.g., rice, grains, corn, and dried fruit) will likely need the impact and shearing action of a rotor/centrifugal mill. These can be very efficient at size reduction but may require additional mixing of the resultant fine powders to ensure homogeneity.
- Dry, medium-hard to hard, brittle matrices (e.g., dried plant material, tree nuts, and vitamin tablets) fracture and break effectively with the crushing, friction and/or impact action of ball mills, disc mills, impact mills or mortar grinders.
- Candy, which comes in numerous forms, can pose unique challenges overcome via use of heating, cooling, or addition of liquid (discussed later).
- Compressed tablets (coated and uncoated) and capsules with dry components can generally be milled at room-temperature with short milling cycles (< 5 s) using mortar, rotor, or impact mills.
- Soft-shell capsules (gelcaps) and dosage units consisting of or containing gelatin, pastes, oils or

liquids are generally more effectively homogenized at cryogenic temperatures.

- As a general rule, maximize mass as much as reasonably possible.
- Mass correction factors (MCF; see 3.4.6) are needed when material is mixed with water and possibly if changes occur while processing (e.g., evaporation).
- Frozen materials are assumed to be thawed and at room temperature unless processed via heating or cooling as discussed separately.

2.2 Table 1 shows procedures that are commonly used in FDA's Total Diet Study. The following terminology is used here and throughout this Section 2.2:

- Water - Water that meets specifications for ASTM Type I water [1].
- BF - Baby food.
- MCF - Mass correction factor.

2.2 Table 1 Homogenization equipment and procedures used in FDA's Total Diet Study

Food Product	**Suggested Procedure**
Apple (red), raw (with peel)	Food Processor
Applesauce, bottled	Blender
Apricot, raw	Blender
Asparagus, fresh/frozen, boiled	Food Processor
Avocado, raw	Food Processor
Bagel, plain, toasted	Food Processor
Banana, raw	Food Processor
Beans, kidney, dry, boiled	Food Processor
Beef chow mein, from Chinese carry-out	Food Processor
Beef stew, homemade	Blender
Beef stroganoff with noodles, homemade	Food Processor
Beef, ground, regular, pan-cooked	Food Processor
Beef, meatloaf, homemade	Food Processor
Beef, roast, chuck, oven-roasted	Food Processor
Beef, steak, loin, pan-cooked	Food Processor
Beer	Mix by Hand
Beets, fresh/frozen, boiled	Food Processor
Beverage, carbonated, cola, low-calorie	Mix by Hand
Beverage, carbonated, cola, regular	Mix by Hand
Beverage, carbonated, fruit-flavored, regular	Mix by Hand
Beverage, fruit drink (10% juice), canned or bottled	Blender
BF, applesauce	Blender

Food Product	Suggested Procedure
BF, bananas	Blender
BF, beef and broth/gravy	Blender
BF, carrots	Blender
BF, chicken and broth/gravy	Blender
BF, chicken noodle dinner	Blender
BF, corn, creamed	Blender
BF, fruit dessert/pudding	Blender
BF, green beans	Blender
BF, infant formula, milk-based, high iron, ready-to-feed	Blender
BF, Infant formula, milk-based, low iron, ready-to-feed	Blender
BF, Infant formula, soy-based, ready-to-feed	Blender
BF, juice, apple	Mix by Hand
BF, juice, orange	Mix by Hand
BF, macaroni, tomato and beef	Blender
BF, mixed vegetables	Blender
BF, peaches	Blender
BF, pears	Blender
BF, peas	Blender
BF, rice cereal with apples	Blender
BF, rice infant cereal, instant, prepared with milk	Blender
BF, spinach, creamed	Blender
BF, split peas with vegetables & ham/bacon	Blender
BF, squash	Blender
BF, sweet potatoes	Blender
BF, teething biscuits	Food Processor
BF, turkey and rice	Blender
BF, vanilla custard/pudding	Blender
BF, vegetables and beef	Blender
BF, vegetables and chicken	Blender
BF, vegetables and ham	Blender
Bologna (beef/pork)	Food Processor
Bread, biscuits, refrigerated-type, baked	Food Processor
Bread, cornbread, homemade	Food Processor
Bread, cracked wheat	Food Processor
Bread, muffin, fruit or plain	Food Processor
Bread, rye	Food Processor
Bread, white roll	Food Processor
Bread, white, enriched	Food Processor
Bread, whole wheat	Food Processor

