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Homogenization of food samples for gamma spectrometry using protease

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Abstract
We previously reported a food preparation method for gamma spectrometry during radiological emergencies aimed at developing best compromises between degree of homogenization, accuracy, speed, and minimizing laboratory equipment contamination. We now propose an additional method using the protease bromelain that can homogenize meats and composite meals containing both meat and starch in ~ 1 h without high heat or harsh chemicals. Additionally, we show evidence from studies with environmental samples suggesting the potential utility of these homogenization methods for use with atmospherically deposited radionuclides using naturally occurring ^{7}Be as a model.

Keywords Sample homogenization · Gamma spectrometry · Radiological emergency · Protease · Atmospheric deposition · Ultra-low background Ge detector

Introduction

In gamma spectrometry of nonhomogeneous food and environmental samples, sampling and homogenization together may contribute a larger proportion of the total uncertainty than sample preparation or determination of radionuclides [1, 2], and correct sampling is difficult and often out of the analyst's control [2]. Two aspects of homogenization, mixing and reduction in particle size, improve the accuracy of sample analysis [2]; in gamma spectrometry analysis, homogenization increases accuracy by promoting uniform sample density and distribution of radioactive particles. To achieve a best compromise between speed, accuracy and minimization of contaminated equipment, we previously developed methods for homogenization of food samples with chemicals and enzymes for use during a nuclear emergency [3]. Here, we describe an additional method for homogenization of protein-containing samples using the protease bromelain.

Bromelain, a mixture of enzymes purified from pineapple stem, is used commercially for applications such as tenderizing meat and breaking down gluten in flour [4]. Bromelain preparations contain at least nine distinct proteases as well as cellulases, glucosidases and other components [5, 6]. As a consequence, bromelain alone can homogenize composite meals such as cheeseburgers and breaded chicken patties that contain a large amount of starch. Composite meals containing starch were not well homogenized using the chemical tetramethylammonium hydroxide (TMAH) because starch thickened and formed a tough mass [3]. Due to its low optimum temperature and activity at a wide range of pH (~ 5 to 9) [5, 6], bromelain can effectively homogenize many types of food in 1 h or less without the use of high heat or harsh chemicals.

Samples of food spiked with various radionuclides in acidified solutions of water, such as those in our previous study [3], may not accurately reflect conditions during a radiological emergency. After the Chernobyl and Fukushima nuclear accidents, food became contaminated by atmospherically deposited radionuclides [7, 8]. We used a small number of field-collected leaf samples containing the atmospherically deposited radionuclide ^{7}Be [9, 10] to model homogenization of food samples contaminated by

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radioactive fallout. We counted these samples before and after homogenization using TMAH, which tends to stabilize ions in suspension [3], to investigate the effects of homogenization on detected activity.

In this work, we provide evidence that homogenization using bromelain may be an effective method for preparing samples for gamma spectrometry in a radiological emergency. In addition, we show that TMAH appears to effectively homogenize some environmental samples containing atmospherically deposited ^{7}Be.

Experimental

Food samples

Food used in these experiments was bought at local grocery stores, and included cheeseburgers, breaded nugget-shaped chicken patties, ground beef, chicken thigh, lettuce, and cabbage.

Field-collected environmental samples

Sample 1 (forsythia, unknown cultivar) [11] was collected on 8/5/2017 and counted for 1000 min on 8/10/2017 (0.159 kg; pre-homogenization) and 9/5/2017 (0.773 kg; post-homogenization). Sample 2 (celandine, *Chelidonium majus*) [12] was collected on 9/24/2017, arranged in alternating layers with store-bought green cabbage, and counted for 1000 min on 9/29/2017 (0.293 kg; pre-homogenization) and 10/5/2017 (0.720 kg; post-homogenization). Sample 3 (wild cucumber, *Echinocystis lobata*) [13] collected on 10/25/2017, was placed in a 0.5 L Marinelli beaker adjoining the recessed area, with a layer of store-bought iceberg lettuce on top, so that the celandine would be closest to the detector. Sample 3 was counted on 10/31/2017 (0.285 kg; pre-homogenization), and 12/22/2017 (0.754 kg; post-homogenization). Count time for Sample 3 was 4000 min in both cases. Outer leaves of store-bought lettuce and cabbage potentially contaminated with ^{7}Be were discarded, and only inner leaves were used for analysis, in order to study the effect of non-uniformity in the field-collected samples, only. All samples were collected in Clark's Mills, NY, USA.

