Journal of Food Composition and Analysis 28 (2012) 155–163

Contents lists available at SciVerse ScienceDirect

Journal of Food Composition and Analysis

journal homepage: www.elsevier.com/locate/jfca

Critical Review

Lack of data on folate in convenience foods: Should ready-to-eat products be considered relevant for folate intake? The European challenge

Violeta Fajardo *, Elena Alonso-Aperte, Gregorio Varela-Moreiras

Departamento de Ciencias Farmaceúticas y de la Alimentación, Facultad de Farmacia, Urb. Montepríncipe, Crta. Boadilla Km. 5.3, Universidad CEU San Pablo, 28668 Boadilla del Monte, Madrid, Spain

ARTICLE INFO

Article history:
Received 31 January 2012
Received in revised form 13 July 2012
Accepted 26 July 2012

Keywords:
Food composition
Food analysis
Folate
Folic acid
Fresh-cut products
Ready-to-eat foods
Nutrient composition data
Nutrient database
Folate status

ABSTRACT

Promoting folate intake from natural food sources is a healthy worldwide strategy for attaining safe levels of folate in overall nutritional status and avoiding potential harm from chronic excessive intakes of folic acid from fortified food products or supplements in certain population groups. Over recent years the consumption of ready-to-eat foods, such as packed vegetables or precooked meals, has become a significant part of the diet. Accordingly, the folate composition of these food categories must be investigated. There is a broad lack of folate data in food composition tables and databases, especially for ready-to-eat products. This context warrants the need for providing new data on total folate and individual forms of folate in ready-to-eat commercial products, either to complete food composition tables or databases and/or to achieve regulatory objectives, or to assess population dietary intakes. Currently, intake recommendations for folate in some European countries range from 400 to 500 μg/day for folate for women of childbearing age, 600 μg/day for the second half of pregnancy and 500 μg/day for women who are breastfeeding. For other population groups, the recommended daily intakes (RDI) for folate are established depending on the age and sex of the individuals (children around 150–200 μg/day, and adults and elderly, 300–400 μg/day), but vary considerably among European countries. Moreover, contradictory data exist concerning both recommended and real dietary intake of folate throughout Europe. Despite a wide variety of analytical methods available for food folate measurement (microbiological assay or high performance liquid chromatography (HPLC), with a previous enzyme extraction based on the use of amylase, protease and/or folate conjugase; HPLC coupled with mass spectrometry; alternative protein-binding and immunoassay methods), many procedural complexities continue to result in poor agreement among methods and laboratories. Given the uncertainty involved in accurately measuring folate, the available certified reference materials should be used by laboratories to check the accuracy of folate data. The challenge to improve quantity and quality of folate data in food composition databases exists in most developed countries, and particularly in Europe, in the absence of mandatory food fortification policies for folate.

1. Introduction: folate health

Human life cannot exist without folates, because they are essential cofactors involved in vital functions of cell metabolism such as DNA replication, repair, methylation, synthesis of nucleotides, and metabolism of other vitamins (e.g. vitamins B_2, B_6, B_{12}) and amino acids (e.g. methionine, homocysteine). The generic term "folates" or "folate" refers to a group of water-soluble B vitamins, which include naturally occurring forms present in food, containing one to six additional glutamate molecules linked through a peptide bond to the γ-carboxyl group of glutamine, such as 5-methyltetrahydrofolate (5-CH_3-H_4PteGlu), 5-formyltetrahydrofolate (5-HCO-H_4PteGlu), 5,10-methenyltetrahydrofolate (5,10-CH-H4PteGlu), 10-formyltetrahydrofolate (10-HCO-H_4PteGlu) and 5,10-methylenetetrahydrofolate (5,10-CH2-H4PteGlu) (Blancquaert et al., 2010). Plants, fungi and many microorganisms synthesise folate de novo, but humans and other higher animals require a dietary supply. Thus, low dietary intake is the most common cause of folate deficiency. Important health problems could be related with vitamin scarcity, ranging from megaloblastic anaemia to more recently described complications such as high homocysteine levels and cardiovascular risk (Cui et al., 2010; Rader, 2002), certain cancers such as colorectal and colon (Kim, 2007; Lee and Chan, 2011), neurocognitive decline in the elderly (Morris et al., 2007), and congenital abnormalities in the development of the spinal cord and central nervous system,

* Corresponding author. Tel.: +34 913724811; fax: +34 913510475.
E-mail address: violeta.fajardomartin@ceu.es (V. Fajardo).

0889-1575/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jfca.2012.07.009

known as neural tube defects (NTDs) (Amarin and Obeidat, 2010; Czeizel and Dudas, 1992). Epidemiological evidence suggests that a suboptimal folate intake, considering the updated dietary reference intakes, may be widespread for many worldwide populations (Herrmann and Obeid, 2011; Yeung et al., 2011).

Mass fortification of grain products with "folic acid" (FA), the most oxidised, stable and easily absorbable synthetic form, has been mandatory in the United States and Canada since 1998 (Shakur et al., 2009). As a result, more than 53 countries, mostly in the Americas, have also implemented mandatory fortification. Results from various studies have shown that voluntary fortification is also well accepted in many European countries (Berry et al., 2010; Cordero et al., 2010; Samaniego-Vaesken et al., 2010). Despite this nutritional intervention and the recommendation of taking FA supplements, specially by women in childbearing age, NTDs are still an important cause of mortality and morbidity, with a conservative estimated incidence of 4.3 million new cases per year (Herrmann and Obeid, 2011; WHO, 2004). It has been estimated that only about 10% of NTDs are currently being prevented through dietary FA fortification (Bell and Oakley, 2009).

Natural food sources of folates include green leafy vegetables with abundant levels of chlorophyll (spinach and collard greens), legumes, some fruits (oranges and grapefruit juice), yeast, eggs and organ meats such as liver, kidney or tongue (Devi et al., 2008; Johansson et al., 2008).

According to data from the Food and Agriculture Organization of the United Nations (FAO), a desirable trend has been observed in the supply of fruits and vegetables in Europe over the past four decades, with an increase of 60% and 34%, respectively (Elmadfa and Freisling, 2009; EUFIC, 2012). However, with the exception of Greece and Spain, Europeans are still far from meeting the dietary goal of at least three portions of vegetables per day (approximately 250 g). Only Mediterranean countries and Austria exceeded the World Health Organization (WHO) recommendations of at least two portions of fruits per day (Elmadfa and Freisling, 2009; Pozo et al., 2012). Conversely, an undesirable decreasing tendency in the consumption of pulses was observed for European countries (Elmadfa et al., 2009). In general, consumption of folate-rich foods showed a South–North gradient in Europe, with the intake of vegetables, fruits and pulses much higher in the South, and with levels far below the average in the North (Elmadfa et al., 2009).

2. Ready-to-eat food products: fresh packed vegetables and precooked chilled meals

During the past few years, as a response to consumer demands, the so-called ready-to-eat food group, such as fresh cut packed vegetables and precooked chilled meals, is becoming increasingly popular worldwide. *Fresh-cut products* are defined as any vegetable, fruit, or their combination, that has been physically modified from the original form (by trimming, washing, peeling and chopping), but remains in the "fresh state". The standard procedure includes packing inside plastic bags with a controlled atmosphere to prevent perishable contents from decay, without the use of additives or preservatives. Chilled ready-to-eat meals are food products prepared and cooked in advance, and suitable to eat as sold, commonly made of meat, pastry, vegetables, fish or shellfish. All these commercial products have to be stored under refrigeration (~4 °C) (Ahlgren et al., 2004; Martín-Belloso and Soliva-Fortuny, 2011).

