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Multipurpose biological reference materials

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Vielseitig verwendbare biologische Referenzmaterialien

Summary. Reference materials to meet multipurpose needs for analysis of both inorganic and organic constituents in biological investigations are not readily available. A human total diet material is being investigated as a reference material for a wide variety of constituents of interest to human nutrition and health. This material shows a stable assay value for the natural levels of a number of vitamins following freeze-drying or radiation sterilization. This is an important feature in producing materials for long term stability as a Reference Material for natural levels of these constituents. An exception is an increase of 34% in assay value of folic acid upon freeze-drying and an 85% increase upon freeze-drying followed by radiation sterilization.

Introduction

Adequate quality assurance is required for all nutritional, biochemical and environmental investigations involving analyses of biological materials. Several considerations are involved. A multidisciplinary approach is needed for planning the investigations and appropriate quality control (QC) standards are required for checking biochemical analytical methodology for both inorganic and organic constituents. The introduction of the available reference materials Non-fat Milk Powder (NBS SRM 1549), Bovine Serum (NBS RM 8419) and Mixed Diet (NBS RM 8431) have helped mainly in meeting the needs of inorganic analysis. Even here, there is a need for developing additional reference materials, especially to the elemental coverage for neglected elements such as B, F, Li, Si, Sn and to some extent Ni. For the organic nutrients, not many alternatives are presently available for QC purposes. One exception is the recently released Coconut Oil standard (NBS SRM 1536) certified for fat soluble vitamins.

In this context, the attempt by Tanner et al. [1] to demonstrate the suitability of currently available biological reference materials (BRM) as potentially useful organic nutrient control materials is an important step. While the needs for developing materials for both organic and inorganic constituents has been recognized by the analytical and nutritional communities, until recently only a limited effort has been extended to meet these needs. Matrix suitability considerations present serious challenges in selecting materials to qualify as multipurpose standards that satisfy the diverse QC requirements of both organic and inorganic analysis.

Multipurpose BRM's, are difficult to develop, but can play a pivotal role in proper use of reference materials. Their impact may be envisaged in a 3-stage process: (1) Preparation of a few carefully chosen matrices representative of natural concentration levels of inorganic nutrient, toxicant and environmental-indicator elements on the inorganic side, and proximates, vitamins, pesticides and other constituents of interest on the organic side, certified for as many constituents as possible. Such a material would satisfy the QC requirements for measurements at typical concentration levels. (2) Identification and preparation of special materials that are naturally enriched in specific constituents, e.g., oyster for zinc, fish for methyl mercury, kidney for cadmium, egg powder for cholesterol and specific organic matrices for biphenyls. (3) Preparation of deliberately spiked materials to meet special needs, e.g., blood and urine with added arsenic and lead for occupational health problems or foods fortified with specific nutrients. These materials with elevated analyte could be used in QC trials to enable measurements by more analytical techniques with poor sensitivity, and where examination of simulated high levels of constituents is of interest.

As a potential solution to the need for multipurpose BRM's, suitability of a human total diet as a common reference matrix for both organic and inorganic constituents has been explored [2]. The stability of this material for minor and trace elements and certain vitamins has been investigated for a limited time period [3, 4]. The purpose of this communication is to report further investigations on the total diet material. These investigations mainly involved studying the effect of freeze-drying and radiation sterilization on the retention of vitamins in the total diet material. Additional analytical data were obtained on the variation of phytate levels with time.

Experimental

The collection and preparation of the total diet materials has been previously described [2]. Briefly representative portions of foods collected in the FDA Total Diet Study program, are composited to prepare a total daily diet material. Representative Total Diet materials are being collected and composited on a regular basis to provide a baseline monitoring series. Material from one of these collections was used for the freeze-drying studies. Four aliquots of a blended fresh Total Diet Composite were equally divided.

