SESSION 4.

Emerging Techniques for Analysis and New Compositional Data for Nutrients and Bioactive Compounds and Their Bioavailability

S4-S-1

Stable Isotope Dilution Assays as Tools for Evaluating Vitamin Contents and Bioactivity

Michael Rychlik

BIOANALYTIK Weihenstephan, Research Center for Nutrition and Food Sciences, Technische Universität München, Alte Akademie 10, D-85354 Freising, Germany

For examining vitamins, exact quantitations in natural matrices are essential. Up to date, one of the most accurate quantitation methods is the stable isotope dilution assay (SIDA), which is based on the use of isotopically labelled analogues of the analytes as internal standards. The merits of SIDA include the ideal compensation for losses, superior specificity and enhanced sensitivity due to the "carrier effect" [1]. Therefore, SIDAs are superior to other assays for vitamins that occur in low concentrations and are labile during analysis. One of these groups of vitamins is the folates.

In countries without mandatory fortification, intake of this vitamin group is considered to be below the human dietary recommendations. As folates play a crucial role as coenzymes in the metabolism of one-carbon groups, folate deficiency is considered to increase the risk of neural tube defects and is suspected of being associated with the development of certain forms of cancers, alzheimer's disease and cardiovascular disease.

For quantitation of the vitamin group of folates, a recently developed SIDA was applied to several unfortified as well as fortified foods. Detection of the isotopologic vitamers tetrahydrofolate (THF), 5-methylTHF, 5-formylTHF, 10-formylfolate and folic acid was achieved by LC-MS/MS [2].

However, to establish accurate dietary recommendations, also the bioavailability of a nutrient has to be considered. For evaluating folate bioavailability, different approaches can be pursued. Of these, we chose two short-term designs.

In a first investigation, we examined bioavailability in a large-scale human study including 26 volunteers with application of 3 model foods and folic acid as the reference dose. Folates were quantified in the test foods, in the plasma of the volunteers as well as in their urine. A newly developed SIDA for folate catabolites in urine was also applied [3].

Calculation of area under the curve data of the post-dose plasma folate levels revealed a folate bioavailability of 20%, 73 % and 33% in Camembert cheese, Spinach and wheat germs, respectively, relative to folic acid.

A second approach was a double label isotope study, in which deuterium and 13C-labels were used to differentiate oral doses from analytical internal standards.

By applying LC-MS/MS, we were able to detect the signals of unlabelled, 13C-labelled and deuterated folates in blood plasma. Using this approach, we were able to show that folate vitamers show different absorption behaviours.

As folate bioavailability widely varies for different foods and different vitamers, further human studies are necessary to compile valid dietary recommendations. Differently labelled folates are perfect tools for this task.

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Keywords: Stable isotope dilution assays; Vitamins; Folates; Bioavailability

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Corresponding author: Assoc. Prof. Michael Rychlik / Email: michael.rychlik@wzw.tum.de

S4-O-2

Bioavailability of the Most Predominant 5-Methyl Tetrahydrofolate (5-MTHF) in Humans using the Emerging LC-MS/MS Technique for Vitamer Determination

Shyamala Vishnumohan[1], Jayashree Arcot[1] and Russell Pickford[2]

[1] Food Science and Technology, School of Chemical Sciences and Engineering, The University of New South Wales, Sydney 2052, Australia; [2] Bioanalytical Mass Spectrometry Facility, The University of New South Wales, Sydney 2052, Australia

Rationale and Objective

It is not known to what extent naturally occurring folates in the mixed diet is bioavailable. Absorption would be best studied in a population not exposed to any folate fortificant. The objective of the study was to understand the bioavailability of dietary 5-methyl tetrahydrofolate in a mixed diet using the LC-MS/MS technique for analysis.

Materials and Methods

A randomized trial (12 weeks) where 22 human subjects aged 18-25 were given a pharmaceutical dose of 400 μg 5-MTHF or a diet containing ~400 μg/day were studied for the relative bioavailability of 5-methylTHF. Analyses of 5-methylTHF in the plasma and RBC was performed using a 13C5 5-MTHF for analysis on the LC-MS/MS in the positive ion mode electrospray ionization. Reliability of the method was tested using certified reference materials. 45 Indian foods used in the study of varied matrices were analysed using LC-MS/MS technique. Relative bioavailability of 5-MTHF was calculated by comparing the response to food folate in relation to supplemental 5-MTHF as indicated by biomarkers.

Results

The folate intakes were reported to be 429±68.7 (g/day when the individual foods from the diet were analysed. The relative bioavailability of food folate predominant in 5-MTHF was 41% based on the serum folate indicator and 47% based on erythrocyte folate status. A mean increase of 60% was observed in the erythrocyte folate levels of the subjects consuming diets predominant in 5-MTHF in 12 weeks.