Chemicals, enzymes and conditions

Bromelain sourced from pineapple stem (2500 GDU/g; Acros Organics) was supplied by Fisher Scientific (Suwanee, GA, USA). Bromelain was added dry to food samples and used at a final concentration of 10 g kg^{-1}. For samples spiked with ^{131}I, we added a Tris (tris(hydroxymethyl)aminomethane)-HCl buffer (0.2 M Tris–HCl pH 8.0 (Fisher Scientific); 0.5 M sodium thiosulfate, and 1 mM sodium iodide as carrier) in place of water to ~ 40% of final volume to prevent volatilization [3]. Homogenization of samples in pH 8.0 buffer may take longer (up to 30 min) than homogenization of unbuffered samples.

Homogenization of spiked food matrices using bromelain

For this study, samples were handled as previously reported [3], with the following modifications. Samples (~ 250 g) cut into cubes ≤ 1 in. were pre-warmed at 37 °C in water or buffer added to ~ 40% of the final volume, leaving ~ 10% of final volume for additional liquid to rinse containers for complete transfer. After adding dry enzyme and stirring in with a spatula, heat was turned up until the mixture reached 42 °C (see Fig. 1). Although the optimum temperature for bromelain activity is ~ 60 °C [5, 6], bromelain loses activity quickly at temperatures above 55 °C [6]. Samples were heated with stirring for 20–60 min at 42 °C. For some fatty or tough meat samples, stirring at higher heat (60–70 °C) for 20–30 min (see Fig. 2) as a final step is required to achieve thorough homogenization. Tender and thin-sliced meats were mostly homogenized after ~ 20 min at 42 °C, while solid, tough or fatty meats requiring an additional heating step took up to 1.5 h for thorough homogenization.

Homogenization of environmental samples using tetramethylammonium hydroxide

Homogenization of food samples using tetramethylammonium hydroxide (TMAH) was described previously [3]. However, our method was modified for use with field-collected vegetation, because some plant parts such as woody branches and stems were resistant to TMAH homogenization. We cut leaves from stems using scissors (see Fig. 3). This step also maximizes the ^{7}Be-contaminated surface area of the samples.

Radionuclides

For spiking food samples, we bought reference standard solutions containing ^{60}Co, ^{131}I, ^{137}Cs, and ^{241}Am radionuclides from Eckert & Ziegler Analytics, Atlanta, GA, USA. Standard solutions were traceable to the National Institute of Standards and Technology (Gaithersburg, MD, USA) and were diluted to desired activity concentrations before use.

Fig. 1 Two replicates of breaded nugget-shaped chicken breast patties (**a**) were prepared by chopping the patties and adding them to ~ 250 g in tared glass beakers. **b** Water was added to ~ 490 ml volume, and stirred in with a spatula, then the beaker was placed on a heating block and pre-warmed to 37 °C. Bromelain (**c**) was added to a final concentration of 0.01 g/ml. Spikes were added and the mixture was heated with stirring at 42 °C for ~ 30 min, then allowed to cool and transferred to 0.5 L Marinelli beakers

