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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Elemental Analysis Manual

for Food and Related Products

2.2 Food Homogenization

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This section provides information to assist the analyst on physically preparing the analytical sample prior to taking an analytical portion for analysis. Obtaining representative analytical portions is critical for accurate and reproducible results. EAM methods assume the analyst has made the analytical sample homogeneous with respect to the size of the analytical portion. Homogenized foods will vary greatly in texture and viscosity and may separate into liquid and solid components. Analytical samples that have been stored after being homogenized usually require re-homogenization before acquiring an analytical portion. The analyst must carefully observe the physical characteristics of the homogenate and choose an appropriate means of obtaining a representative analytical portion (*e.g.*, pouring directly from container possibly during active mixing, using a 1, 5 or 10 mL pipette or using a spatula).

Note:
If non-homogeneity is known or suspected then replicate analytical portions should be analyzed and the results assessed.

2.2.1 LABORATORY HOMOGENIZATION EQUIPMENT

[Food] Blenders[1] - 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 a number of possible speeds.

Food Processors[2] - 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 Homogenizers[3] - 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.

Cryogenic Mills - Cryogenic mills take advantage of the fact that sample materials become brittle at cold temperatures (dry ice at -78 or liquid nitrogen at -196 ^{o}C) and virtually any matrix can be homogenized quickly to a fine powder.

Impactor mill
One type of commercially-available cryogenic mill is an impact mill that uses an oscillating magnetic field to shuttle a stainless steel impactor back and forth against two stationary stainless steel end plugs in a polycarbonate-wall sample vial. The vial assembly is immersed in liquid nitrogen throughout the milling procedure. (add reference)

Disk mill
FDA has experience using a custom-made disk mill that has been used at NIST for SRM production.[4] At cryogenic temperature, the apparatus is filled with pieces of frozen sample then left for at least 12 hours immersed in liquid nitrogen. After this, the mill is placed in a machine that oscillates the mill much like a paint shaker except the movement is only in a two-dimensional swirling motion. An internal puck and a concentric mill ring swirl and pulverize the brittle sample material. This process happens relatively quickly (within 5 minutes) after removal

from the liquid nitrogen. The apparatus is immediately returned to a cryogenic environment (typically above a liquid nitrogen bath) for further manipulation of the resulting powder.

Other
Some homogenization equipment not initially developed for a cryogenic application can nevertheless be used in this manner by freezing sample material in a conventional freezer or in liquid nitrogen then quickly homogenizing the frozen material before it thaws.

Another approach is homogenize the sample material along with some dry ice. After homogenization, the dry ice is allowed to sublime away.

Mortar Grinders - 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.

Rotor Mills - Rotor mills use a stainless steal beater rotating at a high speed (3000 — 28000 rpm) to cut up the sample by impact and shearing.

2.2.2 HOMOGENIZATION PROCEDURES

Diversity is to be expected when homogenization procedures are used. This results from differences in foods, analytical methods, available equipment, etc. Therefore, a great deal of analyst discretion is necessary. Below are comments and examples of procedures that have been useful at FDA.

Conventional processes such as use of blenders and food processors at room temperature are particularly suited to processing large numbers and quantities (kg) of samples. However, some matrices can be particularly difficult to process. Candy poses some unique problems and is given special attention in the EAM (see sections 2.2.2.2 and 2.2.2.3).

Cryogenic homogenization where sample materials become brittle at liquid nitrogen temperatures (-196 ^{o}C) are universally-applicable to virtually any matrix and all materials

become powders that are very easy to work with. However, cryogenic work also requires added effort to work in cold temperatures to keep the materials in powder form, where cloud formation hinders vision, and extra cold safety procedures are needed.

A simple procedure to keep cryogenically homogenized material in a sub-zero environment is to work in a conventional ice-chest type cooler with at least 5 cm of liquid nitrogen and on a raised surface that sits just above the nitrogen. As the nitrogen boils, the movement of dry gaseous nitrogen up and out of the cooler provides a bath of cold, dry nitrogen that is cloud free. The temperature at the sample location would be estimated to be in the -50 to -100 °C range.