The market for ready-to-eat food products has shown substantial growth in Europe over the most recent decades. The main reasons are: (i) customers' claims for fresh, safe and additive-free foods, (ii) facilitation of fruit and vegetable consumption by consuming these preparations everywhere, (iii) the integration of women into the workforce, and (iv) convenience due to lack of time for cooking, as these preparations require little preparation time and potentially retain their original nutritional properties. In addition, the demand for frozen, dried or sterilised products is being substituted by the consumption of precooked refrigerated products since they are considered healthier and of higher quality (Ahlgren et al., 2005; Martín-Belloso and Soliva-Fortuny, 2011). Within this context, it should be pointed out that many chilled ready-to-eat products could be highly processed and energy-dense, with a high content of salt and saturated fats, and somewhat micronutrient-poor due to nutrient loss during processing of the foods. In any event, there is a lack of information about their final nutrient composition.

2.1. Availability of fresh-cut products

Fresh-cut food products were first introduced in Europe in the 1980s, to facilitate caterers and restaurants. Nowadays, although food service is not to be ignored, retail is the major distribution channel for fresh-cut vegetables and fruits. Supermarket departments carry several hundreds of varieties today, and production and commercialisation of fresh-cut fruits have grown rapidly in recent years. However, fresh-cut vegetables, salad made from iceberg lettuce in particular, dominate the production of minimally processed foods (Garrett, 2002; Zhang, 2007).

The largest European fresh-cut product markets are United Kingdom (UK), France and Italy. The Netherlands, Switzerland, Italy and Spain have already established a profitable market and show strong development also in fresh-cut goods. Particularly, Spanish sales reached 70.6 million kg of fruit and vegetable fresh-cut products in 2010, representing an increase of 6% over the previous year (Fernández, 2009; Martín-Belloso and Soliva-Fortuny, 2011). The global consumption of vegetables and fruits has remained constant over the last seven years, but fresh-cut products have gained more acceptances, representing at present roughly a 5% of all fruits and vegetables consumed in Spain (1.5–2 kg per capita per year). However, these figures are still far lower than those of the leader of fresh-cut production and consumption in the world, the United States (30 kg per capita per year) (Alonso, 2007).

2.2. Availability of chilled ready-to-eat meals

The concept of chilled ready-to-eat meals came from convenience quality food served at home from fine restaurants, as a consequence of the increase in disposable income within Europe during the 1980s. It is therefore very common to display convenience products which tend to have a "healthier" look and are more flavoursome than comparable frozen food products. Technological advances and cultural shifts have transformed the chilled prepared foods sector into one of the most dynamic departments in the food market (ECFF, 2011; Parker, 2010).

The overall changing offer and the lack of data for these kinds of food preparations make it difficult to accurately estimate real intake in terms of nutrients and bioactive compounds. However, it is well-recognised that the UK is also the leader in Europe in this sector, followed by France, Germany, Italy and Spain (ACNielsen, 2006; Bourgeois, 2010). In Spain, pizzas are the most important supply (44.3% of total sales and 51.4% in value), followed by *gazpacho* (a cold tomato soup), tortillas, Mediterranean recipes (*paella*, *cocido*, *callos*, etc.), salads, bagels, soups, sauces and sandwiches. Consumption of these products has shown a positive evolution of 8%, both within consumers and foodservice, bringing the total consumption of chilled ready-to-eat meals to 10 kg per capita in 2009 (MARM, 2006; Mercasa, 2010).

Surprisingly, in spite of the increasing consumption of these food products in developed countries during recent decades,

especially in Europe, there is a lack of information about their final nutrient composition.

3. Importance of folate status and achievements

The evidence linking folic acid (FA) to NTDs prevention was the basis for establishing revised recommendations for folate. In 1998, The US Food and Nutrition Board advised that all women intending to become pregnant should consume a daily dose of 400 μg of synthetic FA, in the form of either fortified foods or supplements, in addition to naturally occurring folates from food (IOM, 1998). Similarly, European institutions raised recommendations to levels ranging from 400 to 500 μg/day for folate in target populations (IOM, 2000). In Spain, folate recommended dietary intakes have been set at 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., 2010). For other population groups, the recommended daily intakes (RDI) for folate are established depending on the age and sex of the individuals (children around 150–200 μg/day, and adults and elderly, 300–400 μg/day), but vary considerably among European countries, making it difficult to compare actual intakes when they are expressed as a percentage of the recommended intake (IOM, 2000; Moreiras et al., 2010).

In any case, to cope with these recommendations, pharmacological supplementation, mandatory or voluntary fortification of staple foods with FA, and the advice to increase the intake of folate rich foods have been introduced in different countries. It should be noticed that the latest RDI for folate are now commonly expressed in μg of dietary folate equivalents (DFEs) rather than in μg of folate, because this expression takes into account the greater bioavailability of FA compared to naturally occurring food folate. 1 μg of folate found naturally in food is equal to 1.0 μg DFE. However, there is the fact that the synthetic monoglutamate form of FA used in supplements is considered to be more available than food folate, when consumed independently of food: 1 μg of FA in a supplement is equal to 2.0 μg DFE. FA added to food for fortification purposes is also more active than folate naturally occurring in food, but slightly less active than FA taken as a supplement: 1.0 μg of fortification folate is equal to 1.67 μg DFE (Shuaibi et al., 2009).

Dietary surveys often underestimate the real intake of food items and, consequently, of nutrient amounts. Moreover, as regards folate intake, these studies do not always include the contribution of FA from fortified foods and from FA supplements (BfR, 2007; Black et al., 1991). In this sense, recently published studies have found contradictory results within Europe. Elmadfa and Freisling (2009), in the European Nutrition and Health Report 2009 (ENHR), postulated that mean intakes of folate were below the recommended levels in practically all of the participating countries and all population groups (Elmadfa and Freisling, 2009; Elmadfa et al., 2009). On the contrary, Dhonukshe-Rutten et al. (2009) observed that Finland, UK, Ireland, France, Italy, Spain and Germany, had a mean folate intake at or above their respective national recommendations, whereas in Norway, Sweden, Denmark and The Netherlands, mean folate intake levels were lower than recommendations. Most of the published data indicated that the average folate intake ranged from 200 to 350 μg/day and men had a higher mean folate intake than women in Europe (Dhonukshe-Rutten et al., 2009; Flynn et al., 2009; Park et al., 2011; Tabacchi et al., 2009). In particular, Varela-Moreiras et al. (2010) concluded that folate did not reach 80% of the recommended nutrient intake for adults from both sexes aged 20–40 years in Spain, observations that may be indicative of insufficiency. The Spanish diet provides roughly 233 μg/person/day of folates, more than 50% from vegetables, fruits and legumes (Pozo et al., 2012).

Another way to ascertain an individual's nutritional status is serum folate, a marker of recent dietary intake. Dhonukshe-Rutten et al. (2009) stated that folate status ranged from 6.3 to 20.1 nmol/L in Europe. Within countries, most revealed a comparable folate status: a "low" level was defined as lower than 10 nmol/L, 10–15 nmol/L was established as "moderate" and above 15 nmol/L as "favourable". Consequently, the highest levels were observed in UK, Germany and Spain. A moderate folate status was found in Finland, Ireland, Czech Republic, Portugal and Italy, whereas a low status was shown in Norway, Sweden, The Netherlands and Greece (Dhonukshe-Rutten et al., 2009). However, some of these data are controversial, since the high folate intake reported by Park et al. (2011) and the significant vegetable consumption described by EUFIC (2012) for Greek population are not in accordance with the statement of "low" folate status in Greece (Dhonukshe-Rutten et al., 2009). According to the revealed daily intakes from the European Nutrition and Health Report 2009 (ENHR), folate status is unsatisfactory for most of the European populations (Elmadfa et al., 2009; Park et al., 2011).

