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Original Article

Analysis and evaluation of voluntary folic acid fortification of breakfast cereals in the Spanish market

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ABSTRACT

Folic acid (FA) is a relevant factor in the prevention of a number of pathologies; thus supplementation and/or fortification strategies using FA have been widely introduced as a result. In Spain, there is a lack of reliable data to assess the impact of the increasing number of FA fortified foods. The objective of this work was to evaluate FA fortification levels in breakfast cereal products in Spain. Seventy-three breakfast cereals were analysed for total folate (TF) content. Adequacy was evaluated vs. labelled values (LV), recommended intakes (RI) and tolerable upper intake levels (ULs). Mean TF content ranged from 253 to 427 μg/100 g (76–128 μg/30 g) in different cereal matrix categories. Higher TF content was found in wheat and bran/whole-wheat cereals. As for commercial types, low-fat cereals contained the highest TF levels (445–630 μg/100 g). By consuming these, children (1–9 years old) and women of childbearing age could meet 40–160% and 20–40% of their RIs, respectively, with a standard serving size (30 g). However, children 1–6 years old are at higher risk of excessive FA intake, since low-fat cereals contain more than 50% of their ULs. Our conclusion is that overage (the addition of excess vitamin content) is a practice in FA fortified breakfast cereals. This could be a potential risk for children, but a benefit for women of reproductive age. Physiological status and age are therefore critical factors to take into account to give pertinent advice in consuming FA fortified foods.

1. Introduction

Folic acid (FA) is the synthetic form of a naturally occurring water soluble B-group vitamin, generically known as "folate". It is an essential nutrient involved in the prevention of macrocytic anaemia (Wills, 1931). In the second half of the last century, it was identified as a potential relevant factor in the prevention of cardiovascular disease (Rader, 2002), colorectal cancer (Kim, 2004), neurocognitive decline (Morris et al., 2007), and congenital abnormalities affecting the development of the spinal cord and central nervous system, known as neural tube defects (NTDs) (Czeizel and Dudas, 1992; MRC, 1991). The evidence linking FA to NTDs prevention led to the introduction of public health strategies to increase folate intake: pharmacological supplementation, mandatory or voluntary fortification of staple foods with FA, and the advice to increase the intake of folate-rich foods. In 1998, mandatory fortification of wheat flour and other cereal products with FA was introduced in the US and Canada (IOM, 1998). At present, more than 40 countries have implemented this policy, but most European countries, including Spain, only require addition of this vitamin on a voluntary basis (CDC, 2008).

It has been stated that nutritional folate requirements for disease prevention may be higher than those needed for prevention of the classical vitamin deficiency syndromes (IOM, 1998), for which an adequate folate status can be achieved through regular diet. However, scientific evidence is still inconclusive (Wald et al., 2006; Rader, 2002) and the only established recommendations at present are aimed at women of childbearing age for reducing their risk of having a NTDs affected pregnancy. In 1998, the US Food and Nutrition Board advised that all women capable of becoming pregnant should consume a daily dose of 400 μg of synthetic FA, either in the form of fortified foods or supplements, in addition to naturally occurring folates from food (IOM, 1998). In Spain, the recommended dietary intakes have been set for FA at the same level (400 μg/day) for women of childbearing age, 600 μg/day for the second half of pregnancy and 500 μg/day for women who are breastfeeding (Moreiras et al., 2008a).

Folic acid is a monoglutamate highly stable form of folate, which is more easily absorbed in the intestinal tract than the natural vitamers. Traditionally, it has been considered a safe vitamin; nonetheless, excessive intakes could derive in the masking of vitamin B12 deficiency in the elderly, a condition that

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has been proven to cause irreversible neurological damage (Morris et al., 2007). In the past few years, serious concerns have arisen concerning other potential risks, mainly in vulnerable groups such as children and the elderly (Kim, 2007). FA does not occur in nature and its metabolism and bioavailability in humans is not completely understood (Wright et al., 2007). Recent studies using labelled folates coupled with sensitive LC–MS/MS techniques have hypothesised that the liver could be the initial site of FA metabolism in humans, and that because of its low capacity for FA reduction, saturation could take place at certain intake levels, resulting in unmetabolised FA entering the systemic circulation (Wright et al., 2007). A number of studies highlight the chronic presence of increased unmetabolised FA in the bloodstream as a potential risk factor for other deleterious effects (Kalmbach et al., 2008; Troen et al., 2006). Nevertheless, the values for tolerable upper daily intake limits (UL) were established by taking into consideration only data related to the masking of B12 deficiency (EC, 2000). For all these reasons, European authorities are still reluctant to implement mandatory FA fortification (SACN, 2006).

It is quite contradictory to observe that, regardless of these findings, there is only limited information on food folate and FA content. Folate data in food composition tables and databases are scarce or incomplete: moreover, several studies suggest that current data values are derived from assay procedures that underestimate folate content in foods (Gregory III, 1998; Arcot and Shrestha, 2005). Fortification of staple foods with FA has added difficulty to this task (Pawlosky et al., 2003; Johnston and Tamura, 2004) and despite the increasing number of FA fortified products available in Spain, limited work has been done to identify these products and assess their actual FA content (Perez Prieto et al., 2006).