2.2.2.1 GENERAL PROCEDURES

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[5].

BF - Baby food.

MCF - Mass correction factor.

2.2 Table 1 Examples of homogenization equipment and procedures commonly used[a]

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	Blender

bottled	
BF, applesauce	Blender
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

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.] [6]	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
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
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
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
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
Wine, dry table, red/ white	Mix by Hand
Yogurt, low fat, fruit-flavored	Blender
Yogurt, plain, low fat	Blender

[a] Based on instructions used in FDA's Total Diet Study.

2.2.2.2 CANDY PROCEDURES

Some candy products, such as vitamins, are expected to be homogeneous because they are carefully produced to provide specific amounts of vitamins/nutritional components. Preparation

of an analytical portion for these products is relatively straightforward. It should be taken from a homogenized composite prepared from a minimum of 20 units.

Other candy products can be exceptionally nonhomogeneous. In addition to intentional variations (e.g, swirls and layers in the candy), particulates are sometimes dispersed in varying amounts either within or coated on the surfaces of individual pieces. This nonhomogeneity, which is particularly troublesome for sub-gram analytical portion masses, can cause analytical results to vary widely and make difficult the interpretation of results or even raise questions about the quality of results.

Nonhomogeneity is managed by combining several candies into a well-mixed composite from which a small analytical portion can be taken. The exact number of units used will depend on the characteristics of the sample as received (product packaging, number of subsamples, etc.). This applies for all varieties of hard and soft candy, powdered candy products, and fruit candy products, and especially candies that have chili or other ingredients that may be the source of the contaminant of interest.

Product Types

Type I: Semi-solid or soft candy (e.g., chocolate-based, fruit-based, including tamarind, tejocote, apple, etc.) with or without visible particulate ingredients.

Type II: Hard candy (including suckers) with or without visible particulate ingredients whether in the candy or on the surface of the candy.

Type IIA: Hard candy (including suckers) with supplemental (but separate) ingredients (e.g., salt, chili).

Type III: Powdered sugar or flavored salt products with or without other particulate ingredients (e.g., chili).

Homogenization Approaches

1. Blending with nitric acid and heating.[7,8] (Supporting information[9]).

A food processor is required and a laboratory homogenizer may be needed. For a ~6 - liter stainless steel food processor bowl, the sample + added water should be limited to ~2 liters to allow proper blending action. Vary these amounts accordingly for different bowl sizes.

Use a mass correction factor (MCF; *see §3.4.6 in Section 3.4*) to correct the analytical portion mass. Determine the masses of the materials after heat treatment cycle to account for any loss of liquid through vaporization.

Water + concentrated nitric acid (for Type I products)
Add ~1 kg analytical sample to the food processor bowl with approximately the same mass of water (ASTM Type I[5]). Note that chocolate-based products, which have high fat content, should

be blended first without water. The candy-water mixture can be left over night to soften or partially dissolve. If the sample will not disperse by blending, heating may be required (*e.g.*, on a hot plate). Blend the sample in the food processor until a visually homogenous composite is obtained. This mixture needs to have paste-like consistency to disperse particles and to permanently suspend all ingredients. This paste-like composite must be stable (*i.e.*, no phase separation) until an analytical portion is removed for acid treatment. If blending with water fails to form a paste-like consistency for Type I product, then treat the product as you would for a Type II product.

Weigh ~40 g of product into a nitric-acid-washed beaker. Glass beakers, polypropylene (translucent), or polymethylpentene (transparent) beakers can be used (heat stable to ~150° C). To prevent deformation, a water bath should be used to heat plastic beakers. Add concentrated nitric acid (Metals grade) (20 mL initially) and heat to approximately 100° C until the product has been largely solubilized. Be careful not to heat the mixture too rapidly for this may cause excessive foaming and loss of mixture. The beakers should be covered with watch glasses to minimize contamination. More nitric acid may be added to achieve a more complete dissolution.