During the FA postfortification era, the entire distribution of serum and red blood cell folate concentrations has shifted to levels of sufficiency and even excess in some populations which have implied mandatory fortification such as the United States (Yang et al., 2010). The recent 2010 analysis of the data from the National Health and Nutrition Examination Surveys (NHANES) shows that only 2.7% of the population had daily intakes above the tolerable upper limit (UL) of synthetic FA (1000 μg for adults aged $\geq$ 19 years) set by the Institute of Medicine (IOM, 1998). Therefore, these researchers gave some assurance about the right level of FA for fortification since it is unlikely that US adults, who consume fortified food and supplements averaging up to 400 μg FA/day, would exceed UL for FA. However, Yeung et al. (2011) suggested that the majority of usual daily intakes from US children exceeded their age-specific UL by consumption of different sources of FA. This evidence raises doubts about the safety of mandatory FA fortification, since some authors hypothesise possible long-term adverse effects of excess FA, such as cancer and tumour promotion, epigenetic hypermethylation, miscarriages, multiple births, and the well-documented masking of vitamin B_{12} deficiency (EFSA, 2009; Shakur et al., 2009; Johnston, 2008).

Taking into account all these aspects, the promotion of folate intake from natural food sources continues to be a potential health strategy to reach a safe and adequate nutritional status. Within this framework, the expansion in the detailed consumption of ready-to-eat foods, as a partial or total replacement of traditional items for lunch and dinner, results in an increasing concern about the nutrient density of these products, especially for folates, in vegetable products and recipes. Accordingly, the nutritional composition of these food categories should first be investigated in order to further estimate their contribution in vitamins and other nutrients and their impact on dietary habits for European populations (Lee and Chan, 2011).

4. Determination of folate content in ready-to-eat products

The need for a critical evaluation of all kind of dietary folate sources in order to give more reliable folate content in foods and commercial products has been emphasised by several authors (Dhonukshe-Rutten et al., 2009; Flynn et al., 2009; Johansson et al., 2008). However, as has been discussed above, there is only limited information on food folate and folic acid content. The European Food Information Resource Network of Excellence (EuroFIR) project has set up a working group (BDECA) to create a unified food composition database because of the lack of a reference European nutrient database (http://www.eurofir.eu) (Bouckaert et al., 2011).

Moreover, The European Prospective Investigation into Cancer Nutrient Database (ENDB) program was initiated by the *International Agency for Research on Cancer* to provide standardised nutrient databases with the collaboration of 10 European countries. The ENDB has been completed for a first set of 26 priority nutrients, and has yet to be completed with folate data, considered as a prerequisite in the extension of the ENDB project (Bouckaert et al., 2011; Martínez et al., 2009). Nonetheless, folate data in food composition tables and databases is still scarce or incomplete, especially for novel products such as cooked or ready-to-eat meals. In addition, several studies suggest that current data values are derived from assay procedures that underestimate folate content in foods (Arcot and Shrestha, 2005; Samaniego-Vaesken et al., 2010). For all these reasons there is a need at present not only to improve, but also to provide new data on total folate and individual forms of folate in mixed diets which contain ready-to-use commercial products, to supply data for food composition tables or databases and/or to support regulatory purposes (Ros et al., 2009; Uusitalo et al., 2011).

The information and values showed in food composition tables and databases are either based on chemical analyses, or estimated from previous data. For example, the selection of data from tables from other countries is less expensive and time consuming than the production of new values, but some problems can arise (Bouckaert et al., 2011). Many studies have compared food composition values collected from different sources and have demonstrated the difficulties in making international comparisons due to variations in nomenclature, composition of foods and analytical procedure employed. To avoid these limitations, systematic studies for the development and optimisation of accurate methods are required to: (i) elaborate complete food composition tables, databases and nutritional intervention studies, (ii) assess their adequacy in diets, (iii) add nutritional information in the food products, and (iv) verify by food control laboratories that the information provided on nutritional labelling is correct (Elmadfa and Meyer, 2010; Johansson et al., 2007; Martínez et al., 2009; Olivares et al., 2006).

Several methods have been reported to determine folate content in foods, based predominantly on microbiological assay and high performance liquid chromatography (HPLC), with a previous enzyme extraction in both cases, based on the use of amylase, protease and/or folate conjugase (AOAC, 2006; Arcot and Shrestha, 2005; Gujska et al., 2010; Póo-Prieto et al., 2011). Currently, HPLC coupled with mass spectrometry (LC–MS–MS) is increasingly applied (Phillips et al., 2008; Vishnumohan et al., 2011). Alternative protein-binding and immunoassay methods exhibit unique advantages due to the inherent biospecific nature of the detection molecule (Alaburda et al., 2008; Martin et al., 2010). Besides, the novel photosynthetic proteins-based devices, called biosensors, have been successfully developed in food analysis applications with promising expectations for the detection of folate content (Lavecchia et al., 2011). Despite the plethora of analytical strategies available for food folate measurement, many procedural complexities continue to result in poor agreement among methods and laboratories (Koontz et al., 2005; Soongsongkiat et al., 2010).

The vast majority of methodologies published to date for folate content quantification are focused on raw and frozen vegetables, fruits, whole meals, and fortified food products (Hefni et al., 2010; Johansson et al., 2008). In contrast, considerable lesser approaches have been reported so far dealing with fresh-cut and chilled ready-to-eat vegetable products. The following sections of this review present an overview of the main methodologies reported so far in the literature to determine folate concentrations in variety of foods, particularly, vegetables, fruits, and ready-to-use products. It should also be noted that although some of the described techniques have not yet found a widespread application for folate analysis in ready-to-eat products, it is most likely that their use will be extended in the future (Della Lucia et al., 2011; Elmadfa and Meyer, 2010; Martín-Belloso and Soliva-Fortuny, 2011; Martínez et al., 2009; Olivares et al., 2006).

4.1. Microbiological assay

For decades, the most common procedure used to express folate data in food composition databases has been the widely accepted microbiological assay, which relies on the folate-dependent growth of *Lactobacillus casei* subspecies *rhamnosus*, as measured by turbidity. This provides "total folate". Although there are other organisms suitable for the quantification of folate content, *L. rhamnosus* responds to the widest variety of folate forms, including 5-methyltetrahydrofolate and the formyl derivatives (Arcot and Shrestha, 2005; Bouckaert et al., 2011). The Association of Official Analytical Chemists (AOAC) has approved this method for total folate analysis in foods as a standardised technique, after validated inter-laboratory studies (AOAC, 2006). Table 1 summarises the most relevant published studies on folate content determination in vegetables, fruits and meals by microbiological assays.

Although microbiological analysis is considered as the premium method for folate quantification, there are some associated problems: the different response of the organism to the folate forms, improper maintenance of assay organisms, erroneous

Table 1
Folate content determination in ready-to-eat vegetables, fruits and precooked meals by microbiological assay.