Food Product	Suggested Procedure
Broccoli, fresh/frozen, boiled	Food Processor
Brownie	Food Processor
Brussels sprouts, fresh/frozen, boiled	Food Processor
Butter, regular (salted)	Food Processor
Cabbage, fresh, boiled	Food Processor
Cake, chocolate snack with chocolate icing	Food Processor
Cake, chocolate with icing	Food Processor
Cake, yellow, with white icing, prepared from mixes	Food Processor
Candy, caramel	Separately weigh equal masses of water and candy. Heat water to boiling in a 2-quart stainless steel bowl. Add candy with continuous stirring using large stainless steel spoon. After candy melts, pour into appropriate containers. [MCF = 0.5]
Candy, hard [Hard candy: Based on sugars cooked to the hard-crack stage, including suckers (known as boiled sweets in British English), lollipops, jawbreakers (or gobstoppers), lemon drops, peppermint drops and disks, candy canes, rock candy, etc.]	Separately weigh equal masses of water and candy. Heat water to boiling in a 2-quart stainless steel bowl. Add candy with continuous stirring using large stainless steel spoon. After candy melts, pour into appropriate containers. [MCF = 0.5]
Candy, milk chocolate bar, plain	Food Processor. Avoid inducing elevated temperatures that cause phase separation.
Cantaloupe, raw/frozen	Blender
Carrot, fresh, peeled, boiled	Food Processor
Cauliflower, fresh/frozen, boiled	Food Processor
Celery, raw	Food Processor
Cereal, corn flakes	Food Processor
Cereal, cream of wheat (farina), enriched, cooked	Food Processor
Cereal, crisped rice	Food Processor
Cereal, fruit-flavored, presweetened	Food Processor
Cereal, oat ring	Food Processor
Cereal, oatmeal, plain, cooked	Food Processor
Cereal, raisin bran	Food Processor
Cereal, shredded wheat	Food Processor
Cheese, American, processed	Food Processor
Cheese, cheddar, natural (sharp/mild)	Food Processor
Cherries, sweet, raw	Blender
Chicken breast, oven-roasted (skin removed)	Food Processor
Chicken nuggets, fast-food	Food Processor
Chicken potpie, frozen, heated	Food Processor
Chicken, fried, fast-food	Food Processor
Chicken, fried – homemade	Food Processor

Food Product	Suggested Procedure
Chili con carne with beans, homemade	Food Processor
Coleslaw with dressing, homemade	Food Processor
Collards, fresh/frozen, boiled	Food Processor
Cookies, Chocolate chip	Food Processor
Cookies, sugar	Food Processor
Cookies, sandwich with crème filling	Food Processor
Corn, cream style, canned	Blender
Corn, fresh/frozen, boiled	Food Processor
Corn, hominy grits, enriched, cooked	Food Processor
Corn/tortilla chips	Food Processor
Cottage cheese, 4% milk fat	Blender
Crackers, butter-type	Food Processor
Crackers, graham	Food Processor
Crackers, saltine	Food Processor
Cream cheese	Food Processor
Cream substitute, non-dairy, liquid/frozen	Blender
Cream, Half & half	Blender
Cucumber, peeled, raw	Food Processor
Cucumber, pickles, Dill	Food Processor
Cucumber, pickles, sweet	Food Processor
Doughnut, cake-type	Food Processor
Egg, cheese, and ham on English muffin, fast-food	Food Processor
Eggplant, fresh, peeled, boiled	Food Processor
Eggs, boiled	Food Processor
Eggs, fried	Food Processor
Eggs, scrambled with oil	Food Processor
English muffin, plain, toasted	Food Processor
Fish sandwich on bun, fast-food	Food Processor
Fish sticks or patty, frozen, oven-cooked	Food Processor
Frankfurter (beef/pork), boiled	Food Processor
Frankfurter on bun, fast-food	Food Processor
French fries, frozen, heated	Food Processor
Frozen meal - Salisbury steak, heated	Food Processor
Frozen meal - turkey, heated	Food Processor
Fruit cocktail, canned in light syrup	Blender
Granola with raisins	Food Processor
Grapefruit, raw	Blender
Grapes, red/green, raw	Food Processor
Green beans, fresh/frozen, boiled	Food Processor
Ham, cured (not canned), baked	Food Processor
Hamburger, quarter-pound on bun, fast-food	Food Processor

