Protocol

Conversion of Foods to Powders for Metabolomics

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Materials and Methods

Grinder (Kitchen Aid,170W, Model No. BCG111OBO), Freeze dryer/lyophilizer (Labconco, Model : Free Zone Plus 6), knife sharpener (Chef's choice diamond hone 436-3), Detergent (Dri-Clean® from Decon Labs: 0.5 Oz per gallon in water), Gloves (Versaflex VF500M, which were worn during all steps), paper towels (Torks CB530 C fold), air tight plastic jars (Thermo Scientific™ Samco™ Wide-Mouth Bio-Tite™ 90mL [3 oz] 53mm Specimen Containers, catalog No.13-711-51), weighing paper (Fischer scientific catalog No. 09-898-12C), Reynolds heavy duty aluminum foil, metal sieve (Rotex Cat. No. 30SS8H, 8" diameter, stainless steel).

Pictures

Pictures were taken of each food item and package label.

General procedure for cleaning of glassware and metal

All glassware was washed with tap water, scrubbed using Scotch-Brite® Heavy Duty Scrub Sponge (soaked in detergent), and then rinsed with glove-rubbing under tap water. Each cleaning step was 20s. Aluminum trays were cleaned in the same way.

Before each use, the knife (Fig. 14) was sharpened, wiped with a paper towel, washed with water, and wiped again. Immediately after use, detergent and water washing was done followed by drying with a paper towel.

Cleaning of freeze dryer

The freezer dryer was shut down, and the power cord was removed from the wall. The ice collection chamber was allowed to reach room temperature. The collected water was drained via the drain pipe. The residue was wiped off using paper towels. The metallic and glass surfaces were washed with tap water, scrubbed using Scotch-Brite® Heavy Duty Scrub Sponge (soaked in detergent) and then rinsed thoroughly with tap water. With strong aromatic samples like garlic, onion and basil, additional wiping was done three times using paper towels soaked in methanol followed by two times with isopropanol to get rid of the odor.

Sample washing, cutting and freezing

Samples were washed for 30s per item or per batch under cold tap water. Water was removed from larger items (e.g. apples) by placing them on aluminum foil to drip, and then drying with a paper towel. For smaller items like strawberries, they were placed in a metal sieve which was tapped to remove most of the water. Cutting of larger foods was done by knife on a glass cutting board into small pieces (1 cm^3), that were averaged by mixing (see below) prior to storage in a -80C freezer for 12h and then grinding to a powder. Chick peas, black beans, soy beans were processed without cutting. Garlic was minced.

Sample averaging (mixing)

Each food powder was formed from 6 food items or batches unless a food already came averaged, e.g. a bag of chick peas. In the case of 6 food items or batches, averaging (mixing) was done when the items were cut up into small pieces (1 cm^3). All of the pieces were placed in an aluminum foil pan and this pan was doubly wrapped with aluminum foil (Figs 5-7). It was then shaken rigorously and randomly (all directions) for 30s.

Sample Lyophilization

Cut food that had been kept in the -80C freezer for 12h was immediately placed in a 2L freeze drier flask and dried in the dark to a final vacuum of 5×10^{-3} mbar, and until the flask reached room temperature to the touch.

Grinding food to a powder

The food pieces from the lyophilizer were immediately transferred to the grinder and pulverized for 30 sec x 3 = 1.5 min with a break of 10s with manual tapping between each grinding step to bring down the powdery food from the sides. Before the powder was removed from the grinder, the grinder was allowed to stand for 10 minutes to enable complete settling of the powder so it did not disperse into the room upon opening.

Overview of procedure with pictures

Fig. 1 Overview of general steps involved in making freeze dried sample (model example: chick peas)

(i)Procurement of chick peas from store; ii) wash and transfer to an aluminum tray; (iii & iv) wrap tray in aluminium foil; (v) shake to mix (average) the contents well; (vi) transfer to -80C freezer; (vii) transfer to freeze drying flask and freeze dry; (viii) freeze drying done; (ix & x) transfer to grinder; (xi) grind to form powder; (xii) powder taken on weighing paper; (xiii) powder transfer to plastic jar; (xiv) enclose jar in a secondary container (freezer grade Ziplock bag); and (xv) store in a -80C freezer.

1) Apples:

Six apples were from Whole Foods Market. A picture of the package label and the produce were taken (Fig. 2 & 3).