Food products	*n*	Extraction method	Total folate content (μg/100 g)	Reference
Fast food (hamburguer, pizza, sandwich, and Mexican food) Breakfast items (sausage and steak biscuit)	56	Tri-enzyme	165–401	Johnston et al. (2002)
Vegetables	22	Chicken pancreas	100–425	Iwatani et al. (2003)
Korean foods (vegetables, legumes, and fruits)	111	Tri-enzyme or folate conjugase	2–2112	Yon and Hyun (2003)
Commercially packaged fresh spinach	ND	Di-enzyme	84–225	Pandrangi and Laborde (2004)
Foods (strawberry, orange juice, spinach, pinto beans, macaroni, and meat and vegetable pizza)	6	Variable Interlaboratorial study	3.7–244	Koontz et al. (2005)
Fijian foods (local vegetables and poultry products)	18	Tri-enzyme	3–256	Devi et al. (2008)
Indian foods (rice, legume, vegetable, milk, and wheat based preparations)	43	Tri-enzyme	256–130	Vishnumohan et al. (2009)
Indian fruits	33	Tri-enzyme and single enzyme	10–328	Akilanathan et al. (2010)
Broccoli, spinach, potato, lentil, soy, strawberry, egg and yolk hen, beef liver, and white bread	9	Folate congujase (rat or human plasma)	15–350	Bassett and Sammán (2010)
Dried fruit (Sultanas, Sunmuscat, currants, prunes, and apricots)	41	Tri-enzyme	15–73	Bennett et al. (2011)

n: number of samples; ND: non specified.

dilution of assay media, or folate contamination of reagents and glassware. In addition, the technique itself is labour-intensive, time-consuming and it cannot distinguish between folate forms.

Differentiation is crucial in folate analysis, since the vitamers show diverse bioavailabilities and stabilities. For that purpose, HPLC approaches have recently been developed (Arcot and Shrestha, 2005).

4.2. High performance liquid chromatography (HPLC)

Chromatographic techniques involve two distinct steps after digestion of the samples: separation and purification of deconjugated extract, and detection and quantification of eluted monoglutamates (Arcot and Shrestha, 2005; Martin et al., 2010).

HPLC coupled with ultraviolet absorption (UV), fluorescence detection (FDA) or electrochemical detection (ED) offers the possibility of profiling inherent polyglutamyl folates. However, these technologies are limited by sensitivity or lack of selectivity and require complicated clean-up procedures as well as expensive affinity columns (AC) using folate-binding protein (FBP) for purification (Finglas et al., 1999; Martin et al., 2010). In order to overcome these barriers, the more recent use of HPLC, liquid chromatography–mass spectrometry (LC–MS) and/or liquid chromatography–tandem mass spectrometry (LC–MS/MS) presents the potential for measuring individual folate mono-glutamates with accuracy, precision, high sensitivity and selectivity at trace-level analyte, in complex samples, in a short time. However, HPLC approaches have not been adequately validated for folate content determination in food analysis. Moreover, the summation of individual folates (obtained by HPLC or LC–MS/MS) has been used in compilation to provide total folate. This should be avoided unless the methods have been well-validated and adequate documentation is provided (Bouckaert et al., 2011; Wang et al., 2010). In LC–MS the addition of a stable isotope as international standard is inevitable, not only to correct for recovery losses, but also to compensate for variations in ionisation yields due to matrix interferences. Besides, these assays require standards for all the isomers (Freisleben et al., 2003a).

Although there are no reference procedures solely for folic acid (FA) determinations in food, chromatographic techniques have been routinely used for folate quantification (Table 2). Despite the development of several HPLC and LC–MS methods for food folate analysis (Careri et al., 2002; Freisleben et al., 2003a), there is still a need for better resolution and sensitivity when analysing complex food matrices containing different folate derivatives at very low levels. The application of a novel chromatographic technique, ultra performance liquid chromatography (UPLC), for determining folates in foods is therefore of great interest, because it can provide much higher resolution compared with "traditional" HPLC. However, UPLC operates commonly at elevated column temperatures (up to 90 °C), which may constrain the application of this technique for folate analysis due to thermal instability of some folate derivatives (Jastrebova et al., 2011).

Another difficulty associated with HPLC methodologies is the presence of different endogenous constituents in food extracts which may interfere with folates. Therefore, there is also a need to improve selectivity of the HPLC methods (Nilsson et al., 2004).

4.3. Other methods

Bio-specific procedures or ligand binding methods are sensitive, rapid and specific techniques for folate analysis that can be used as an alternative to HPLC and microbiological assays. Natural folate-binding proteins (FBPs) in enzyme-linked ligand sorbent assay (ELLSA) (Hansen and Holm, 1988) or anti-folic acid antibody in enzyme-linked immunosorbent assay (ELISA) (Alaburda et al., 2008) have also been used to measure folate quantities in food products (Hoegger et al., 2007; Lermo et al., 2009). Folate immobilised in microplates is used to capture an FBP or antifolate antibody to which a reporter enzyme has been coupled. Competition between free folate in the sample and bound folate for the enzyme-labelled folate receptor reduces the amount of enzyme bound in the wells that is subsequently measured colorimetrically (Finglas et al., 1988). This methodology offers several advantages relative to an antibody, including innate specificity, monomeric binding stoichiometry, and cross-reactivity profile towards folate

Table 2
HPLC technologies for folate content determination in ready-to-eat vegetables, fruits and precooked meals.

Food products	Extraction method	HPLC technology	Reference
Peas, spinach, apples, fruit juice, egg yolk powdered milk, yeast, wheat flour, liver beef, and fillet	Tri-enzyme	HPLC-FD	Ndaw et al. (2001)
Spinach, orange juice, refined flour, and non-fat dried milk (NFDM)	Tri-enzyme	HPLC-UV/HPLC-FD	Doherty and Beecher (2003)
Spinach, wheat bread, and beef	Di-enzyme	LC–MS/MS	Freisleben et al. (2003a)
Carrot, spinach, broccoli, orange juices, meat, and cereals	Tri-enzyme	HPLC–MS/MS	Freisleben et al. (2003b)
Green and red sweet peppers	Tri-enzyme	LC–MS	Phillips et al. (2006)
Spinach and lettuce varieties	Tri-enzyme	LC–MS	Johansson et al. (2007)
Beans (green, white, black-eyed, kidney, jack, and mung), soybeans, peas (green and black-eyed), chickpeas, lentils (green and red), and peanuts	Di-enzyme	LC–MS/MS	Rychlik et al. (2007)
Broccoli, cauliflower, carrot, pepper, curled parsley, Brussels sprouts, lettuce, spinach, cabbage, onion tops, and potatoes	Tri-enzyme	Reverse phase-HPLC-FD	Holasová et al. (2008)
Broccoli, asparagus, leeks, apples, peaches, pears, and potatoes	Tri-enzyme	LC–MS	Phillips et al. (2008)
Precooked vegetarian ready meals (Based on rice or pasta)	Mono, di, tri-enzyme	HPLC-FD and LC–MS	Johansson et al. (2008)
Egyptian foods (legumes: chickpeas, cow peas, lentils, kidney and faba beans; cereals: corn flour, Egyptian wheat flour, Russian wheat flour, and rice; vegetables: spinach, lettuce, garlic, green peas, green peppers, carrots, potato, cucumber, tomato, onion, and Jew's mellow; and fruits: strawberry and banana)	Tri-enzyme and di-enzyme	Reverse phase-HPLC-FD	Hefni et al. (2010)
Broccoli, strawberry, white grape, orange, tomato, raspberry, banana, and kiwifruit	Mono-enzyme	HPLC	Martin et al. (2010)
Cabbage, mustard, spinach, and broccoli	Mono-enzyme	HPLC-FD	Della Lucia et al. (2011)
Beetroots, strawberries, orange juice, egg yolk, dry baker's yeast, and soft drink	Mono-enzyme	UPLC and HPLC	Jastrebova et al. (2011)

HPLC: high-performance liquid chromatography; HPLC-FD: high-performance liquid chromatography-fluorescence detection; HPLC-UV: high-performance liquid chromatography-ultraviolet detection; LC–MS/MS: liquid chromatography–tandem mass spectrometry; HPLC–MS/MS: high-performance liquid chromatography–tandem mass spectrometry; LC–MS: liquid chromatography–mass spectrometry; UPLC: ultra performance liquid chromatography.

vitamers. However, as with the microbiological assay, it is not possible to distinguish between the single vitamers or show different responses to them (Arcot and Shrestha, 2005).

