

samples. Neutron activation is suitable for almost all media. X-ray methods are used especially for environmental samples. For the speciation of organic Hg compounds, selective organic extractions with different chromatographic methods are the most extensively used.

**Determination of folates in foods — challenges and advances of the use of HPLC.** Liisa Vahteristo,\* Velimatti Ollilainen, Pekka Koivistoinen & Pertti Varo.

*Department of Applied Chemistry and Microbiology, FIN-00014 University of Helsinki, Finland.*

There is increased need for specific data on folates and their vitamers distribution in foods. Many procedures for folate determination do not meet these needs, but are chiefly meant for measuring total folates or added folic acid. Use of HPLC enables separation and quantitation of the most abundant folate vitamers in their monoglutamate forms (Gregory, 1984).

In this study reduced folate vitamers were determined in some foods, e.g. liver and liver products, after heat extraction and deconjugation of polyglutamate forms of folate moiety into folate monoglutamates using hog kidney conjugase. These folate forms were separated using reversed-phase high performance liquid chromatography and quantitated using fluorescence detection. The methods used provided data on the amounts and the distribution of the folate vitamers present. The amounts determined in e.g. pork and beef liver were much higher than expected from literature. Folate content of some other foods were much more in accordance with previous findings.

The data on folate content of foods should be carefully evaluated for its validity, and in many cases new analysis should be performed before such data can be used, for example for the estimation of the dietary intake of folate. Additional data on folate forms present in food, determined using HPLC, provide more in-depth information of the chemical nature of this vitamin. But careful validation of the method and specific identification procedures are essential for HPLC methods intended for folate analysis.

1. Gregory III, J. F. (1984) *J. Assoc. Off. Anal. Chem.*, 67(5), 1015–19.

\*To whom correspondence should be addressed.

**New components included in future food composition tables.** Gary. R. Beecher.

*Food Composition Laboratory, Beltsville Human Nutrition Research Center, ARS/USDA, Beltsville, MD 20705, USA.*

Nutrients and food components for which data are essential are dependent on the food supply and health status of the population of each country or region. Several regions of the world have diseases resulting from inadequate intakes of several micronutrients including iodine, iron and vitamin A active components. Several

debilitating diseases, in 'well-nourished' regions of the world have strong dietary associations. With regard to cancer and cardiovascular disease (CVD), many nutrients and food components with antioxidant activity are being investigated as preventive agents. These components include carotenoids, flavonoids, phytate, tocopherols, selenium and vitamin C. Several food component, coumestrol, isoflavonoids and lignans, either have anti-estrogenic activity or are converted to compounds with this activity in the gastro-intestinal tract and are proposed to reduce the risk of hormone related cancers. The risk of CVD also is altered by dietary levels of total fat, individual fatty acids, trans fatty acids and cholesterol as well as dietary fiber and its fractions. Low folic acid intake has been implicated in the risk of several diseases. Dietary levels of calcium, boron and vitamins D and K are important determinants for osteoporosis. The intake of sodium is one of the primary risk factors to stroke. Example of priority setting and justification for the addition of nutrients to databases will be discussed.

**The selenium content of human milk and infant formulae in Finland.** Päivi Ekholm,\* Maija Ylinen & Pertti Varo.

*University of Helsinki, Department of Applied Chemistry and Microbiology, Post Box 27, Viikki-D, FIN-00014 University of Helsinki, Finland.*

The aim of this study was to monitor the effects of selenium (Se) fertilization (started in 1985) on the Se content of human milk and infant formulae.

The samples of human milk were received from the Helsinki University Central Hospital. Each sample was a pool of milks from several donors (median 20). The infant formulae were sampled from food stores in the Helsinki area. Se was determined from freeze-dried samples by the electrothermal atomic absorption method.

The Se content of human milk was 0.08 mg/kg DM in 1994, more than twice as high as in the mid 1970s. The present level in Finland is nearly equal to that prevalent in many other countries. The mean Se content of infant formulae was 0.06 mg/kg DM in 1994, which is three times higher than in the mid 1970s. The Se content of infant formulae has remained lower than that of human milk.

The present Se content of human milk is an indication of the improved Se status of the Finnish population. The Se fertilization has increased the Se content of all Finnish foods effectively, including the infant formulae.

\*To whom correspondence should be addressed.

**Trends in the cadmium contents of bovine and porcine liver between 1982–1995.** Merja Euroola\* & Pertti Varo.

*University of Helsinki, Department of Applied Chemistry and Microbiology, P.O. Box 27, Viikki, General Chemistry, SF-00014 University of Helsinki, Finland.*

The aim of the study was to monitor retrospectively the development of the cadmium (Cd) contents of bovine and porcine livers from the beginning of 1980.

The samples were purchased in connection with the long-term selenium monitoring programme and have been freeze-dried and stored vacuum packed at +4°C. The samples of bovine liver were obtained from 16 retail food stores in the Helsinki area and the samples of porcine liver from a communal slaughter house. The samples were combined into four pooled samples per sampling period. The samples were analyzed by electrothermal atomic absorption spectrometry.