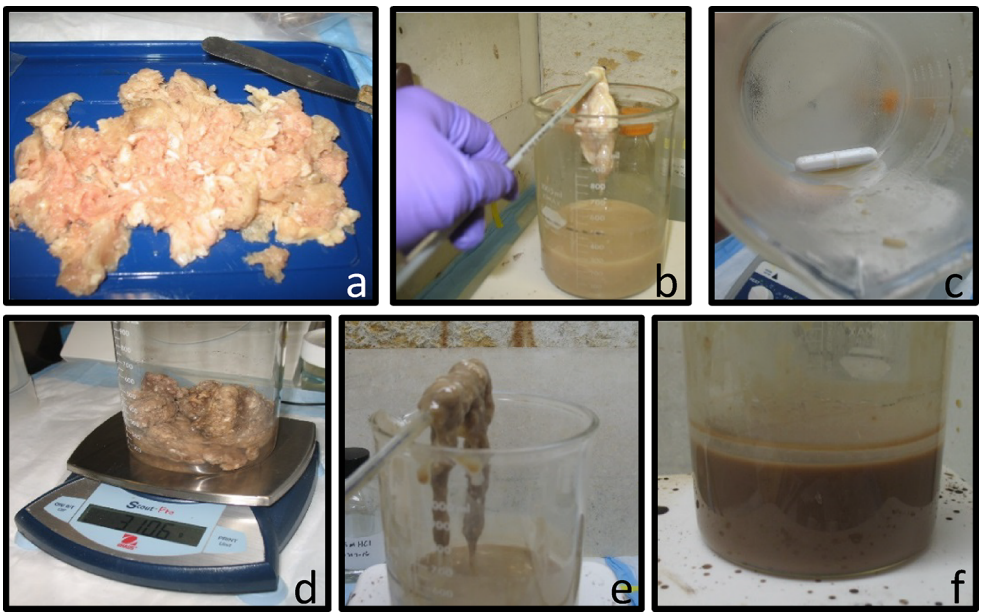

Fig. 2 Homogenization of high-fat samples with increased heat. Chicken thigh cut into ~ 1 inch pieces (**a**) formed a fatty mass (**b**) during homogenization with bromelain at 42 °C. Heating to 60 °C melted fat and allowed thorough homogenization (**c**) within ~ 1 h. Likewise, ground beef buffered to pH 7.5–8.0 (**d**) formed a fatty mass that prevented stirring (**e**). Heating to 70 °C allowed homogenization to proceed (**f**)

Gamma spectrometry analysis

Gamma spectrometry analysis of spiked food samples was performed as previously described [3]. For field-collected leaf samples containing ^{7}Be, we used an ultra-low background germanium (Ge) gamma spectrometer with 140% relative efficiency (laboratory code GE12; from Mirion Technologies (Canberra), Meriden, CT, USA) with a full