Discussion and Conclusion

Taking into account the folate intakes reported in the present study (429±68.7 (g/day) and the net bioavailability of 5-MTHF reported based on the biomarkers (44%), the selected population is consuming 188 μg/day of bioavailable folate which is nearly twice the suggested recommended intake 100 μg/day (Gopalan *et al,* 2002).

Keywords: Folate; LC-MS/MS; 5-MTHF; Bioavailability; Biomarkers

Corresponding author: Dr. Jayashree Arcot / Email: j.arcot@unsw.edu.au

Metrology and Methods for Multielement Content of Canadian Food Items by Neutron Activation

Amares Chatt, Weihua Zhang

SLOWPOKE-2 Facility, Trace Analysis Research Centre, Department of Chemistry, Dalhousie University, Halifax, Nova Scotia, B3H 4J3, Canada

Most of the food composition tables used around the world lack reliable data on multielement content of food items. There are various reasons for that including the lack of appropriate analytical methods and rigorous metrology of the data obtained. We have developed several neutron activation analysis (NAA) methods for the determination of a number of elements in individual food items and duplicate diets. The methods include: cyclic instrumental neutron activation analysis (CINAA) for selenium, instrumental NAA (INAA) for several elements, epithermal INAA (EINAA) for iodine, preconcentration and radiochemical NAA for iodine, radiochemical NAA (RNAA) for selected elements, preconcentration NAA (PNAA) for uranium and thorium, and instrumental and preconcentration NAA for aluminum. We have designed comprehensive quality assurance programs of our methods and estimated overall and expanded uncertainties. We have applied these methods to measuring multielement content of about 140 Canadian food items and 20 duplicate diets. Details of the methods and results will be presented.

Keywords: Multielement, Food, Neutron activation, Metrology

Corresponding author: Prof. Amares Chatt / Email: a.chatt@dal.ca

Investigation of Thai Plants as Potential Sources of Fructans and Inulin Main Fractions

Kunchit Judprasong, Siriporn Tanjor, Pongtorn Sungpuag, Prapasri Puwastien

Institute of Nutrition, Mahidol University, Salaya, Putthamonthon, Nakhon Pathom 73170, Thailand

Fructans and inulin-type fructans are polysaccharides which have been used as potential sources of dietary fibre in food products. These substances were claimed to have health benefits such as relieving constipation, decreasing risk of osteoporosis and atherosclerosis, reducing plasma cholesterol concentrations and stimulating the immune system of the body. Natural inulin and oligofructose were found in varieties of plants, especially starchy roots, fruits, and vegetables. These data in foods consumed in Thailand, however, are limited. Therefore, the objective of this study was to determine the levels of fructans, fructo-oligosaccharides (FOS: 1-kestose (1-kestotriose; GF2), nystose (1,1-kestotetraose; GF3), and 1F-β-fructofuranosylnystose (1,1,1-kestopentaose; GF4)) and total sugar (glucose, fructose and sucrose) in potential food sources.. Food samples were randomly purchased from three main markets in the Bangkok metropolitan area. These included 12 kinds of starchy roots and tubers, 11 kinds of fruit vegetables, 7 kinds of fruits, 9 kinds of spices and 4 kinds of seeds. Each food sample was extracted by hot water, and then fructans and fructo-oligosaccharides were hydrolysed by inulinase based on AOAC method 997.08. Released sugars were further derivatised by oxymation and silylation reactions. High-temperature gas chromatography was used to determine individual sugars, before and after enzyme hydrolysis. Starchy roots and tubers contain fructans in trace amounts to 19.4±3.9 g/100 g fresh weight (FW) and contain FOS in trace amounts to 5.2±1.0 g/100 g FW. Fructans and FOS were found in spices at the levels of trace amounts to 29.2±5.6 g/100 g FW and trace amounts to 1.6±1.4 g/100 g FW, respectively and in seeds were found at trace amounts to 2.4±2.8 g/100 g FW and not detectable, respectively. In conclusion, great headed garlic, Chinese garlic, garlic and Jerusalem artichoke (Keantawan) showed relatively high concentrations of fructans (29.2±5.6, 24.3±1.9, 23.0±4.6 and 19.4±3.9 g/100 g FW, respectively), whereas Jerusalem artichoke is dominant in FOS (5.2+1.0 g/100 g FW). In contrast, shallot and curry leaf tree demonstrated small amounts of fructans (9.4±1.9 and 3.6±1.0 g/100 g FW, respectively). Other studied foods contained small amounts (<1.0 g/100 g FW). Most of the studied foods contained total sugar less than 5.0 g/100 g FW, except relatively high levels were found in banana and dragon fruit (7-10 g/100 g FW). Great headed garlic, Chinese garlic, garlic and Jerusalem artichoke are potential sources of fructans and inulin-type fructans.