Biosensors incorporating surface plasmon resonance (SPR) optics provide a viable analytical alternative in applications related to food composition, safety and compliance (Indyk, 2011; Lavecchia et al., 2011). The modern photosynthetic proteins-based devices, an SPR biosensor immunoassay utilising an anti-folic acid monoclonal antibody, is a viable alternative technique that unambiguously quantifies supplemental FA, due to its specificity and sensitivity to folic acid (Indyk, 2010).

Capillary electrophoresis (CE) is a relatively new separation approach which has been used in food analysis for folate quantification. It provides high efficiency and resolution, ease of automation and speed. Uysal et al. (2010) demonstrated that this technique should be applicable to food analysis laboratories that deal with folic acid or folate determinations. After a simple extraction procedure it enabled an easy, sensitive, selective, accurate and precise determination of FA in lentils.

Researchers, dieticians, and analysts should understand that methods for folate analyses in foods have a relatively high analytical uncertainty compared to other nutrient determinations such as total fat, individual fatty acids, or nitrogen (for protein content), even when performed using a carefully validated method at a highly experienced laboratory. In terms of techniques used up to now, only data obtained using the standardised microbiological assay for total folate (fully validated by inter-laboratory studies) and FA by HPLC can be considered of an acceptable quality level. However, Puwastien et al. (2005) established a wide inter-laboratory performance difference in food folate assays due to the high variability of the methods employed for folate extraction and detection. Although some researchers have reported good agreement between total folate values determined by HPLC and microbiological methods, Arcot and Shrestha (2005) observed consistently lower values (less than 50%) for total folate analysed by the HPLC method. Similarly, European Interlaboratory comparison also found HPLC results much lower (30–40%) than microbiological results (Arcot and Shrestha, 2005; Kariluoto et al., 2001). Koontz et al. (2005) showed that interlaboratory folate analyses by microbiological assays have an extraordinarily high intra- and inter-laboratory variation for non-fortified foods (cooked pinto beans, spinach, strawberries) due to several factors such as incubation time, sample storage and preparation conditions, incubation temperature, pH, growth medium, and sterilisation procedures (Arcot and Shrestha, 2005). Targeting fresh-cut products, there is only one study referring to commercially packed "fresh" spinach, which revealed that the higher the temperature and storage time, the greater the losses of folate (Pandrangi and Laborde, 2004).

Based on these findings, a range of suitable food certified reference materials are available and should be used by laboratories to check the accuracy of the data (Bouckaert et al., 2011). Consequently, it is critical to include quality control samples in each run and enough sample replicates to obtain an estimate of uncertainty in all measured values.

Therefore, there are many critical points in folate food analysis, aggravated by the fact that the vitamin is also highly sensitive to physical or chemical factors. Particularly, in ready-to-eat vegetable food products, this fact is strongly relevant since the predominant folate form is the labile 5-methyltetrahydrofolate (5MTHF) (Xue et al., 2011).

Ready-to-use products, especially precooked meals, are prepared and processed before their distribution in the market. In this context, it should be pointed out that folates are extremely sensitive to destruction by heat, oxidation and UV light. Besides, the degree of loss can be influenced by environmental factors, including pH, O_2 content, metal ion concentrations, antioxidant levels, and product:water ratio. Therefore, the presence of reducing agents in the food, such as ascorbic acid, can increase folate retention during thermal procedures (Eitenmiller and Landen, 1999; Gregory III, 1989). In order to reduce folate loss during manufacture or determination, it is important to understand how folates are affected by different factors during each step, such as soaking and cooking practices (Ros, 2010; Vishnumohan et al., 2011; Xue et al., 2011).

Methods of foodstuff processing used in small- and large-scale cooking service systems often include: blanching, steam-boiling, boiling, *sous-vide*, oven baking and microwave cooking. Loss of folate during heat treatments, such as boiling, blanching and steam-boiling has been shown to be substantial, and the extent seems to depend on the time and amount of water used (Jägerstad et al., 2004; Stea et al., 2006). Likewise, other forms of cooking that minimise the direct contact of food with the cooking water, such as pressure cooking (Dang et al., 2000), microwave cooking (Klein, 1989), *sous-vide* (Petersen, 1993) and oven baking (Stea et al., 2006) have been found to be preferable in terms of folate retention (Mckillop et al., 2002). These works show that losses during processing occur simply by leaching of folates into water used for washing, blanching or cooking, and due to oxidation or other pathways of degradation (Dang et al., 2000; Mckillop et al., 2002; Scott et al., 2000). This is also confirmed by studies that measure both folate content in the drained food samples and remaining water after treatment (Dang et al., 2000). Other studies indicated that a large portion of the folates present in vegetables and legumes are lost during canning (Xue et al., 2011). Moreover, the content of the most predominant natural folate form, 5MTHF, in food groups such as vegetables and fruits, decreased with prolonged cooking time. Sterilisation causes folate oxidation, promoted by matrix heat destruction, increased water penetrability, and folate diffusivity (Indrawati et al., 2004; Patring et al., 2005; Stea et al., 2006).

5. Conclusions

The only well-documented benefit of proper folate status is the prevention of megaloblastic anaemia and the risk reduction of NTDs (Mastroiacovo and Leoncini, 2011). Consequently, strategies focused on fortification of food products with FA have been implemented or advised in several countries. Traditionally, FA has been considered a safe vitamin, but researchers confirm that excessive intakes could derive in the masking of vitamin B_{12} deficiency in the elderly (Mills et al., 2003) and other still unexpected concerns, including cancer and tumour promotion, epigenetic hypermethylation, interference with antifolate treatment, miscarriages, and multiple births (Bailey et al., 2010). To avoid these consequences, health promotion campaigns advise increasing the dietary intake of naturally occurring folate from vegetables, fruits and legumes to achieve the recommended daily intakes in general population.

The availability of a wide range of fresh-cut products and chilled ready-to-eat meals in the food market must be taken into consideration. It is important to estimate their benefit on vitamin and nutrient intakes and to analyse their impact on dietary habits. Within this context, it is necessary to increase the quantity and to improve the quality of food composition databases, and it is very important to focus on the production of new analytical data. Currently, in spite of the increasing consumption of ready-to-use foods, there is only limited information on food folate and FA content (Bouckaert et al., 2011). Results from these scientific advances could be used to accurately assess dietary folate intake of the population. Data analysed will also assist dietary studies to estimate and evaluate the adequacy of folate intakes of the

population, to formulate experimental diets for folate bioavailability studies, and to revise dietary recommendations for the population. In addition, data may help the health authorities in planning and executing strategies for intervention programmes (Finglas, 2005).

Acknowledgements

Violeta Fajardo is recipient of a Postdoctoral contract from the Subprograma Juan de la Cierva (Ministerio de Ciencia e Innovación, Spain).