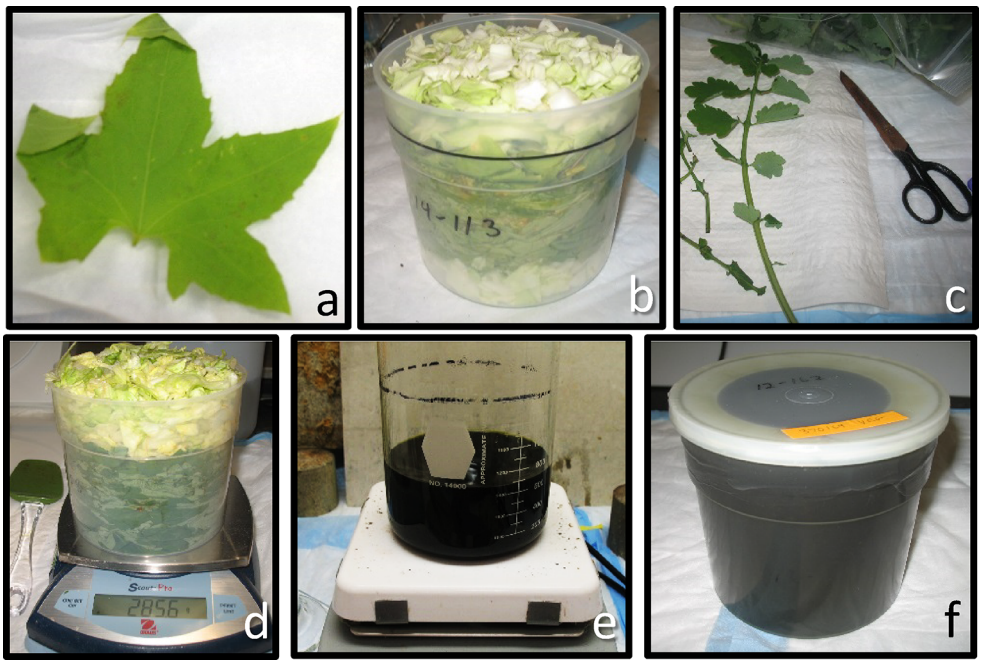

Fig. 3 Preparation of Samples 2 and 3 for gamma spectral analysis. Wild cucumber (**a**) and celandine (**c**) leaves were cut from stems using scissors and placed in 0.5 L Marinelli beakers. Chopped cabbage was added to Sample 2 in random layers (**b**). For Sample 3, celandine was added to the top of the recessed area of the Marinelli where it would be closest to the detector and covered with a layer of chopped lettuce (**d**). The samples were counted and transferred to a glass beaker and TMAH was added to 25% final volume (**e**). The samples were heated to 90 °C with stirring for 1 h and transferred to another Marinelli beaker for counting (**f**)

muon shield. Wadsworth Center's Nuclear Chemistry Laboratory is in the basement of the Corning Tower and has a 33-meter water equivalent overburden. Its counting room is fitted with 6-in.-thick pre-World War II steel walls, helping to reduce background radiation to very low levels necessary for detection of ^{7}Be. Additional Ge gamma spectrometers and efficiencies used to analyze spiked samples were described previously [3]. Gamma acquisition was performed using Genie 2000 software (Mirion Technologies). Energy lines used for gamma spectral analysis were as follows: 477.6 keV (^{7}Be); 661.7 keV (^{137}Cs); 1332.5 keV (^{60}Co); 364.5 keV (^{131}I); 59.5 keV (^{241}Am). We used 0.5-L Marinelli beakers (Ga-Ma & Associates, Ocala, FL, USA), sealed with electrical tape or Container Seal (Electron Microscopy Sciences, Hatfield, PA, USA), for all samples. The Monte Carlo simulation code GESPECOR version 4.2 [3] (CID Media GmbH, Hasselroth, Germany) was used to calculate density and coincidence summing corrections.

Results and discussion

Homogenization of spiked food matrices using bromelain

To evaluate the efficacy of homogenization using bromelain for recovery and detection of gamma activity, we prepared replicate samples of identical matrices spiked with various radionuclides and homogenized using bromelain. We chose matrices representing composite meals (breaded nugget-shaped chicken breast patties and cheeseburgers) and meat, only (sliced ham), and used a range of activity levels from ~ 100 to ~ 600 Bq, with three different levels for ^{131}I (~ 100, 175, and 600 Bq). We analyzed three replicates of each matrix spiked with ^{60}Co, ^{137}Cs and ^{241}Am, and two replicates of each matrix spiked with ^{131}I, for a total of 15 samples. Each sample was counted on two detectors. Table 1 shows calculated biases and z-scores for all 15 samples.

Activity from spiked samples using bromelain gave slightly lower biases and z-scores (average bias of 0.83 and an average z-score of 0.03, see Table 1) than homogenization using TMAH and/or enzymes in our previous study [3]. This difference may be due to use of ^{60}Co in place of ^{134}Cs, whose larger bias may be due to inadequate correction for coincidence summing. Again, the low biases and z-scores of the samples suggest that little or no

Table 1 Biases and z-scores of activity from food samples spiked with ^{60}Co, ^{137}Cs, ^{131}I and ^{241}Am and homogenized using bromelain

Sample Radionuclide (*N*)	Total activity (Unc.) (Bq)	Known activity (Unc.) (Bq)	Bias (%)	z-score
Sliced ham				
^{60}Co (3)	96.9 (2.4)	98.7 (3.6)	− 1.81	− 0.19
^{137}Cs (3)	129.9 (2.8)	131.1 (3.8)	− 0.98	− 0.11
^{131}I (2)	589.9 (16.9)	586.1 (3.4)	0.65	− 0.01
^{241}Am (3)	561.8 (25.5)	567.0 (3.4)	− 0.91	− 0.00
Cheeseburgers				
^{60}Co (3)	98.0 (2.4)	98.7 (3.6)	− 0.71	− 0.07
^{137}Cs (3)	130.0 (2.8)	131.1 (3.8)	− 0.90	− 0.10
^{131}I (2)	108.7 (4.1)	107.5 (3.7)	1.06	− 0.01
^{241}Am (3)	562.7 (24.9)	567.0 (3.4)	− 0.76	0.06
Chicken patties				
^{60}Co (3)	97.5 (2.4)	98.7 (3.6)	− 1.18	− 0.12
^{137}Cs (3)	128.5 (2.7)	131.1 (3.8)	− 2.12	− 0.24
^{131}I (2)	167.3 (5.5)	166.0 (3.7)	0.78	0.02
^{241}Am (3)	578.9 (26.3)	567.0 (3.4)	2.10	0.04
Average			0.83	0.03