Food Product	Suggested Procedure
Hamburger, quarter-pound with cheese on bun, fast-food	Food Processor
Honey	Mix by Hand
Ice cream, light, vanilla	Blender
Ice cream, regular, vanilla	Blender
Jelly, any flavor	Blender
Juice, apple, bottled	Mix by Hand
Juice, grape, frozen concentrate, reconstituted	Blender
Juice, grapefruit, bottled	Blender
Juice, orange, frozen concentrate, reconstituted	Blender
Juice, prune, bottled	Mix by Hand
Juice, tomato, bottled	Mix by Hand
Lamb chop, pan-cooked with oil	Food Processor
Lasagna with meat, homemade	Food Processor
Lemonade, frozen concentrate, reconstituted	Blender
Lettuce, iceberg, raw	Food Processor
Lima beans, immature, frozen, boiled	Food Processor
Liver (beef/calf), pan-cooked with oil	Food Processor
Luncheon meat (ham)	Food Processor
Luncheon meat, salami, (not hard)	Food Processor
Macaroni and cheese, prepared from box mix	Food Processor
Macaroni, boiled	Food Processor
Margarine, regular (salted)	Food Processor
Martini	Mix by Hand
Mayonnaise, regular, bottled	Food Processor
Milk shake, chocolate, fast-food	Blender
Milk, chocolate, low fat, fluid	Blender
Milk, evaporated, canned	Blender
Milk, low fat (2%), fluid	Blender
Milk, skim, fluid	Blender
Milk, whole, fluid	Blender
Mixed vegetables, frozen, boiled	Food Processor
Mushrooms, raw	Food Processor
Mustard, yellow	Mix by Hand
Noodles, egg, enriched, boiled	Food Processor
Nuts, mixed, no peanuts, dry roasted	Blender
Oil, olive/safflower	Mix by Hand
Okra, fresh/frozen, boiled	Food Processor
Olives, black	Food Processor
Onion, mature, raw	Food Processor
Orange (navel/Valencia), raw	Blender
Pancakes from mix	Food Processor

Food Product	Suggested Procedure
Peach, canned in light/medium syrup	Blender
Peach, raw/frozen	Blender
Peanut butter, creamy	Food Processor
Peanuts, dry roasted, salted	Food Processor
Pear, canned in light syrup	Blender
Pear, raw (with peel)	Food Processor
Peas, green, frozen, boiled	Food Processor
Peas, mature, dry, boiled	Food Processor
Pepper, sweet, green, raw	Food Processor
Peppers, green, stuffed, homemade	Food Processor
Pie, apple, fresh/frozen	Food Processor
Pie, pumpkin, fresh/frozen	Food Processor
Pineapple juice, frozen concentrate, reconstituted	Blender
Pineapple, canned in juice	Blender
Pinto beans, dry, boiled	Food Processor
Pizza (carry-out), cheese & pepperoni, regular crust	Food Processor
Pizza (carry-out), cheese, regular crust	Food Processor
Plums, raw	Food Processor
Popcorn, popped in oil	Food Processor
Popsicle, fruit-flavored	Blender
Pork and beans, canned	Blender
Pork bacon, oven-cooked	Food Processor
Pork chop, pan-cooked with oil	Food Processor
Pork roast, loin, oven-roasted	Food Processor
Pork sausage (link/patty), oven-cooked	Food Processor
Potato chips	Food Processor
Potato, baked (with peel)	Food Processor
Potato, boiled (without peel)	Food Processor
Potato, french-fried, fast-food	Food Processor
Potatoes, mashed, from flakes	Food Processor
Potatoes, scalloped, homemade	Food Processor
Pretzels, hard, salted	Food Processor
Prunes, dried	Food Processor
Radish, raw	Food Processor [*Alternative: Food Processor with equal masses of water and food. MCF = 0.5*]
Raisins	Food Processor [*Alternative: Food Processor with equal masses of water and food. MCF = 0.5*]
Rice, white, enriched, cooked	Food Processor
Salad dressing, French, regular	Mix by Hand
Salad dressing, Italian, low-calorie	Blender