10% Nitric Acid (for Types II, IIA, and III products)
Add ~1 kg of the analytical sample to a beaker. For Type IIA candies, also add enough powder from packets to correspond with the candy used to make the composite. Add an equal mass of 10% nitric acid. Heat the mixture (to approximately 100° C) until the sample mass is largely dissolved. This step may need to be left over night to maximize dissolution.

The remaining particulate materials should be subjected to further homogenization with a laboratory homogenizer such as a laboratory homogenizer. This final mixture should be highly acidic and should be largely a solution with respect to the analyte. Many candy ingredients and some natural products may not completely dissolve after the solubilization procedure. Silicon dioxide, chili flakes, and titanium dioxide are common additives that will not dissolve in this type of procedure. However, lead metal and other metallic ingredients should be completely dissolved. This liquid should now be in a satisfactory state for removing the analytical portion. If particulates persist in the product, then stir vigorously while removing the analytical portions.

2. Melting by stirring while heating on hotplate (for all candy Types)

No specific notes are given. Analyst discretion according to the sample characteristics with a trial-and-error approach is needed to choose apparatus, settings, timing, etc.

3. Microwave-assisted melting and cryogenic blending using laboratory homogenizer (for all candy Types)

FDA obtained excellent results for 0.3-0.5 g analytical portions of homogenized vitamins in candy form. The following procedure has been published[10]:

> *For microwave assisted melting, a 250 mL polypropylene cup with the candy was placed in the microwave oven, power was set to 300 W and applied for ~25 sec. If the material had not melted after 25 sec, then power was re-applied repeatedly for*

10 sec intervals until melted. The analytical portion was removed and stirred with a Teflon spatula until its appearance was uniform.

Although this was a quick and easy procedure for compositing candy-like samples, the heating and cooling cycle sometimes changed the physical properties such that the matrix became very hard and rubbery. This made removing the analytical portion extremely difficult even when a metal spatula was used.

For cryogenic homogenization, an analytical mill was used (A11 Basic with stainless steel beater, IKA Works, Inc, Wilmington, NC). The impact beater in this mill spins up to 28000 revolutions/min that equates to a circumferential speed of 53 m/sec.

After vitamin candies (at least 20) were added to the mill cup, liquid nitrogen was slowly added until the candy was covered. Immediately after the first portion evaporated, more liquid nitrogen was added to ensure the candy was completely frozen. As soon as the second portion of liquid nitrogen evaporated, the cup was attached to the mill and the candy was ground for 2-3 sec. This short grinding time was sufficient and prevented sample heating. The analytical portion was quickly transferred to to a container for digestion.

Although reduced to a fine powder immediately after grinding, candy-like samples solidified into a soft solid mass after a few hours. However, the analytical portions were easily removed with a plastic spatula.

4. Cryogenic blending using freezer/mill (for all candy Types)

FDA obtained excellent results homogenizing lollipops/suckers coated with chili powder. The following procedure has been published[11]:

Several lollipops were placed in a polyethylene bag and cooled in a conventional freezer (-20 °C) to make them brittle. While still in the bag, they were tapped using a hammer to break them into pieces and separate the candy from the paper "sticks". The bag was then shook to mix the pieces then several were removed to produce the composite.

For cryogenic homogenization, the sample vial assembly was filled (~40 to 90 g candy) and assembled. It was then mounted in the mill and mechanically lowered into the mill's internal liquid nitrogen chamber (pre-filled with liquid nitrogen). The assembly remained immersed in liquid nitrogen throughout the entire milling process.

The vial assembly was pre-cooled in liquid nitrogen for 20 minutes then homogenized using three cycles of 1-min grinding time at an oscillation rate of 10 per second with 2-min cooling time between cycles. After homogenization, the sample vial assembly was removed from the homogenizer and put in a conventional

freezer and warmed to -20 °C before opening and sample transfer for analysis or storage.

The homogenized candy was a fine white powder as long as it remained frozen. When warmed to room temperature, the powder particles immediately coalesced and were very hygroscopic, forming a sticky material that was not easy to work with. To overcome this problem, the material was kept in a cryogenic environment.