Average values represent counts on two detectors for each sample of three samples per matrix for ^{60}Co, ^{137}Cs and ^{241}Am spikes, and two samples per matrix for ^{131}I spike. Bias is calculated as % deviation from known; z-score is calculated as the difference between the measured and known activity divided by the square root of the sum of variances. *N*, number of samples; Unc., uncertainty at 95% confidence level

radioiodine was lost to volatilization, and that the homogenization was adequate for the radionuclides used.

Homogenization of ^{7}Be in environmental samples using TMAH

To investigate effects of homogenization on detection of ^{7}Be activity, we used an ultra-low background Ge spectrometer (see Experimental section) to count samples before and after homogenization with TMAH. Judging by the samples' appearance and texture, use of TMAH resulted in thorough homogenization of the collected leaf samples (see Fig. 3). Sample 1 consisted of forsythia leaves, only. For Samples 2 and 3, collected wild cucumber and celandine leaves were mixed with cabbage and lettuce, respectively. For Sample 3, we intentionally introduced high detection bias by placing the celandine leaves containing ^{7}Be in the Marinelli adjoining the recessed area where they would be closest to the detector, and the lettuce at the top (Fig. 3d). We homogenized the sample with TMAH (Fig. 3e and f) and counted again. Detected activities and uncertainties for ^{7}Be are given in Table 2.

As shown in Table 2, ^{7}Be samples had an average percent change of 7.16 ± 5.49 Bq/kg (95% CL) after homogenization. For the randomly arranged sample consisting of forsythia leaves, only (Sample 1), detected activity increased after homogenization, but not significantly. The forsythia leaves were not mixed with other material, and the percent difference after homogenization was $< 2\%$, indicating that distribution of radioactive particles was relatively homogeneous prior to addition of TMAH. The two samples mixed with presumably uncontaminated material (inner leaves only of store-bought cabbage and lettuce) represent nonhomogeneous mixtures in terms of distribution of radioactive particles, and possibly in terms of density as well. Detected activity increased significantly for Sample 2 after homogenization, and decreased significantly after homogenization for the high bias arrangement of Sample 3. Sample 2 had the largest change in activity after homogenization, possibly

Table 2 ^{7}Be activity of three field-collected leaf samples before and after homogenization with TMAH

Sample no.	^{7}Be activity (uncertainty) (Bq/kg)		% change
	Pre-TMAH	Post-TMAH	
1	1.29 (0.17)	1.31 (0.27)	− 1.53
2	2.69 (0.29)	3.15 (0.33)	− 14.6
3	1.38 (0.12) bias high	1.31 (0.25)	5.34
		Average	7.16
		Sd	5.49

Activities and uncertainties are given at 95% confidence. Percent difference in activity before and after TMAH was calculated as activity (pre-homogenization − activity post-homogenization)/(activity post-homogenization) × 100

because the cabbage leaves were thicker and firmer and made the sample's density less uniform than that of the other samples. Overall, homogenization significantly changed the detected activity of non-homogeneous samples, with the percent difference after homogenization from − 14.6 to 5.34%.

The difference in detected activity we observed in environmental samples before and after homogenization suggests that sizeable error might be introduced due to nonhomogeneity of food samples contaminated by radioactive fallout. Depending on the circumstances, the accuracy we achieved with nonhomogeneous samples prior to homogenization might be acceptable in an emergency. However, the contrast between our results with spiked food samples and those with nonhomogeneous environmental samples is striking. Thorough homogenization becomes more important to accuracy at low detection limits [2]. In a nuclear emergency, if consumption of contaminated food became necessary, accurate determination of radionuclides at very low levels would be important to protect public health. The US EPA sets the maximum contaminant level for combined beta and photon emitters in drinking water as the activity yielding a dose of 4 mrem/y to the body or any critical organ. For the fallout radionuclides ^{131}I and ^{137}Cs, these concentrations correspond to 0.11 and 7.4 Bq/L, respectively [14].

