Determination of Iodine Content in Dairy Products by Inductively Coupled Plasma Mass Spectrometry

Kathryn A. Vance*, Amir Makhmudov, Gulchekhra Shakirova, Hanna Roenfanz, Robert L. Jones, and Kathleen L. Caldwell

Centers for Disease Control and Prevention, National Center for Environmental Health, Division of Laboratory Sciences, Inorganic and Radiation Analytical Toxicology Branch 4770 Buford Hwy NE, MS F-18, Atlanta, GA 30341, USA

ABSTRACT

A probing study to establish a reliable and robust method for determining the iodine concentration using the ELAN® DRC™ II ICP-MS was performed in combination with a sample digestion and filtration step. Dairy products from locally available sources were evaluated to help determine the possibility and need for further evaluations in relation to the U.S. population's iodine intake. Prior to analysis, the samples were aliquoted and digested for 3 hours at 90±3 °C. Dilution and filtration were performed, following the digestion. The sample extract was analyzed, and the results were confirmed with NIST SRM 1549a Whole Milk Powder. Further experimentation will need to be performed to optimize the method for projected sample concentration and throughput.

INTRODUCTION

Iodine is an essential nutrient for normal growth and development (1). Iodine deficiency affects approximately 1.88 billion people globally and is the leading, most preventable, cause of mental retardation in the world (1). The body uses iodine in the synthesis of thyroid hormones, which are necessary for normal growth and development throughout a person's lifetime (2). Adequate iodine nutrition is essential during fetal and newborn development because of the rapid and fundamental growth taking place (3). Table I shows the recommended median Urine Iodine (UI) values for the population as determined by the World Health Organization (WHO) (4). This table shows the range of adequate levels for the average human (100–199 µg/L) and for pregnant women and women of childbearing age (150–250 µg/L). Iodine deficiency can lead to many developmental and health issues, which are referred to as iodine deficiency disorders (IDD) (6). Deficiency during pregnancy can cause miscarriage, still birth, or congenital abnormalities such as cretinism (7). During infancy and childhood, deficient individuals can experience stunted physical growth, goiter, and varying degrees of mental impairment (7).

To combat iodine deficiency, many countries have chosen food fortification. The most common of these is iodized salt (7). Even with the prevalence of iodine deficiency, only 70% of the world's salt is iodized (8). In the United States, only 50–60% of the population uses iodized salt. Additionally, only 15% of dietary iodine intake in the American diet comes from iodized salt (9). The main sources of iodine in U.S. diets are found in dairy products (10).

Dairy products supply over 60% of the dietary iodine intake in the American diet (1). Even though dairy products are the primary source of iodine in the diet, little work has been done to evaluate these products (10, 12). Because of the many constituents of the American diet and the wide range of iodine content of products, continuous monitoring of the iodine status of the U.S. population is a necessary public health measure.

Due to the excretion of more than 90% of dietary iodine in urine, the iodine status of the U.S. population is evaluated by measuring urinary iodine (UI) concentration on a population scale (13). The National Health and Nutrition Examination

TABLE I
Criteria for Assessing Median Urinary Iodine Values for the Population, Pregnant Women, and Women of Childbearing Age (6)

Iodine Status	Urinary Iodine Concentration
Excessive Intake	> 300 µg/L
More than Adequate Intake	200-299 µg/L
Adequate Intake	100-199 µg/L
Mild Deficiency	50-99 µg/L
Moderate Deficiency	20-49 µg/L
Severe Deficiency	< 20 µg/L
Iodine Status for Pregnant Women/ Women of Childbearing Age	**Urinary Iodine Concentration**
Adequate Intake	150-249 µg/L
Inadequate Intake	<150 µg/L

*Corresponding author.
E-mail: xky1@cdc.gov
Tel: 770-488-4164

Survey (NHANES) monitors iodine status of the U.S. population. Since the beginning of NHANES in the early 1970s, the population's iodine status has slowly declined (1), as seen in Figure 1. While the population median UI is adequate, some vulnerable populations, like pregnant women in certain U.S. regions, fall in the less-than-adequate range (1).

Center for Disease Control's (CDC's) efforts to accurately assess the U.S. iodine status remains a critical component in ensuring adequate iodine intake. Determining the iodine content in various products contributing to this status can inform efforts to ensure adequate nutrition of the population.

The purpose of this study was to develop a reliable and reproducible method that can be used to examine iodine content in dairy products and to help characterize the products which have the largest influence on the iodine status of the U.S. population.