2.2.2.3 PILLS, CAPSULES, SUPPLEMENTS, ETC.

To ensure an accurate representation of the sample, an analytical portion is taken from a composite made from at least 20 units. This procedure is recommended by the US Pharmacopeia and commonly used by others. [12,13]

As reported[10], FDA obtained excellent results for 0.3-0.5 g analytical portions of homogenized pills/vitamins/supplements.

The analytical mill used in this study was an analytical mill A11 Basic with stainless steel beater, IKA Works, Inc, Wilmington, NC. The impact beater in this mill spins up to 28000 revolutions/min which equates to a circumferential speed of 53 m/sec.

Tablets and hard shelled capsules were reduced to a fine powder in 2-3 sec.

Gel caps are usually filled with a paste or oil and required cryogenic grinding. After adding them to the mill cup, liquid nitrogen was slowly added until they were covered. Immediately after it evaporated, more liquid nitrogen was added to repeat this step. As soon as the second portion of liquid nitrogen evaporated, the cup was attached to the mill and samples were ground for 2-3 sec. This short grinding time was sufficient and prevented sample heating.

The (gel cap) composite was quickly transferred to to a container for, e.g., digestion. Using this procedure, even fish oil capsules became brittle and were quickly reduced to a fine powder. Note that thawed samples tended to have a thick pasty consistency with finely ground pieces of the gel capsule dispersed throughout the oil.

REFERENCES

1. Wikipedia contributors (2014) Blender (device), *Wikipedia, The Free Encyclopedia* [accessed June 11, 2014]. Available internet.
2. Wikipedia contributors (2014) Food processor, *Wikipedia, The Free Encyclopedia* [accessed June 11, 2014]. Available internet.
3. GlobalSpec (2014) Homogenizers, Laboratory, *GlobalSpec, Inc* [accessed June 11, 2014]. Available internet.

4. Zeisler, R., Langland, J.K., and Harrison, S.H. (1983) Cryogenic homogenization of biological tissues, *Anal. Chem.* **55**, 2431-2434.

5. ASTM D1193-06 (2011) Standard Specification for Reagent Water, ASTM Book of Standards Vol. 11.01. [accessed June 12, 2014]. Available internet.

6. Wikipedia contributors (2014) Confectionery, *Wikipedia, The Free Encyclopedia* [accessed June 11, 2014]. Available internet.

7. Jones, J. W. and Boyer, K. W. (1979) Sample Homogenization Procedure for Determination of Lead in Canned Foods, *J. Assoc. Off. Anal. Chem.* **62**, 122 – 128.

8. Suddendorf, R. F., Wright, S. K., and Boyer, K. W. (1981) Sampling Procedure and Determination of Lead in Canned Foods, *J. Assoc. Off. Anal. Chem.* **64**, 657 – 660.

9. Jacobs, R., Castro, C., Peiffer, G., Shippey, K., Wong, J., Yee, S., and Palmer, P. (2005) Determination of Lead in Mexican Candy and Flavored Salt Products, *FDA Laboratory Information Bulletin #4346* [accessed June 11, 2014]. PDF available FDA internet.

10. Mindak W.R., Cheng, J., Canas B.J., and Bolger P.M. (2008) Lead in women's and children's vitamins, *J. Agric. Food Chem.* **56**, 6892-6896.

11. Cunningham, W.C. (2008) Study of cryogenic procedures for preparation of food for element analysis, *J. Food Comp. Anal.* **21,** 35-44.

12. United States Pharmacopeia (USP) (2003) USP-26-NF21 Supplement 2, 2003. The Official Compendia of Standards, United States Pharmacopeia Convention, Rockville, MD.

13. Roseland, J. M., Holden, J. M., Andrews, K. W., Zhao, C., Schweitzer, A., Harnly, J., Wolf, W. R., Perry, C. R., Dwyer, J. T., Picciano, M. F., Betz, J. M., Saldanha, L. G., Yetley, E. A., Fisher, K. D., and Sharpless, K. E. (2008) Dietary supplement ingredient database (DSID): Preliminary USDA studies on the composition of adult multivitamin/mineral supplements. *J. Food Compos. Anal.* **21**, S69-S77.