These were washed under running cold tap water with mild glove rubbing for 30s each. After washing the apples were placed on aluminum foil and then dried further with a paper towel. Each apple after this drying was sliced from the outside (including skin) up to the core. For each apple individually, the core, stem and the bottom were discarded and the edible part was chopped into small pieces and transferred to a separate aluminum foil (Fig. 4). The foil was wrapped and transferred to a -80C freezer, and the same process was done for the next four apples (to minimize browning). After the 6th apple, all fractions (last one plus 5 apples in foil from the freezer) were combined in an aluminum tray for

wrapping and shaking followed by storage at -80C for 12h (Fig.7). The apple pieces (overall about 200 g) were transferred to a 2L freeze dryer flask as shown in Fig. 9 and freeze drying was done. For this apple sample, freeze drying time was 50 h. The pieces were ground followed by transfer to weighing paper and then a plastic jar, which was labelled including date and kept at-80C.

Fig. 2

Fig.3

Fig.4

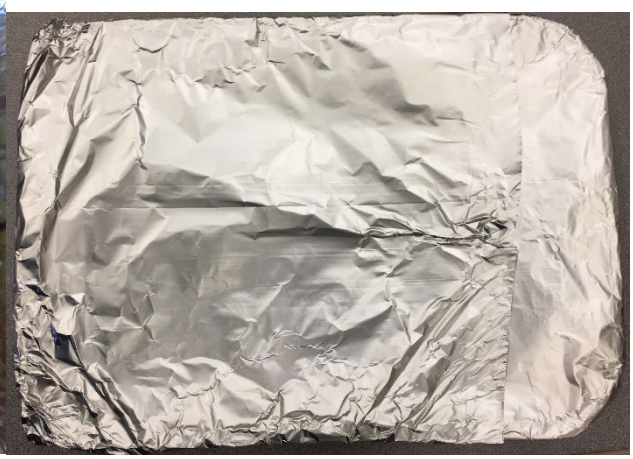

Fig.5

Fig.6

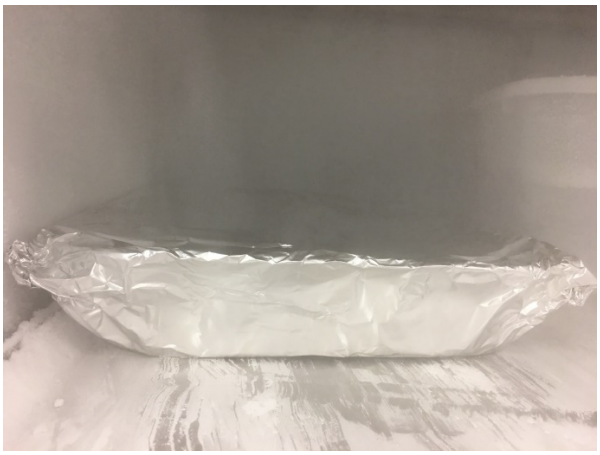

Fig.7

Fig.8

Fig.9

2) Bananas:

Six bananas were from Stop & Shop. The bananas were peeled and chopped and immediately cut into small pieces and transferred to an aluminum tray followed by wrapping and shaking then keeping in a -80C freezer for 12h. The banana pieces (about 200g) were lyophilized. For this sample, freeze drying time was 96h. The pieces were ground and the resulting powder was transferred to weighing paper and then a plastic jar. It was labelled and dated and kept at -80C.

3) Tomatoes:

Six tomatoes were from Whole Foods Market.

The washed and dried tomatoes were chopped into small pieces and transferred to an aluminum tray for averaging followed by keeping in a -80C freezer for 12h. These tomato pieces (about 200g) were lyophilized. For this sample, freeze drying time was 120h. The pieces were ground and the powder was transferred a weighing paper and then a plastic jar. It was labelled and dated and kept at -80C.

4) Lettuce:

Six heads of lettuce were from Whole Foods Market. Each item was washed and drained with tapping on a metal sieve followed by chopping and transfer to a separate aluminum tray. Six trays were filled, and from each of these a small fraction (~30g) was taken into a new aluminum tray followed by wrapping and shaking and then keeping in -80C freezer for 12h. The lettuce pieces (about 80g) were freeze-dried. For this sample, freeze drying time was 120h. The pieces were ground and the resulting powder was transferred to weighing paper and then a plastic jar. It was labelled and dated and kept at -80C.

5) Strawberries:

Three boxes of Strawberries were purchased from Star Market. The berries were washed and drained with tapping on a metal sieve. They were chopped into small pieces and transferred to an aluminum tray followed by wrapping, shaking and keeping in a -80C freezer for 12h. After this, the strawberry pieces (about 200g) were freeze-dried. For this sample, freeze drying time was 120h. The pieces were ground (Fig. 10, 11, 12) and the resulting powder was transferred to a weighing paper as shown in Fig. 13 and then to a plastic jar. It was labelled and dated and kept in -80C freezer.

Fig.10

Fig.11

Fig.12

Fig. 13

6) Carrots:

Six carrots were from Whole Foods Market. These were washed, transferred to aluminum foil and dried with paper towels. They were chopped into small pieces and transferred to an aluminum tray. The remaining procedure was the same as for strawberries. For this sample, freeze drying time was 120h.

