ELSEVIER

Journal of Food Composition and Analysis 18 (2005) 387–397

JOURNAL OF FOOD COMPOSITION AND ANALYSIS

www.elsevier.com/locate/jfca

Original Article

International inter-laboratory analyses of food folate

Prapasri Puwastien[a,*], Naruemol Pinprapai[a], Kunchit Judprasong[a], Tsunenobu Tamura[b]

[a] *Institute of Nutrition, Mahidol University, Salaya, Putthamonthon 4, Nakorn Pathom 73170, Thailand*
[b] *Department of Nutrition Sciences, University of Alabama at Birmingham, Birmingham, AL-35294, USA*

Received 10 June 2003; received in revised form 2 December 2003; accepted 11 February 2004

Abstract

An international inter-laboratory performance of food folate assay was evaluated using soybean flour, fish powder and breakfast cereal which were prepared as test materials. These materials were sent to 34 laboratories, which were asked to use their routine methods of food folate analysis, and 26 laboratories (76%) worldwide returned their assay data. Although trienzyme extraction has been recommended for folate extraction before the assay, this method of folate extraction was used in only nine laboratories, and eight laboratories still performed a single enzyme treatment using folate conjugase. Of these 26 laboratories, 20 used microbiological assay (17 used *Lactobacillus casei*), four used an HPLC-UV detection method, one LC-MS and one radiobinding assay for folate analysis, indicating a wide variety of folate detection methods. Among 17 laboratories where *L. casei* microbiological assay was performed, the inter-laboratory coefficient of variations of these test materials was 24%, 35% and 24% for soybean flour, fish powder and breakfast cereal, respectively, indicating that a valid comparison of the values between the laboratories may be difficult. Our observations suggest that for food folate analysis, it is important to standardize the methods of folate extraction and detection, and the use of reliable reference materials should be encouraged among laboratories worldwide.

Keywords: Food folate; Inter-laboratory evaluation; Reference material; Trienzyme extraction; Microbiological assay; Quality control

1. Introduction

During the last decade, the importance of adequate folate intake has become well recognized in the reduction of the rate of neural tube defects and in the possible prevention of cardiovascular

*Corresponding author. Tel.: +66-24410217; fax: +66-24419344.
E-mail address: nuppw@mahidol.ac.th (P. Puwastien).

0889-1575/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.jfca.2004.02.011

disease by lowering plasma homocysteine concentrations (MRC Vitamin Study Research Group, 1991; Hankey and Eikelboom, 1999). Thus, it is important to accurately assess folate intake in the general population; however, this is difficult because food folate data in the literature are known to be notoriously inaccurate. The problem of food folate data became more apparent after trienzyme extraction (use of three enzymes including protease, α-amylase and folate conjugase) was recognized as the preferred method of food folate extraction compared to traditional conjugase treatment alone (De Souza and Eitenmiller, 1990; Martin et al., 1990). In the last several years, the use of this method has become popular (Tamura et al., 1997; Pfeiffer et al., 1997; Lim et al., 1998; Tamura, 1998; Rader et al., 1998, 2000; Aiso and Tamura, 1998; Konings, 1999; Eitenmiller and Landen, 1999; Konings et al., 2001; DeVries et al., 2001; Johnston et al., 2002a, b; Iwatani et al., 2003; Doherty and Beecher, 2003; Yon and Hyun, 2003; McKillop et al., 2002, 2003; Han and Tyler, 2003; Pentieva et al., 2003), although results were not always satisfactory (Shrestha et al., 2000; Ndaw et al., 2001).

At the Third International Food Data Conference held in Rome in 1999, the working group on "Folate Bioavailability" reached a consensus that the use of certified reference materials of known folate content should be encouraged in addition to the improvement in folate assay methods. We thought that the development of reference materials at a low cost was important, since commercially available certified reference materials are expensive especially for laboratories in the developing countries where resources are limited. We evaluated an inter-laboratory performance of food folate assay using three test materials, which were prepared for the study presented here.

2. Materials and methods

2.1. Test materials

Three dry food items, soybean flour, fish powder, and breakfast cereal, were selected as test materials for this study. These items are readily available and inexpensive, and represent different food types and matrices. Soybean is an oil-seed legume with a relatively high folate content, and fish powder contains high protein with a moderate amount of fat and folate. Breakfast cereal contains a large amount of carbohydrates with a small amount of fat, with fortified folic acid (pteroylglutamic acid, about 300 μg per 100 g) being the major folate.

2.2. Preparation and evaluation of test materials

Soybean flour and breakfast cereal were provided by Nestle, Thailand, and fish powder was prepared by Overseas Merchandize Inspection, Thailand. Each food item (2–3 kg) was ground by the producers until fine powders were obtained, and the powders were then passed through a standard sieve (35–50 mesh) and thoroughly mixed before packaging. Portions of samples (about 15 g each) were placed in laminated aluminum foil bags under vacuum.

Seven packages of each test material were analyzed for folate content by microbiological assay in duplicate for the evaluation of sample homogeneity (between-sample variation within the same test material). The stability of folate in these test materials was evaluated after 1 week at room temperature (30–32°C), which generally represented the shipping conditions to various