Scientific work groups across Europe have underlined that this lack of reliable data on folate content of fortified foods makes the evaluation of dietary folate intake levels among vulnerable population groups usually unfeasible (Finglas, 2005; Dragsted et al., 2009). As stated in an International Life Sciences Institute's (ILSI) workshop, held in 2005 (Bologne, Italy), "New Horizons for the safe addition of micronutrients to food", FA fortified food composition data has been considered a key research need in order to apply sound risk-benefit analysis methodologies (Dragsted et al., 2009).

The objective of this study was to evaluate voluntary FA fortification levels in commonly available breakfast cereal products in Spain and assess their adequacy for increasing the daily folate intake in different population age groups.

2. Materials and methods

The use of an updated food composition database developed by our group, which includes FA fortified products available in the Spanish market (Samaniego-Vaesken et al., 2009), allowed us to identify breakfast cereals as the most representative food group.

Briefly, assayed total folate values (TF) were compared with FA label values (LV) and used to calculate the theoretical adequacy of these products to both Spanish recommended folate intakes (RI) (Moreiras et al., 2008a) and tolerable upper intake levels (ULs) (EC, 2000) for different age groups on a basis of manufacturer's average recommended standard serving size (30 g).

2.1. Food sample trienzyme extraction

A total of 73 commercial breakfast cereal products were purchased at local supermarkets and retail stores and analysed for TF content. Two different batches of each sample were independently grounded, processed and analysed in triplicate under subdued light, minimising contact with air. All assays were completed within a month of the product's acquisition and reagents were purchased at analytical grade. Taking the high starch matrix composition into account, extraction and enzyme treatments were carried according to a previously described trienzyme extraction method (Martin et al., 1990) with modifications (Póo-Prieto et al., 2006). Shortly, 0.5–1 g of sample was homogenised in 10 vol. of 0.026 M Tris–HCl extraction buffer (pH 7.4) containing sodium ascorbate (1% w/v) in polyallomer centrifuge tubes (Beckman Instruments, Germany). Tubes were capped and autoclaved for 15 min at 120 °C (1.034 bar). Homogenates were then cooled and sequentially incubated in a shaking water bath at 37 °C with a 20 mg/mL α-amylase solution (Bacillus sp (EC.3.2.1.1), Sigma) and chicken pancreas conjugase (Difco, Detroit, MI, EEUU) (Keagy, 1985) for 4 h, followed by a 2 mg/mL protease solution (type XIV, Streptomyces griseus, Sigma) for 1 h. Enzyme activity was stopped in a boiling water bath for 5 min. Homogenates were cooled on ice and centrifuged for 20 min at 36,000 × *g* at 4 °C. Finally, supernatants were filtered through sterile syringe filters (Millex-AA, 0.8 μm, Milipore) and stored at −20 °C until further analysis.

2.2. Microbiological assay of folates

TF was determined in each extracted sample by a method that relies on *Lactobacillus casei* ssp. *rhamnosus* (ATCC 7469) folate-dependant growth (Horne and Patterson, 1988; Tamura, 1990).

An automatic microplate reader fixed at λ = 600 nm (DigiScan Reader, Asys Hitech, Austria) and sterile 96-well microplates were used. Enzyme blanks were assayed to account for potential endogenous folate contribution. Sterile plastic, glassware, water and reagents were used. Standard stock solutions were prepared by dissolving FA (Sigma) in 0.01 mol/L NaOH (20 μmol/L) and concentrations were determined in pH 7.0 buffered solutions, using UV absorption at λ = 282 nm for FA and a molar extinction coefficient (ε) of 27,000 $mol^{-1}\,cm^{-1}$ (Blakley, 1969). Suitable volumes of the stock solution were diluted with water to construct an 8-point calibration curve.

2.3. Quality control and statistical analysis

A Standard Reference Material, FA fortified infant formula (SRM 1846, NIST) (Sharpless et al., 1997), was used to monitor inter- and intra-assay variation. A subsample of SRM 1846 was analysed with every batch to monitor repeatability. Recovery experiments were performed using commercial wheat flour spiked with FA standard solutions prepared as described above, at a 1.5 μg AF/g level.

Statistical analysis was performed using SPSS 15.0 work package. For each sample, two different batches were analysed and TF values obtained in each were compared with Student's *t*-test for independent samples, with a significance level set at $p < 0.05$.

3. Results and discussion

3.1. Quality control of the trienzyme-microbiological assay

Intra- and inter-assay precision values obtained for SRM 1846 were (mean ± S.D.) 1.45 ± 0.6 mg/kg ($n = 6$) (%CV = 4.1) and 1.5 ± 0.8 mg/kg ($n = 6$) (%CV = 5.7), respectively. Values were higher than the mass fraction of 1.29 ± 0.28 mg/kg determined by microbiological assay with *L. casei* for this material (Sharpless et al., 1997). The reason for this difference may be due to that SRM 1846 was developed with an extraction method that does not include the trienzymatic extraction, therefore yielding a lower folate release from the food matrix. Recovery values for FA spiked wheat flour were 87.5 ± 5% (CV = 5.7%, $n = 10$).