In the period 1982–1995 the mean Cd level of bovine liver was 0.17 mg/kg DM and of porcine liver 0.07 mg/kg DM. Cd contents varied between 0.02–0.49 and 0.02–0.21 mg/kg DM respectively. No significant trends were observed during this period. On the whole the concentrations were lower than those reported in the 1970s.

\*To whom correspondence should be addressed.

**Microwave acid digestion for the determination of mercury in foodstuffs by CVAAS.** Jari Toivo\* & Merja Eurola.

*University of Helsinki, Department of Applied Chemistry and Microbiology, Post Box 27, Viikki-D, University of Helsinki, FIN-00014, Finland.*

The objective of this study was to develop a microwave acid digestion technique prior to an amalgamation cold vapor atomic absorption spectrometric (CVAAS) detection method for the determination of total mercury in different food matrices.

Microwave acid sample digestion was carried out in sealed vessels. The heating time, acid volume and type were varied to determine the optimal conditions for decomposition. In the CVAAS method an amalgamation technique and tin (II) chloride reduction were used.

The proposed procedure for the decomposition of dried samples was to heat a 200 mg sample for 1.5 min. (power 600 W) in 3.5 ml HNO<sub>3</sub>. The results of the certified samples were in good agreement with the certified values. The precision of the proposed method was good (RSD < 5%). Microwave heating of 3.0 min in 5.0 ml HNO<sub>3</sub> was needed to yield satisfactory recoveries for very fat and protein-rich non-dried sample materials. The limit of detection was 5.2 ng/L in analytical conditions used.

Using microwave acid sample digestion combined with an amalgam-CVAAS-detection the total mercury can be determined accurately, precisely and very rapidly in food samples. However, the decomposition conditions required strongly depend on the sample matrix.

\*To whom correspondence should be addressed.

**Factors influencing the determination of folate in foods.** Caroline Martin,\* Louise O'Mahony & Tony Sheehy.

*Department of Nutrition, University College, Cork, Ireland.*

Many factors can influence the determination of food folate concentrations. The objective of this study was to

investigate the thermal and pH-stability of folates, the influence of conjugase type on polyglutamate deconjugation, and the implications of the choice of calibrant used in the microbiological assay. The thermal stability of folic acid (FA), folinic acid (5 CHO THFA), 5-methyl tetrahydrofolic acid (5CH<sub>3</sub> THFA), 7,8-dihydrofolic acid (DHFA) and 5, 6, 7, 8 tetrahydrofolic acid (THFA) was determined using a Cary 1E UV-visible wavelength spectrophotometer. Solutions (approximately 10–100 nm) were incubated in a universal buffer (ph 4, 5 to 9, 0) at 37°C and 70°C for 4 h. After 4 h at 37°C, concentrations of all folates were > 80% of initial values. Increasing the incubation temperature to 70°C reduced the concentrations of FA and 5-CHO THFA slightly at pH 4, 5–6, 0. At this temperature, 5-CH<sub>3</sub> THFA, DHFA and THFA were extremely unstable above pH 7, 0. After 4 h, concentrations were < 30% of their initial values. Free and polyglutamyl folate concentrations measured in a variety of foods differed significantly depending on whether human plasma, chicken pancreas or hog kidney conjugase was used for polyglutamate deconjugation. The growth response of *Lactobacillus rhamnosus* (ATCC 7469) to 5-CHO THFA, 5-CH<sub>3</sub> THFA, DHFA and THFA at concentrations typical of those used in the microbiological assay (0–1, 4 ng/4 ml medium) was 58.2, 43.6, 26.1 and 14.9% respectively, of the response to FA. This represents a serious limitation of the microbiological assay when samples contain complex mixtures of folates. In conclusion, careful optimization of extraction pH, temperature and time, conjugase type and method of standard curve calibration are needed to generate reliable food folate data.

\*To whom correspondence should be addressed.

**Determination of carotenoids in fruits and vegetables by LC.** Erik J. M. Konings\* & Harry H. S. Roomans.

*Inspectorate for Health Protection, Food Inspection Service, PO Box 2516, 6201 GA Maastricht, The Netherlands.*

In many epidemiologic studies, an increased intake of fruits and vegetables was associated with a reduced risk of lung and other cancers. We elaborated a method for the determination of lutein, zeaxanthin,  $\alpha$ -carotene,  $\beta$ -carotene,  $\beta$ -cryptoxanthin and lycopene in fruits and vegetables.

After extraction carotenoids were dissolved and a fraction was injected onto the LC-system.

The isocratic LC-system consisted of a Vydac column. Carotenoids were detected using a diode array detector, and quantified by means of the internal standard method. Carotenoids in the saponified mixture were calculated against a mixed standard solution which also was saponified.

The separation of the individual carotenoids on the analytical system was satisfactory. The interference of other components was small. A considerable amount (40%) of lycopene was destroyed by hastalloy-frits.