EXPERIMENTAL

Instrumentation

A model ELAN® DRC™ II inductively coupled plasma mass spectrometer (ICP-DRC-MS) with a quartz concentric spray chamber, quartz concentric nebulizer Type C 2.0 mm i.d. (Precision Glass Blowing, Centennial, CO, USA), and a 2.0 mm i.d. quartz injector with nickel or platinum sampler and skimmer cones were used (PerkinElmer, Inc., Shelton, CT, USA). The ICP-MS was fitted with an ESI SC-4 DX autosampler (Elemental Scientific Inc., Omaha, NE, USA) and DXi-FAST micro-peristaltic pump sample introduction system (Elemental Scientific Inc., Omaha, NE, USA). Sample preparation dilutions were performed using a Digiflex™ semi-automatic liquid handler (Titertek, Huntsville, AL, USA). Table II shows the method parame-

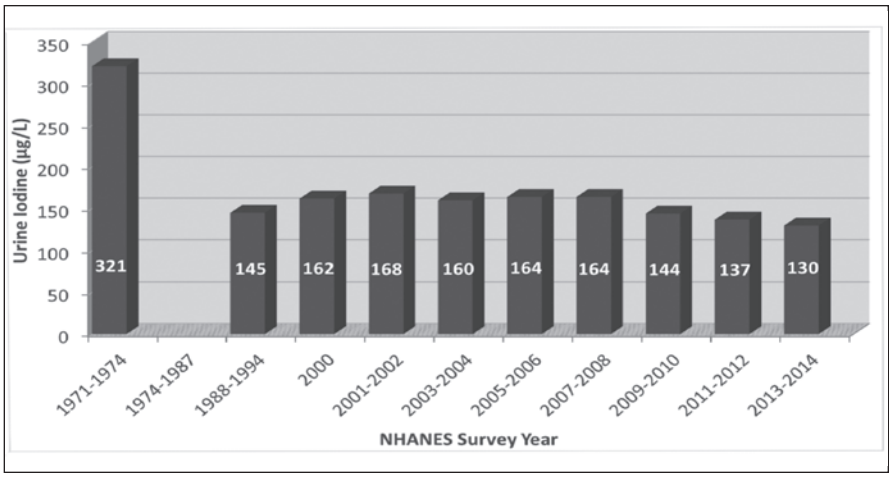


Fig. 1. NHANES data for the median urinary iodine status from 1971–2014. Note: 1974-1987 NHANES data was not collected for urine iodine (11).

TABLE II
Method Parameters for the Determination of Iodine in Milk by ICP-MS

*Division of Laboratory Sciences Method for Determination of Iodine in Urine

CDC Division of Laboratory Sciences 3002 Method Parameters*	
Parameters	Value/Description
ICP-MS	ELAN® DRC™ II, DXi-FAST Peristaltic Pump, Fomblin Oil Pump
Autosampler	ESI SC4-DX with ULPA-filtered Cabinet
FAST	1 mL loop, Teflon® Ctator, CTFE rotor
Nebulizer and Spray Chamber	Quartz Concentric and Quartz Cyclonic
Plasma Argon Flow Rates :	Plasma (15), Aux (1.2), Neb (~0.95) (L/min)
	RF Power = 1450 W (1150-1600)
Analysis Timing and Groupings	Sweeps/Reading = 70
	Readings/Replicate = 1
	No. of Replicates = 3
	Dwell/Integration Times: 30 ms/^{185}Re, ^{127}I
Calibration Range (µg/L)	5 Log-normal Distributed Dalibration Standards,
	Matrix-matched I: 8.0 – 3000.0 µg/L
Sample Introduction	Nebulizer Liquid Flow Rate Constant at 320 µL/min (3 rpm, blk/blk tubing)
	FAST Carrier Solution = Sample Diluent
	0.4% (v/v) TMAH,
	1% EtOH, 0.01%APDC, 0.05% Triton® X-100, 5 µg/L Re
	SC4-DX Rinse Solution = 0.4% (v/v) TMAH,
	1% EtOH, 0.01% APDC, 0.05% Triton x-100
Analysis – ELAN DRC II	Sample Flush = 3 s
	Read Delay = 37 s
	Analysis Time = 4.6 min
	Wash Delay = 100 s
Washout Timing – SC4-DX/FAST	Loop Fill (1 mL) = 3 s
	AS Probe Rinse 1 and 2 = 0 s, 10 s
	FAST Valve and Loop Rinse:
	1 Load / Inject Valve Cycle, 4 Loop Rinses (3 s each)

ters. All sample mixing was performed using a standard vortex mixer (Fisher Scientific, Fairlawn, NJ, USA). A water bath capable of maintaining temperatures of 90 ± 3 °C for over 3 hours, (VWR®, Radnor, PA, USA) was used for sample digestion prior to analysis.

