Concurrent Session 14: Folate

**Folate: Analytical methods for foods**

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**Background** – Analytical methods have been a limitation in the study of folates due to their inability to distinguish accurately between the added form (folic acid) and naturally occurring forms in foods. This is critical in view of the need for accurate data in establishing folate composition, requirements and assessing the bioavailability of the vitamin. The complexity, diversity and instability of folates are substantial obstacles encountered in the development and selection of analytical methods. The analysis of folates is further complicated due to the difficulties in sample preparation which include extraction, deconjugation and extract purification. Currently, there are three main analytical methods: Microbiological assay (MA), High pressure liquid chromatography (HPLC) and biospecific procedures including enzyme protein binding assays (EPBA), enzyme linked immunosorbent assays (ELISA) and radioassays (RBPA). Folate in foods is commonly measured by microbiological assay which is based on the assumption that *L.rhamnosus* (most commonly used folate dependant microorganism) has identical growth responses to the mono-, di- and triglutamyl folate structures present in foods. However there is much debate over this assumption as some investigators report that *L.rhamnosus* response is similar to all forms (O’Broin et al., 1975; Shane, Tamura and Stokstad, 1980) while others do not (Phillips and Wright 1982; Goli and Vanderslice, 1992).

High pressure liquid chromatography separation techniques with ultraviolet and/or fluorescent detection have been documented to detect the different forms of folate. In many instances these methods lack specificity and have failed to reach the required detection limits due to matrix interference by the presence of breakdown products that arise during extraction (Shane, 1986). Recently, LC-MS methods have been reported as an acceptable and accurate approach to the analysis of folates in foods and biological materials which are based on the separation power of reversed phase chromatography coupled with the superior detection capability of mass spectrometry (Rychilik et al., 2003).

**Objectives** –The main objective is to critically review the current status of folate analysis methods. This session will provide a discussion on 1. Strengths and limitations of the various methods of analysis, 2. Considerations in the selection of existing analytical methods and 3. Further research needs concerning folate analysis and an added review on the liquid chromatography mass spectrometry methods available today. Quantification of folates is performed using C$^{13}$ isotopically labelled internal standards.

**Review** – Though the microbiological assay is most commonly used, it is time consuming, needs great care and skill. It cannot however quantify the different forms of food folates. In addition, whether microorganisms respond to the different forms differently is still under question. Immunoassay techniques are quick, easy and cheaper but are not suitable for food folate determination. HPLC has proven to be a better analytical technique and more recently liquid chromatography mass spectrometry (LC-MS) techniques offer better sensitivity and specificity to accurately quantify folates in foods and biological samples. It appears that the method of choice would depend on the purpose.

**Conclusions** – The LC-MS/MS techniques offer an accurate, reproducible and reliable method for profiling and quantifying the folate forms present in foods. This new method provides enhanced sample throughput of 36 samples/12 hours.

**References**