References

ACNielsen, 2006. Consumers and ready-to-eat meals: a global ACNielsen report. ACNielsen http://www.acnielsen.com.
Ahlgren, M., Gustafsson, I.B., Hall, G., 2004. Attitudes and beliefs directed towards ready-meal consumption. Food Service Technology 4, 159–169.
Ahlgren, M.K., Gustafsson, I.B., Hall, G., 2005. The impact of the meal situation on the consumption of ready meals. International Journal of Consumer Studies 29, 485–492.
Akilanathan, L., Vishnumohan, S., Arcot, J., Uthira, L., Ramachandran, S., 2010. Total folate: diversity within fruit varieties commonly consumed in India. International Journal of Food Sciences and Nutrition 61, 463–472.
Alaburda, J., De Almeida, A.P., Shundo, L., Ruvieri, V., Sabino, M., 2008. Determination of folic acid in fortified wheat flours. Journal of Food Composition and Analysis 21, 336–342.
Alonso, P., 2007. Situación de la IV gama en España. Horticultura Internacional 58, 54–58.
Amarin, Z.O., Obeidat, A.Z., 2010. Effect of folic acid fortification on the incidence of neural tube defects. Paediatric and Perinatal Epidemiology 24, 349–351.
AOAC, 2006. Association of Official Analytical Chemists. Method 2004.05. Total Folates in Cereals and Cereal Foods. Microbiological Assay-Trienzyme Procedure.
Arcot, J., Shrestha, A., 2005. Folate: methods of analysis. Trends in Food Science and Technology 16, 253–266.
Bailey, R.L., Mills, J.L., Yetley, E.A., Gahche, J.J., Pfeiffer, C.M., Dwyer, J.T., Dodd, K.W., Sempos, C.T., Betz, J.M., Picciano, M.F., 2010. Unmetabolized serum folic acid and its relation to folic acid intake from diet and supplements in a nationally representative sample of adults aged >=60 y in the United States. American Journal of Clinical Nutrition 92, 383–389.
Bassett, M.N., Sammán, N.C., 2010. Folate content and retention in selected raw and processed foods. Archivos Latinoamericanos de Nutricion 60, 298–305.
Bell, K.N., Oakley, G.P., 2009. Update on prevention of folic acid-preventable spina bifida and anencephaly. Birth Defects Research. Part A: Clinical and Molecular Teratology 85, 102–107.
Bennett, L.E., Singh, D.P., Clingeleffer, P.R., 2011. Micronutrient mineral and folate content of Australian and imported dried fruit products. Critical Reviews in Food Science and Nutrition 51, 38–49.
Berry, R.J., Bailey, L., Mulinare, J., Bower, C., 2010. Fortification of flour with folic acid. Food and Nutrition Bulletin 31, S22–S35.
BfR, 2007. Bundesinstitut für Risikobewertung. Folate status of the population in the European community and strategies for change. http://www.bfr.bund.de/cm/343/folate_status_of_the_population_in_the_european_community_and_strategies_for_change.pdf.
Black, A.E., Goldberg, G.R., Jebb, S.A., Livingstone, M.B., Cole, T.J., Prentice, A.M., 1991. Critical evaluation of energy intake data using fundamental principles of energy physiology: 2. Evaluating the results of published surveys. European Journal of Clinical Nutrition 45, 583–899.
Blancquaert, D., Storozhenko, S., Loizeau, K., De Steur, H., De Brouwer, V., Viaene, J., Ravanel, S., Rébeillé, F., Lambert, W., van der Straeten, D., 2010. Folates and folic acid: from fundamental research toward sustainable health. Critical Reviews in Plant Sciences 29, 14–35.
Bouckaert, K.P., Slimani, N., Nicolas, G., Vignat, J., Wright, A.J.A., Roe, M., Witthöft, C.M., Finglas, P.M., 2011. Critical evaluation of folate data in European and international databases: recommendations for standardization in international nutritional studies. Molecular Nutrition & Food Research 55, 166–180.
Bourgeois, A., 2010. Chilled and frozen ready meals – UK – May 2010 Mintel Oxygen: http://oxygen.mintel.com/sinatra/oxygen/display/id=480766.
Careri, M., Bianchi, F., Corradini, C., 2002. Recent advances in the application of mass spectrometry in food-related analysis. Journal of Chromatography A 970, 3–64.
Cordero, A., Mulinare, J., Berry, R.J., Boyle, C., Dietz, W., Johnston, R.J., Leighton, J., Popovic, T., 2010. Additional opportunities to prevent neural tube defects with folic acid fortification. Morbidity and Mortality Weekly Report (MMWR) 59, 980–984.
Cui, R., Iso, H., Date, C., Kikuchi, S., Tamakoshi, A., 2010. Dietary folate and vitamin B6 and B12 intake in relation to mortality from cardiovascular diseases. Stroke 41, 1285–1289.
Czeizel, A.E., Dudas, I., 1992. Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. New England Journal of Medicine 327, 1832–1835.
Dang, J., Arcot, J., Shrestha, A., 2000. Folate retention in selected processed legumes. Food Chemistry 68, 295–298.
Della Lucia, C.M., Da Silva, E.R., Ribeiro, S.M.R., Pinheiro-Sant'ana, H.M., Brandao, S.C.C., 2011. Optimization of method to analyze folate in leafy vegetables by high performance liquid chromatography with fluorescence detection. Quimica Nova 34, U335–U409.
Devi, R., Arcot, J., Sotheeswaran, S., Ali, S., 2008. Folate contents of some selected Fijian foods using tri-enzyme extraction method. Food Chemistry 106, 1100–1104.
Dhonukshe-Rutten, R.A.M., De Vries, J.H.M., De Bree, A., Van Der Put, N., Van Staveren, W.A., De Groot, L.C.P.G.M., 2009. Dietary intake and status of folate and vitamin B12 and their association with homocysteine and cardiovascular disease in European populations. European Journal of Clinical Nutrition 63, 18–30.
Doherty, R.F., Beecher, G.R., 2003. A method for the analysis of natural and synthetic folate in foods. Journal of Agricultural and Food Chemistry 51, 354–361.
ECFF, 2011. http://www.ecff.net (accessed 19.07.11).
EFSA, 2009. European Food Safety Authority Scientific Cooperation Working Group on analysis of risks and benefits of fortification of food with folic acid. ECSO Report on Analysis of Risks and Benefits of Fortification of Food with Folic Acid. Parma, Italy European Food Safety Authority.
Eitenmiller, R.R., Landen, W.O., 1999. Folate. In: Vitamin Analysis for Health and Food Science, 2nd ed. CRC Press, Boca Raton, FL, pp. 411–466.
Elmadfa, I., Freisling, H., 2009. Nutritional status in Europe: methods and results. Nutrition Reviews 67, S130–S134.
Elmadfa, I., Meyer, A., Nowak, V., Hasenegger, V., Holm Totland, T., Andersen, L.F., et al., 2009. European Nutrition and Health Report 2009. In: Elmadfa, I. (Ed.), Forum of Nutrition, Vienna.
Elmadfa, I., Meyer, A.L., 2010. Importance of food composition data to nutrition and public health. European Journal of Clinical Nutrition 64, S4–S7.
EUFIC, 2012. Fruit and vegetable consumption in Europe—do Europeans get enough? http://www.eufic.org/article/en/expid/Fruit-vegetable-consumption-Europe.
Fernández, M., 2009. IV Gama: el sector planta cara a la crisis y sigue creciendo. AlimarketIn: http://www.alimarket.es (accessed 19.07.11).
Finglas, P.M., 2005. Folate: From Food to Functionality and Optimal Health. Institute of Food Research http://ec.europa.eu/research/quality-of-life/ka1/ka1-volume1.pdf, http://ec.europa.eu/research/quality-of-life/ka1/ka1-volume2.pdf.
Finglas, P.M., Faulks, R.M., Morgan, M.R.A., 1988. The development and characterization of a protein-binding assay for the determination of folate: potential use in food analysis. Journal of Micronutrient Analysis 4, 295–308.
Finglas, P.M., Wigertz, K., Vahteristo, L., Witthöft, C., Southon, S., De Froidmont-Görtz, I., 1999. Standardisation of HPLC techniques for the determination of naturally-occurring folates in food. Food Chemistry 64, 245–255.
Flynn, A., Hirvonen, T., Mensink, G.B., Ocké, M.C., Serra-Majem, L., Stos, K., Szponar, L., Tetens, I., Turrini, A., Fletcher, R., Wildemann, T., 2009. Intake of selected nutrients from foods, from fortification and from supplements in various European countries. Food & Nutrition Research 53, 1–51.
Freisleben, A., Schieberle, P., Rychlik, M., 2003a. Comparison of folate quantification in foods by high-performance liquid chromatography-fluorescence detection to that by stable isotope dilution assays using high-performance liquid chromatography–tandem mass spectrometry. Analytical Biochemistry 315, 247–255.
Freisleben, A., Schieberle, P., Rychlik, M., 2003b. Specific and sensitive quantification of folate vitamers in foods by stable isotope dilution assays using high-performance liquid chromatography–tandem mass spectrometry. Analytical and Bioanalytical Chemistry 376, 149–156.
Garrett, E.H., 2002. Fresh-cut produce: tracks and trends. In: Lamikanra, O. (Ed.), Fresh-cut Fruits and Vegetables: Science, Technology and Market. CRC Press, Boca Ratón, (Chapter 1), pp. 1–10.
Gregory III, J., 1989. Chemical and nutritional aspects of folate research: analytical procedures, method of analysis, stability, and bioavailability of dietary folates. Advances in Food Nutrition Research 33, 1–101.
Gujska, E., Michalak, J., Czarnowska, M., 2010. Determination of folates in some commercial milk and fermented dairy beverages using high-performance liquid chromatography. Milchwissenschaft-Milk Science International 65, 425–428.
Hansen, S.I., Holm, J., 1988. A competitive enzyme-linked ligand sorbent assay (ELLSA) for quantitation of folates. Analytical Biochemistry 172, 160–164.
Hefni, M., Öhrvik, V., Tabekha, M., Witthöft, C., 2010. Folate content in foods commonly consumed in Egypt. Food Chemistry 121, 540–545.
Herrmann, W., Obeid, R., 2011. The mandatory fortification of staple foods with folic acid. Deutsches Ärzteblatt International 108, 249–254.
Hoegger, D., Morier, P., Vollet, C., Heini, D., Reymond, F., Rossier, J., 2007. Disposable microfluidic ELISA for the rapid determination of folic acid content in food products. Analytical and Bioanalytical Chemistry 387, 267–275.
Holasová, M., Fiedlerová, V., Vavreinová, S., 2008. Determination of folates in vegetables and their retention during boiling. Czech Journal of Food Science 26, 31–37.
Indrawati, A.C., Messagie, I., Nguyen, M.T., Van Loey, A., Hendrickx, M., 2004. Comparative study on pressure and temperature stability of 5-methyltetrahydrofolic acid in model systems and in food products. Journal of Agricultural and Food Chemistry 52, 485–492.
Indyk, H.E., 2010. The determination of folic acid in milk and paediatric formulae by optical biosensor assay utilising folate binding protein. International Dairy Journal 20, 106–112.
Indyk, H.E., 2011. An optical biosensor assay for the determination of folate in milk and nutritional dairy products. International Dairy Journal 21, 783–789.