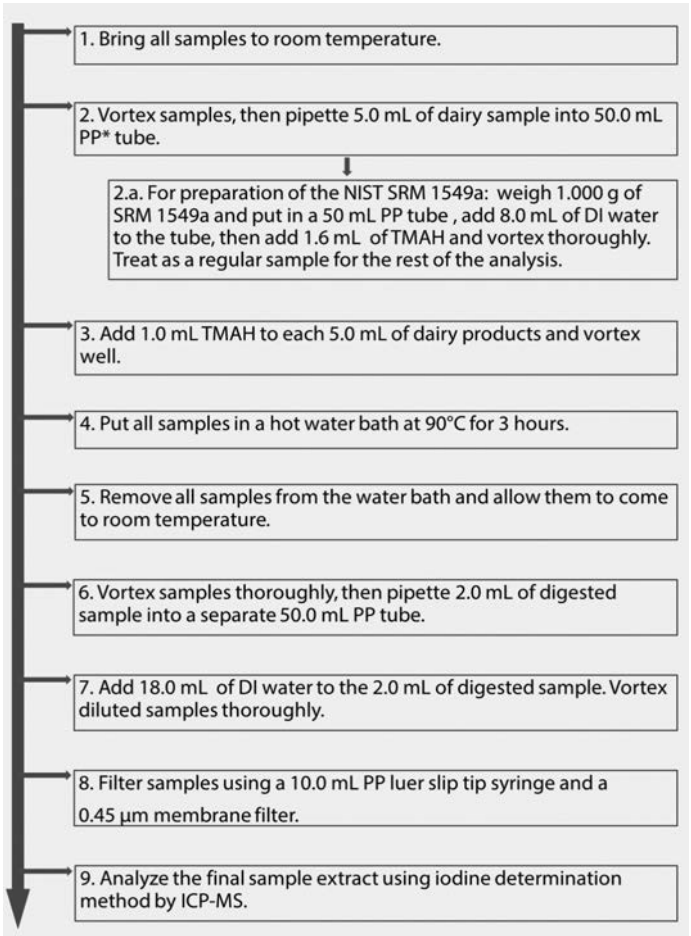
Materials and Reagents

All solutions were prepared using ≥18 MΩ•cm deionized (DI) water from a NANOpure® Diamond™ Ultrapure Water System (Barnstead International, Dubuque, IA, USA). High purity argon gas (>99.999% purity, Specialty Gases Southeast, Atlanta, GA, USA) was used for the ICP-MS plasma and the nebulizer gas. Tetramethylammonium hydroxide (TMAH), ethyl alcohol (Ethanol, USP dehydrated 200 proof), sulfamic acid (GFS Chemicals, Columbus, OH, USA), ammonium pyrrolidine dithiocarbamate (ADPC), (laboratory grade, Fisher Scientific, Fairlawn, NJ, USA) and Triton® X-100 (JT Baker Chemical Company Phillipsburg, NJ, USA) were used. Standard solutions of rhenium (Re) and iodide, traceable to the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA) were purchased from Inorganic Ventures (Inorganic Ventures, Christiansburg, VA, USA) and High Purity Standards (Charleston, SC, USA). For this study, NIST Standard Reference Material (SRM) 1549a Whole Milk Powder was used and all dairy samples (whole milk from varying brands) were purchased at local sources.

Sample Digestion and Preparation

During sample preparation, a 5.0-mL portion of each dairy product was mixed via vortex, then added to a 50-mL polypropylene tube (PP). Next, 1.0 mL of TMAH was added to each tube. The tubes were capped tightly and mixed well via vortex for 10–15 seconds. All tubes were placed in a preheated water bath at 90 °C for 3 hours. The NIST SRM 1549a Whole Milk Powder was digested with each batch of samples by adding 1.000 g of SRM 1549a to a 50-mL PP tube. Then, 8 mL of >18 MΩ•cm water was added to the tube and mixed well. Further, 1.6 mL of TMAH was added to the tube, capped tightly, mixed well, and added to the water bath at 90±3 °C for 3 hours. All sample tubes were allowed to cool to room temperature following completion of the digestion. After cooling, 2.0 mL of each sample, SRM 1549a and base dairy (material to be used for matrix matching), were transferred to separate 50-mL PP tubes. Each sample tube (containing a 2.0-mL aliquot) was then diluted with 18.0 mL of >18 MΩ•cm DI water. The samples were mixed thoroughly. Following the digestion and dilution, the samples were cooled to room temperature, then filtered using 10-mL plastic Luer lock syringes and membrane filters, pore size 0.45 µm (EMD Millipore, Billerica, MA, USA). The final sample extract was analyzed using the CDC's DLS for iodine determination in urine by the ICP-MS method. See Figure 2 for sample preparation timeline. Some samples were harder to filter than others because of the leftover solids after digestion. These samples required use of several filters and ultimately resulted in a wide range of sample extract volume from the samples.