Conclusions

As previously discussed, refractory hot particles would not be dissolved by chemical and/or enzymatic homogenization [3]. However, our results with environmental samples support the effectiveness of homogenization with TMAH for preparation of gamma spectrometry samples contaminated with radionuclides deposited as aerosols. Taken together, the results presented here suggest that the suite of methods we have developed for chemical and/or enzymatic homogenization of food samples may be useful for gamma spectrometry analysis during a nuclear emergency.

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References

1. Lichon M (2004) In: Nollet LML (ed) Handbook of food analysis, methods and instruments in applied food analysis, vol 3, 2nd edn. Marcel Dekker, Basel
2. Lacorte S, Bono-Blay F, Cortina-Puig M (2012) In: Pawliszyn J (ed) Comprehensive sampling and sample preparation: analytical techniques for scientists, vol 1. Elsevier, Amsterdam, Boston
3. Nishikawa K, Bari A, Khan AJ, Li X, Menia T, Semkow TM, Lin Z, Healey S (2017) Homogenization of food samples for gamma spectrometry using tetramethylammonium hydroxide and enzymatic digestion. J Radioanal Nucl Chem 314:859–870
4. Arshad ZI, Amid A, Yusof F, Jaswir I, Ahmad K, Loke SP (2014) Bromelain: an overview of industrial application and purification strategies. Appl Microbiol Biotechnol 98:7283–7297
5. Matagne A, Bolle L, El Mahyaoui R, Baeyens-Volant D, Azarkan M (2017) The proteolytic system of pineapple stems revisited: purification and characterization of multiple catalytically active forms. Phytochem 138:29–51
6. Schomburg D, Stephan D (1998) In: Enzyme Handbook 16, First Supplement Part 2 Class 3: Hydrolases. Springer, Berlin, Heidelberg 51–58
7. Kaltofen M, Gundersen A (2017) Radioactively-hot particles detected in dusts and soils from Northern Japan by combination of gamma spectrometry, autoradiography, and SEM/EDS analysis and implications in radiation risk assessment. Sci Total Environ 607–608:1065–1072
8. Smith JT, Tagami K, Uchida S (2017) Time trends in radiocaesium in the Japanese diet following nuclear weapons testing and Chernobyl: implications for long term contamination post-Fukushima. Sci Total Environ 601–602:1466–1475
9. Landis JD, Renshaw CE, Kaste JM (2014) Quantitative retention of atmospherically deposited elements by native vegetation is traced by the fallout radionuclides ^{7}Be and ^{210}Pb. Environ Sci Technol 48:12022–12030
10. Kitto ME, Fielman EM, Hartt GM, Gillen EA, Semkow TM, Parekh PP, Bari A (2005) Long-term monitoring of radioactivity in surface air and deposition in New York State. Health Phys 90:31–37
11. Forsythia, Natural Resources Conservation Service Plants Database, United States Department of Agriculture. https://plants.usda.gov/core/profile?symbol=FORSY. Accessed 2 Feb 2018
12. Chelidonium majus *L. Celandine*, Natural Resources Conservation Service Plants Database, United States Department of Agriculture. https://plants.usda.gov/core/profile?symbol=chma2. Accessed 2 Feb 2018
13. *Echinocystis lobata* (Wild Cucumber), Minnesota wildflowers, a field guide to the flora of Minnesota, Minnesota Environment and Natural Resources Trust Fund. https://www.minnesotawildflowers.info. Accessed 2 Feb 2018
14. Drinking Water Requirements for States and Public Water Systems. Radionuclides Small Entity Compliance Guide, United States Environmental Protection Agency. https://www.epa.gov/sites/production/files/2015-09/documents/guide_radionuclides_table-betaphotonemitters.pdf. Accessed 27 Feb 2018