Food Product	Suggested Procedure
Salmon, steaks/fillets, baked	Food Processor
Sauerkraut, canned	Food Processor
Sherbet, fruit-flavored	Blender
Shrimp, boiled	Food Processor
Soup, bean with bacon/pork, canned, condensed, prep with water	Blender
Soup, chicken noodle, canned, condensed, prep with water	Blender
Soup, clam chowder, New England, canned, condensed, prep with whole milk	Blender
Soup, mushroom, prep with whole milk	Blender
Soup, tomato, canned, condensed, prep with water	Blender
Soup, vegetable beef, canned, condensed, prep with water	Blender
Sour cream	Blender
Spaghetti with meat sauce, homemade	Food Processor
Spaghetti with tomato sauce, canned	Blender
Spinach, fresh/frozen, boiled	Food Processor
Squash, Summer, fresh/frozen, boiled	Food Processor
Squash, winter (Hubbard/acorn), fresh/frozen, boiled	Food Processor
Strawberries, raw/frozen	Food Processor
Sugar, white, granulated	Mix by Hand
Sweet potato, fresh, baked	Food Processor
Sweet roll/Danish pastry	Food Processor
Syrup, chocolate	Mix by Hand
Syrup, pancake	Mix by Hand
Taco/tostada with beef and cheese, from Mexican carry-out	Food Processor
Tomato catsup	Blender
Tomato sauce, plain, bottled	Blender
Tomato, raw	Food Processor
Tomato, stewed, canned	Blender
Tortilla, flour	Food Processor
Tuna noodle casserole, homemade	Food Processor
Tuna, canned in oil	Food Processor
Turkey breast, oven-roasted	Food Processor
Turnip, fresh/frozen, boiled	Food Processor
Veal cutlet, pan-cooked	Food Processor
Water, tap	Mix by Hand
Watermelon, raw/frozen	Blender
Whiskey	Mix by Hand

Food Product	Suggested Procedure
Wine, dry table, red/ white	Mix by Hand
Yogurt, low fat, fruit-flavored	Blender
Yogurt, plain, low fat	Blender

2.2.2.2 Processing Heated Samples

Some foods (especially those with high sugar content such a honey or some candies) which are difficult to homogenize at room temperature become more workable when heated.

- Heating procedures can be readily accomplished using common laboratory equipment (hot-plates, heating blocks, hot-water baths, laboratory ovens or microwave ovens).

Maximum temperatures for sample containment vessel materials:	
Polypropylene (PP)	*120°C*
High-density polyethylene (HDPE)	*120°C*
Polymethylpentene (PMP)	*150°C*
Perfluoroalkoxy polymer (Teflon PFA)	*250°C.*
Plastic	*not recommended for hot plate*

- Heating should be applied gradually and only enough to facilitate adequate mixing (to avoid unnecessary chemical reactions).
- Periodic or continuous stirring is recommended to avoid localized overheating. This is a particular caution with hotplates and heating blocks.
- The weight of the composite should be recorded both before and after melting to account for any loss of volatile components (e.g., as water vapor) during the melting process.
- When using microwave ovens, use low-power settings and short (5-10s) cycles with stirring between cycles.
- Heating to high temperatures may not be appropriate with speciation methods.
- Highly viscus foods (*e.g.,* honey, sugar syrups, gelatin-based products) tend to be much more workable if heated (to ≤50°C). Heat slowly to avoid over-heating localized portions of the sample.
- Crystalized sugars in honey will re-dissolve using a water bath (at 50°C) or a microwave in 5-10 s intervals. Stirring is not necessary in this case.
- Many candy products melt when heated and can be homogenized by stirring or blending (during the melting process or quickly afterward). Chocolate-based candies typically melt at ~ 40 to 60°C and hard candies at ~ 120 to 150°C. Temperature should be increased gradually until melting begins then maintained at that temperature until the material is fully melted. Rapid heating can result in spattering and high temperatures can result in sugar caramelization,

charring, etc. If un-melted constituents remain, additional processing of the (warm) melt with probe-type laboratory homogenizers may provide a more homogenous composite.