7) Peaches:

Six peaches were from Stop & Shop. The procedure was the same as for apples. For this sample, the freeze drying time was 120h.

8) Onions:

Six onions were from Whole Foods Market. These were cut in half vertically and the outer two layers were removed. The remaining, edible part was chopped into small pieces and transferred to an aluminum tray. The remaining procedure was the same as for strawberries. For this sample, the freeze drying time was 120h.

9) Spinach:

Six bunches of spinach were from Star Market. These were washed, placed on aluminum foil and dried with paper towels. The bottom one inch part of the each bunch was cut and discarded. The remaining (edible part) was chopped into small pieces and averaging was done in the same as lettuce. About 60 g were lyophilized. For this sample, freeze drying time was 120h. The pieces were ground followed by transferring to weighing paper and then a plastic jar. It was labelled and dated and kept at -80C.

10) Peppers:

Six red peppers were from Whole Foods Market. These were washed, placed on aluminum foil, dried with a paper towel, and cut vertically. The stem along with the seeds were discarded and the remaining edible part was chopped into small pieces and transferred to an aluminum tray. The remaining procedure was the same as for strawberries. For this sample the freeze drying time was 120h.

11) Corn:

Six corn cobs were from Star Market. These were husked and the kernels were exposed. The cobs were washed on a sieve and partly dried by tapping. The kernels (edible part) were removed from the cob using a knife and were kept in an aluminum tray (Fig. 14). Six trays were filled from these six corn cobs. About 30g was taken from each tray and transferred to a new tray. This tray was then wrapped and averaged by shaking followed by keeping in a -80C freezer for 12h. The remaining procedure was same as for strawberries. For this sample, freeze drying time was 96h.

Fig. 14

12) Garlic:

Six bulbs of garlic were from Whole Foods Market. The individual garlic cloves were separated. The outer layer (husk) was removed with the aid of a knife and discarded, and the remaining edible part was placed on aluminum foil. These were minced immediately and transferred to an aluminum tray. Averaging was done by wrapping and shaking followed by keeping in a -80C freezer for 12h. The remaining procedure was the same as for strawberries. For this sample, the freeze drying time was 120h.

13) Basil:

Six bunches of basil were purchased from Star Market.

These were washed and partly dried on a metal sieve. The bottom one inch part of each bunch was cut and discarded and the remaining, edible part was placed on aluminum foil. The remaining procedure was the same as for spinach. For this sample, freeze drying time was 96h.

14) Potatoes:

Six potatoes were from Whole Foods Market. These were washed, placed on aluminum foil, and dried with paper towels. The outer layer (skin) as well as any dark colored spots or parts were removed and discarded, and the remaining, edible part was cut, placed in an aluminum tray, averaged, and frozen. For this sample, freeze drying time was 72h. The remaining procedure same as for strawberries.

15) Chick Peas:

A bag of dry chick peas was from Star Market (Fig.1- i). About 200g of were soaked for 10min in a 1L capacity beaker containing cold water. These then washed on a sieve and partly dried by tapping the sieve. The remaining procedure was same as for strawberries except the chick peas were not chopped. For this sample, freeze drying time was 72h.

16) Common Bean (Black beans):

A bag of dry black beans was from Star Market. The remaining procedure was the same as for chick peas. For this sample, freeze drying time was 72h.

17) Olives:

A bottle of olives (soaked/marinated in brine/proprietary solution) was from Star Market (Fig. 16). The olives (~100g) were separated from the liquid part using a spoon and placed on a sieve (Fig.17) followed by washing and then partly dried by tapping the sieve. The solid core/seed was removed with a knife and discarded, and the edible part was chopped into small pieces and transferred to an aluminum tray (Fig. 18). This tray was wrapped and averaged by shaking, Lyophilization was done (Fig. 19). The remaining procedure was same as for strawberries. This gave a greenish powder (Fig. 20). For this sample, freeze drying time was 168h.

Fig. 16

Fig.17

Fig. 18

Fig.19 Fig.20

18) Soybeans:

A bag of dry soybeans was from Hong Kong supermarket. About 200g beans were soaked for 10min in a 1L beaker containing cold water. The remaining procedure was the same as for chick peas. For this sample, freeze drying time was 72h.

19) Sugar beets:

Six beets were from Star Market. These were washed followed by placing on aluminum foil and drying with a paper towel. The outer layer (skin), stem and the thin root/s were removed and discarded, and the remaining edible part was placed in an aluminum tray. The remaining procedure was the same as for potatoes. For this sample, freeze drying time was 96h.

20) Pears:

Six pears were from Stop & Shop. The procedure was the same as for apples. For this sample, the freeze drying time was 120h.