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Table 1. Assay reproducibility for determination of vitamins in total diet material

Vitamin	Coefficient of variation (%)	
	Fresh	Freeze-dried
Niacin	0.6	6.0[a]
Riboflavin	3.9	0.8
Thiamin	3.3	4.9
Vit B6	2.7	3.8
Vit B12	3.3	7.1
Biotin	11.6	4.0
Folic acid	5.7	18.7
Pantothenic acid	1.9	7.1

[a] Values for approximate vitamin content are given in reference [2]

Table 2. Effect of freeze-drying and radiation sterilization on vitamin content of toal diet material

Vitamin	Ratio to fresh	
	Freeze-dried	Freeze-dried and radiation sterilized
Niacin	0.93	0.97
Riboflavin	0.95	1.04
Thiamin	1.01	1.08
Vit B6	1.04	0.94
Vit B12	1.06	1.09
Biotin	1.07	1.05
Folic acid	1.36	1.85
Pantothenic acid	1.04	1.14

One half of each portion was stored at −50° C until analysis. The second half of each portion was frozen and freeze dried. The frozen samples were then thawed and both the fresh and freeze-dried samples were analysed simultaneously for a number of vitamins at the FDA lab in Washington. Water soluble vitamins were determined by turbidimetric microbiological assays. Vitamin B12 activity, niacin and riboflavin were determined by AOAC methods 43.175−43.282, 43.191−43.199, and 43.209−43.217, respectively [11]. The AOAC vitamin B6 assay was followed except that the ion-exchange separation of isomers, 43.233 was not used and modifications were made in the medium as described in more detail in another paper in this issue [5]. Folacin was extracted with pH 7.8 buffer solution containing 1% ascorbic acid [6] and analysed as described in the companion paper [5].

Pantothenic acid was released from coenzyme A by using simultaneous alkaline intestinal phosphatase and pigeon liver (peptidase) enzymolysis [7]. The released acid was assayed by AOAC method 43.200−43.208 [11]. The pantothenic acid standard solution was also subjected to the enzymolysis step to eliminate the need for blank correction.

Thiamine was assayed by the method of Defibaugh et al. [8] which used Viridescens (ATCC 12706) as the test organism and commercially prepared basal medium.

Biotin was assayed by the method of Wright and Skeggs [9] which used L. plantarum (ATCC 8014) as the test organism and commercially prepared basal medium.

Phytate was analysed on both the fresh and freeze-dried materials by the method of Ellis et al. [10].

In the course of obtaining the various collections of total diet material, a large amount of material was composited from several collections and is being prepared as a Total Diet Standard Reference Material. This SRM material in preparation was also freeze-dried and in addition has been radiation sterilized to improve long term storage from the standpoint of possible microbial degradation. This SRM material contains material from the same collection as the material used in this freeze-drying study. Therefore, a sample of the freeze-dried, radiation sterilized material was analysed simultaneously with the fresh and freeze-dried aliquots to determine if significant changes in vitamin content occured upon radiation sterilization.

Results and discussion

In general good agreement was seen in the results for replicate analysis. Coefficients of variation for the four samples each of the fresh and freeze-dried material shown in Table 1 indicate analytical variation in the range of less than approximately 7%, except for biotin (12%) in the fresh material and a significant increase to greater than 18% for folic acid in the freeze-dried material.

The effect of freeze-drying and radiation sterilization is shown in Table 2. Results for the mean value of the freeze-dried samples ($n = 4$) and the value for the freeze-dried, radiation sterilized sample ($n = 1$) are presented as a ratio to the mean value for the fresh samples ($n = 4$). These data indicate agreement within analytical variation for both freeze-dried and radiation sterilized material with the fresh material for all nutrients, with the exception of folic acid. Folic acid shows an increased assay value of 36% over the fresh value. The value for the freeze-dried, radiation sterilized material was increased by 85% over the fresh value. This increase is well outside analytical variation and full explanation is not known at the present. It could be that the assay organism is not as responsive to the forms of folate in the fresh material, whereas there is some change in the form upon freeze-drying and radiation sterilization. This change in form could lead to either more efficient extraction in the dry material or a higher relative response in the assay. Explanation awaits further investigation. Prolonged observations on the retention of vitamins in both the fresh frozen and freeze-dried material stored at −50° C for periods up to 3 years are underway.

No change was observed in phytate levels of freeze-dried material upon prolonged storage. The value of 1.4±0.2 mg/g remained the same.

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