IOM, 1998. Food and Nutrition Board, Institute of Medicine. Panel on Folate, Other B Vitamins and Choline. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic acid, Biotin, and Choline. National Academy Press, Washington, DC.

IOM, 2000. Institute of Medicine: Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic acid, Biotin, and Choline. National Academy Press, Washington, DC.

Iwatani, Y., Arcot, J., Shrestha, A.K., 2003. Determination of folate contents in some Australian vegetables. Journal of Food Composition and Analysis 16, 37–48.

Jägerstad, M., Jastrebova, J., Svensson, U., 2004. Folates in fermented vegetables—a pilot study. Lebensmittel-Wissenschaft Und-Technologie (Food Science and Technology).37, 603–611.

Jastrebova, J., Strandler, H.S., Patring, J., Wiklund, T., 2011. Comparison of UPLC and HPLC for analysis of dietary folates. Chromatographia 73, 219–225.

Johansson, M., Furuhagen, C., Frølich, W., Jägerstad, M., 2008. Folate content in frozen vegetarian ready meals and folate retention after different reheating methods. LWT—Food Science and Technology 41, 528–536.

Johansson, M., Jägerstad, M., Frølich, W., 2007. Folates in lettuce: a pilot study. Scandinavian Journal of Nutrition 51, 22–30.

Johnston, K.E., Lofgren, P.A., Tamura, T., 2002. Folate concentrations of fast foods measured by trienzyme extraction method. Food Research International 35, 565–569.

Johnston, R.B.J., 2008. Will increasing folic acid in fortified grain products further reduce neural tube defects without causing harm? Consideration of the evidence. Pediatric Research 63, 2–8.

Kariluoto, S., Vahteristo, L., Finglas, P.M., van den Berg, H., Carnovale, E., Jagerstad, M., et al., 2001. Intercomparison of current methods for folate determination in food. In: Proceeding of Fourth International Food Data Conference, 24–26 August, 2001, Bratislava, Slovakia, pp. 40–41.

Kim, Y.I., 2007. Folate and colorectal cancer: an evidence-based critical review. Molecular Nutrition & Food Research 51, 267–292.

Klein, B.P., 1989. Retention of nutrients in microwave-cooked foods. Boletin Asociación Medica de Puerto Rico 81, 277–279.

Koontz, J.L., Phillips, K.M.M.W.K., Exler, J., Holden, J.M., Gebhardt, S.E., Haytowitz, D.B., 2005. Comparison of total folate concentrations in foods determined by microbiological assay at several experienced U.S. commercial laboratories. Journal of AOAC International 88, 805–813.

Lavecchia, T., Tibuzzi, A., Giardi, M.T., 2011. Biosensors for functional food safety and analysis bio-farms for nutraceuticals. In: Giardi, M.T., Rea, G., Berra, B. (Eds.), Bio-Farms for Nutraceuticals: Functional Food and Safety Control by Biosensors. Springer, US.

Lee, J.E., Chan, A.T., 2011. Fruit, vegetables, and folate: cultivating the evidence for cancer prevention. Gastroenterology 141, 16–20.

Lermo, A., Fabiano, S., Hernández, S., Galve, R., Marco, M.P., Alegret, S., Pividori, M.I., 2009. Immunoassay for folic acid detection in vitamin-fortified milk based on electrochemical magneto sensors. Biosensors and Bioelectronics 24, 2057–2063.

MARM, 2006. La alimentación en España. Ministerio de Medio Ambiente y Medio Rural y Marino, Madrid, España.

Martín-Belloso, O., Soliva-Fortuny, R., 2011. Advances in Fresh-cut Fruits and Vegetables Processing. CRC Press, New York.

Martin, H., Comeskey, D., Simpson, R.M., Laing, W.A., Mcghie, T.K., 2010. Quantification of folate in fruits and vegetables: a fluorescence-based homogeneous assay. Analytical Biochemistry 402, 137–145.

Martínez, M.A., Martínez-Victoria, I., Mila, R., Farran, A., Farre, R., Ros, G., Yago, M.D., Audi, N., Santana, C., López, M.B., Ramos, S., Mañas, M., Martínez-Victoria, E., 2009. Building a unified Spanish food database according to EuroFIR specifications. Food Chemistry 113, 784–788.