Fig. 2. Sample Preparation Timeline



Method

The method used for this evaluation was based on the Centers for Disease Control and Prevention's Division of Laboratory Sciences determination of iodine in urine by the ICP-MS method, combined with the sample digestion and preparation technique procedure from Hong Kong Government Laboratory and advisement from the National Institutes of Standards and Technology (14). The method parameters are listed in Table II. A base material was chosen and used throughout the calibration in order to account for and reduce the effect caused by the dairy matrix. The calibration curve materials were matrix-matched via previously aliquoted base dairy. The base dairy material was prepared like all other dairy samples and underwent digestion prior to use. The base dairy material was purchased at a local source and evaluated prior to sample analysis to ensure homogeneity throughout the original container. The calibration range used was 8.0 µg/L to 3000 µg/L. This range was adopted from the already standing method and used during the probing study because the expected sample concentrations were unfamiliar. However, in the future, a smaller range would be acceptable. Two levels of QC material were used based on standards 2 and 5 with concentrations of 20.0 µg/L iodine and 400.0 µg/L iodine, respectively. Because of the time required for proper sample digestion and preparation, analysis was generally performed on the following day. The sample extracts were stored in a refrigerator at 4 °C until analysis.

RESULTS AND DISCUSSION

In this study, the iodine content was determined in 55 different types of dairy products, including 10 whole milk products. The 10 different (brands) whole milks are listed in Table III. These concentrations were surveyed across three separate evaluations of the same products to ensure among-run agreement. Between-run reproducibility for iodine concentration in various dairy products (concentration span) averaged 4.8% RSD. The limit of detection for the method was determined over 10 separate runs to be 2.31 µg/L. The limit of detection was calculated by taking the standard deviation of the same standard over 10 runs (0.77 ppb) and multiplying it by 3. Sample throughput averaged 20 samples per analytical run. Sample preparation and digestion was usually performed the day prior to analysis in order to maximize run time with an average of three analytical runs per day. All digested and diluted samples were stored in a refrigerator at 4 °C. In order to ensure accuracy of the results, all runs contained NIST SRM 1549a Whole Milk Powder. NIST SRM 1549a was also used to determine the percent recovery. The NIST target value was 3334.0 µg/L for the iodine analyte, and the CDC analyses averaged 96.6 % recovery across 20 separate measurements of NIST SRM 1549a Whole Milk Powder. All samples maintained their integrity throughout at least 2 freeze-thaw cycles. The homogeneity of the original sample containers was determined by fully aliquoting one gallon of whole milk into 50-mL polypropylene tubes. The tubes were chosen at random and analyzed for their iodine content. These tubes showed no significant differences (p-value = 0.497) and confirmed the homogeneity of the aliquoted samples.

CONCLUSION

Evaluation of dairy products via ICP-MS served as a robust and reliable method for the determination

TABLE III
Results of Iodine Content in Whole Milk

Sample Identifier	Results 1 (µg/L)	Results 2 (µg/L)	Results 3 (µg/L)	Average (µg/L)	Range (µg/L)	Standard Deviation (µg/L)	RSD (%)
1	219.4	214.8	232.2	222.1	214.8 – 232.2	9.0	4.1
2	305.0	313.1	333.6	317.2	305.0 – 333.6	14.7	4.6
3	294.0	301.0	317.0	304.0	294.0 – 317.0	11.8	3.9
4	491.7	510.9	527.4	510.0	491.7 – 527.4	17.9	3.5
5	678.4	687.3	743.2	703.0	678.4 – 743.2	35.1	5.0
6	218.1	216.7	234.9	223.2	216.7 – 234.9	10.1	4.5
7	292.8	295.9	314.1	300.9	292.8 – 314.1	11.5	3.8
8	505.7	527.8	547.5	527.0	505.7 – 547.5	20.9	4.0
9	33.2	29.3	27.5	30.0	27.5 – 33.2	2.9	9.7
10	514.8	517.0	560.4	530.7	514.8 – 560.4	25.7	4.8

of iodine. The limiting factor in this method is the digestion process. In future work, the digestion process needs to be improved to eliminate the need to separate the digestion process the day before the analysis, while still being able to reasonably maintain acceptable sample throughput. It is also important to look into easier and more economical ways to filter the digested sample instead of using multiple syringes and filters. This step in the process was often hard to perform because of the large solids still left in the milk after digestion. Possible options include larger pore size filters, increased digestion times, or increased dilution factors. In the future, the calibration range will also be reduced to more reasonably accommodate the expected sample range.

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Disclaimers

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention. Use of trade names and commercial sources is for identification only and does not constitute endorsement by the U.S. Department of Health and Human Services or the Centers for Disease Control and Prevention.

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