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Keywords: fructans; Inulin; Fructo-oligosaccharides; Thai food

Corresponding author: Dr. Kunchit Judprasong / Email: nukjp@mahidol.ac.th

The Content of Coenzyme Q10 in Selected Thai Foods: Meat Products, Vegetables, Fruits, Seeds, Legumes, and Spices

Krongkan Kuewdum, Aikkarach Kettawan, Ratchanee Kongkachuichai, Rin Charoensiri

Institute of Nutrition, Mahidol University, Salaya, Putthamonthon, Nakhon Pathom 73170, Thailand

Coenzyme Q10 (CoQ10) is a fat-soluble and vitamin-like substance. It is one of the important mobile electron carriers in the electron transport chain. It also acts as antioxidant and has the potential to reduce risk of chronic diseases. Although CoQ10 can be obtained from both biosynthesis and diet, CoQ10 synthesis decreases with aging. Therefore the amount of CoQ10 content in natural foods and food products is very important. Six groups of Thai food items which are commonly consumed including meat products (8 items), vegetables (9 items), fruits (6 items), seeds (5 items), legumes (8 items), and spices (12 items) were obtained from 5 representative markets in Bangkok, Thailand. They were analyzed for their CoQ10 content using a high performance liquid chromatography (HPLC) system equipped with an electrochemical detector (ECD). To study the effect of cooking, CoQ10 content in meat products, vegetables, seeds, and legumes groups were analyzed in both raw and cooked samples. All food items were analyzed by single composite sample. The richest CoQ10 content in the sample from each group of food items was found in shredded pork (21.83±4.56 μg/g) for meat products; Chinese kale. (*Brassica oleracea var. alboglabra, Bail)* (5.57±1.91 μg/g) for vegetables; ripe papaya*. (Carica papaya, Linn.)* (3.90±1.69 μg/g) for fruits; cowpea. *(Vigna unguiculata* (L.) *Wasp. var. unguiculata Syn. V.)* (13.26 ±4.57 μg/g) for legumes; black sesame. (*Sesamum indicum,* L.) (2.00±1.36 μg/g) for seeds; and kaffir lime peel. *(Citrus hystrix)* (3.88±1.82 μg/g) for spices. Among the 6 groups of food items, legumes and shredded pork showed the highest CoQ10 content. Household cooking affected CoQ10 content in some food items.

Keywords: Coenzyme Q10; HPLC; Electrochemical detector; Selected Thai foods

Corresponding author: Dr. Aikkarach Kettawan / Email: nuakw@mahidol.ac.th

S4-O-6

Evaluation of Ascorbic Acid as a Standard for the Oxygen Radical Absorbance Capacity (ORAC) Assay

Katherine Stockham[1,2], Rohani Paimin[2], John D Orbell[2], Paul Adorno[1], Saman Buddhadasa[1]

[1] *Australian Government, National Measurement Institute, Port Melbourne, Victoria, Australia 3207*

[2] *School of Biomedical and Health Sciences, Victoria University, Werribee, Victoria, Australia 3030*

In recent years antioxidants have been a topic of much research due to the increase in claims by companies that their products contain antioxidants and have potential health benefits. It is believed that these compounds have a role in stabilizing free radical species that can otherwise contribute to a range of diseases such as stroke and the process of aging. Radical species occur naturally in the body as a result of aerobic metabolism, diet, and from exposure to different types of radiation. Many foods and beverages have measurable antioxidant abilities. One of the most commonly used techniques to measure antioxidant capacity in recent years is the Oxygen Radical Absorbance Capacity (ORAC) assay. This assay utilizes the fluorescent probe Fluorescein (FL) and the peroxyl radical donor 2, 2'-azobis (2-amidinopropane) dihydrochloride (AAPH). Peroxyl radicals are generated by thermal degradation of AAPH, and FL decay curves are produced. The area under the curve (AUC) is then used to determine antioxidant capacity of a food or beverage sample. The ORAC assay measures the total antioxidant capacity of the sample arising from a variety of antioxidant classes. Values are traditionally reported as equivalents of a synthetic analogue of vitamin E known commonly as Trolox. This research focuses on the validation of ascorbic acid as a standard for the ORAC assay, and the implications for reporting antioxidant capacity in vitamin C equivalents. Reporting ORAC values in Trolox equivalents can be difficult for consumers to understand, especially as the units used are molar based. The validation of ascorbic acid as an analytical standard for the ORAC assay is novel work and has been conducted on a variety of food matrices, for both water and fat soluble antioxidant capacity. A new mode of reporting ORAC is also proposed based on mass basis rather than molar units, and will be discussed in this presentation. This research is conducted in collaboration with Victoria University and the National Measurement Institute in Port Melbourne and is ongoing.

Keywords: ORAC, Antioxidant capacity, Vitamin C, Trolox, Food

Corresponding author: Dr. Saman Buddhadasa /
Email: saman.buddhadasa@measurement.gov.au