- For some candy, a cooled homogenate can become very hard and/or elastic-like and it may be difficult to remove analytical portions. In such cases, analytical portions can be removed while still melted (but allow to cool before weighing).

2.2.2.3 Processing While Sample is Frozen

Homogenization is facilitated when samples are processed in frozen state because they are substantially more brittle. This effect increases with decreasing temperature. Processing temperatures (in °C) are approximately -20, -80, and -190, which are associated with a conventional freezer, dry ice, and liquid nitrogen, respectively. At -80, and especially at -190, the equipment is more specialized and safety procedures are more complicated. These extra low temperatures are therefore used only when needed. They result, however, in fine homogenous powders for virtually all food matrices.

After processing, homogenate manipulations (e.g., removal of analytical portions and storage of reserve material) will usually need to be performed quickly or in a cold environment so that the powder remains intact. Otherwise, textures may become unworkable (e.g., candy becoming sticky) or components can separate (e.g., capsule pieces separate from oil for liquid gelcap dietary supplements).

Best Practices:

- Safety precautions are imperative, especially at cryogenic temps (<150 °C)
- Pre-freeze sample material in workable sizes.
- Consider working capacity of cold temperature milling vessels when planning work.
- Maintain frozen temperature to the extent possible during processing. Maintaining cryogenic conditions can be challenging.
- At liquid nitrogen temperature, homogenate powder behavior can be very different from in warmer conditions.
- Ideal storage of homogenate is in a -80 °C freezer until analytical work is completed.

Freezing approaches that have been used successfully in FDA laboratories:

- Processing while mixed with dry-ice or liquid nitrogen, which sublime or evaporate, respectively, and leave the homogenized material,
- Pre-freezing material and processing it (quickly) in the frozen state, and
- Processing the sample while immersed in liquid nitrogen.

2.2.2.4 Addition of Liquid

Some foods are easier to homogenize if liquid is added. Examples are peanut butter, cheese, shellfish tissue, raisins, and chocolate-based or fruit-based candy, with or without visible particulate ingredients.

- The addition of liquid can be particularly useful for candy products.
- Water (or dilute non-ionic surfactant, or dilute mineral acid) is typically added in roughly equal proportion to sample material.

- The total volume (sample + water) should be ≤1/3 of the mixing/homogenizing vessel's volume.
- Dilution effects are accounted for via mass dilution factors.
- Blend mixtures until a visually homogenous composite is obtained. This mixture is expected to have paste-like consistency which must remain stable (*i.e.*, no phase separation or precipitation) until analytical portions are removed.
- Chocolate-based products, which have high fat content, should be blended first before adding water.
- The blending process can sometimes be more effective if a candy-water mixture stands for several hours (e.g., overnight) and/or is heated (~40-60°C) to melt, soften, and partially dissolve.
- If a more rigorous approach is desired, add 10% nitric acid to candy (instead of water) and heat the mixture to approximately 100°C until the material is dissolved. This step can be left over night to maximize dissolution. If needed, due to remaining particulate materials, also blend using a laboratory homogenizer such as a high-shear or ultrasonic probe.
- Silicon dioxide, chili flakes, and titanium dioxide are common additives which are nearly insoluble even in nitric acid solutions. If particulates persist, stir vigorously while removing analytical portions.

2.2.3. HISTORY

EAM 2.2 Table 2. History

Version	Revisions Made	Effective Date
1.0	*Analytical Sample to Analytical Portion*	June 2008 (hard copy only)
2.0	Major re-organization. Title changed to *Food Homogenization*; expanded cryogenic discussion; inserted candy and degasification information (brought in from section 2.1); added subsection on *Pills, etc.* (2.2.2.3); Converted to pdf for web posting.	September 2014
3.0	Major reorganization and editing. Organized according to homogenization procedures (formerly according to food type); added *History* section.	September 2021

References

[1] ASTM Subcommittee D1193, "ASTM D1193-06(2018) Standard Specification for Reagent Water," [Online]. Available: https://www.astm.org/Standards/D1193.htm. [Accessed September 2021].