Mastroiacovo, P., Leoncini, E., 2011. More folic acid, the five questions: why, who, when, how much, and how. BioFactors 37, 272–279.

Mckillop, D.J., Pentieva, K., Daly, D., Mcpartlin, J.M., Hughes, J., Strain, J.J., Scott, J.M., McNulty, H., 2002. The effect of different cooking methods on folate retention in various foods that are amongst the major contributors to folate intake in the UK diet. British Journal of Nutrition 88, 681–688.

Mercasa, 2010. Platos precocinados y preparados. Mercasa, Madrid, España.

Mills, J.L., Von Kohorn, I., Conley, M.R., Zeller, J.A., Cox, C., Williamson, R.E., Dufour, D.R., 2003. Low vitamin B-12 concentrations in patients without anemia: the effect of folic acid fortification of grain. American Journal of Clinical Nutrition 77, 1474–1477.

Moreiras, O., Carvajal, A., Cabrera, L., Cuadrado, C., 2010. Ingestas Recomendadas de energía y nutrientes para la población española (revisadas y ampliadas 2006). Departamento de Nutrición. Universidad Complutense de Madrid. In: Tablas de Composición de los Alimentos, 14st ed. Pirámide, Madrid, pp. 228–229.

Morris, M.S., Jacques, P.F., Rosenberg, I.H., Selhub, J., 2007. Folate and vitamin B-12 status in relation to anemia, macrocytosis, and cognitive impairment in older Americans in the age of folic acid fortification. American Journal of Clinical Nutrition 85, 193–200.

Ndaw, S., Bergaentzle, M., Aoude-Werner, D., Lahely, S., Hasselmann, C., 2001. Determination of folates in foods by high-performance liquid chromatography with fluorescence detection after precolumn conversion to 5-methyltetrahydrofolates. Journal of Chromatography A 928, 77–90.

Nilsson, C., Johansson, M., Yazynina, E., Stralsjo, L., Jastrebova, J., 2004. Solid-phase extraction for HPLC analysis of dietary folates. European Food Research and Technology A 219, 199–204.

Olivares, A.B., Bernal, M.J., Ros, G., Martínez, C., Periago, M.J., 2006. Quality of data on folic acid content in vegetables included in several Spanish food composition tables and new data on their folate content. Nutricion Hospitalaria 21, 97–108.

Pandrangi, S., Laborde, L.F., 2004. Retention of folate, carotenoids, and other quality characteristics in commercially packaged fresh spinach. Journal of Food Science 69, 702–707.

Park, J.Y., Nicolas, G., Freisling, H., Biessy, C., Scalbert, A., Romieu, I., et al., 2011. Comparison of standardised dietary folate intake across ten countries participating in the European Prospective Investigation into Cancer and Nutrition. British Journal of Nutrition 1, 1–18.

Parker, P.M., 2010. The 2011–2016 World Outlook for Chilled Microwavable Foods. Icon Groups International Inc., San Diego, USA, p. 192.

Patring, J.D.M., Johansson, M.S., Yazynina, E., Jastrebova, J.A., 2005. Evaluation of impact of different antioxidants on stability of dietary folates during food sample preparation and storage of extracts prior to analysis. Analytica Chimica Acta 553, 36–42.

Petersen, M.A., 1993. Influence of sous vide processing, steaming and boiling on vitamin retention and sensory quality in broccoli florets. Zeitschrift für Lebensmitteluntersuchung und -Forschung A 197, 375–380.

Phillips, K.M., Rasor, A.S., Ruggio, D.M., Amanna, K.R., 2008. Folate content of different edible portions of vegetables and fruits. Nutrition & Food Science 38, 175–181.

Phillips, K.M., Ruggio, D.M., Ashraf-Khorassani, M., Haytowitz, D.B., 2006. Difference in folate content of green and red sweet peppers (*Capsicum annuum*) determined by liquid chromatography–mass spectrometry. Journal of Agricultural and Food Chemistry 54, 9998–10002.

Póo-Prieto, R., Alonso-Aperte, E., Varela-Moreiras, G., 2011. Analysis of folate form distribution in Spanish beers using combined affinity and ion-pair chromatography. Journal of the Institute of Brewing 117, 188–194.

Pozo, S., García, V., Cuadrado, C., Ruiz, E., Valero, T., Ávila, J.M., Varela, G., 2012. Valoración Nutricional de la Dieta Española de acuerdo al Panel de Consumo Alimentario. Fundación Española de la Nutrición (FEN), Madrid, España.

Puwastien, P., Pinprapai, N., Judprasong, K., Tamura, T., 2005. International interlaboratory analyses of food folate. Journal of Food Composition and Analysis 18, 387–397.

Rader, J.I., 2002. Folic acid fortification, folate status and plasma homocysteine. Journal of Nutrition 132, 2466S–2470S.

Ros, G., 2010. Influence of food processing on vitamin content and availability. In: Aperte-Alonso, E., Varela-Moreiras, G. (Eds.), Hot topics en vitaminas y salud. Im&C, Instituto Tomás Pascual, Universidad San Pablo CEU, Madrid, pp. 25–34.

Ros, G., Martínez De Victoria, E., Farran, A., 2009. Spanish food composition database: a challenge for a consensus. Food Chemistry 113, 789–794.

Rychlik, M., Englert, K., Kapfer, S., Kirchhoff, E., 2007. Folate contents of legumes determined by optimized enzyme treatment and stable isotope dilution assays. Journal of Food Composition and Analysis 20, 411–419.

Samaniego-Vaesken, M.L., Alonso-Aperte, E., Varela-Moreiras, G., 2010. Analysis and evaluation of voluntary folic acid fortification of breakfast cereals in the Spanish market. Journal of Food Composition and Analysis 23, 419–423.

Scott, J., Rebeille, F., Fletcher, J., 2000. Folic acid and folates: the feasibility for nutritional enhancement in plant foods. Journal of the Science of Food and Agriculture 80, 795–824.

Shakur, Y.A., Rogenstein, C., Hartman-Craven, B., Tarasuk, V., O'connor, D.L., 2009. How much folate is in Canadian fortified products 10 years after mandated fortification? Canadian Journal of Public Health 100, 281–284.

Shuaibi, A.M., Sevenhuysen, G.P., House, J.D., 2009. The importance of using folate intake expressed as dietary folate equivalents in predicting folate status. Journal of Food Composition and Analysis 22, 38–43.

Soongsongkiat, M., Puwastien, P., Jittinandana, S., Dee-Uam, A., Sungpuag, P., 2010. Testing of folate conjugase from chicken pancreas vs commercial enzyme and studying the effect of cooking on folate retention in Thai foods. Journal of Food Composition and Analysis 23, 681–688.

Stea, T.H., Johansson, M., Jägerstad, M., Frølich, W., 2006. Retention of folates in cooked, stored and reheated peas, broccoli and potatoes for use in modern large-scale service systems. Food Chemistry 101, 1095–1107.

Tabacchi, G., Wijnhoven, T.M., Branca, F., Román-Viñas, B., Ribas-Barba, L., Ngo, J., García-Alvarez, A., Serra-Majem, L., 2009. How is the adequacy of micronutrient intake assessed across Europe? A systematic literature review. British Journal of Nutrition 101, S29–S36.

Uusitalo, U., Kronberg-Kippilä, C., Aronsson, C.A., Schakel, S., Schoen, S., Mattisson, I., Reinivuo, H., Silvis, K., Sichert-Hellert, W., Stevens, M., Norris, J.M., Virtanen, S.M., 2011. Food composition database harmonization for between-country comparisons of nutrient data in the TEDDY Study. Journal of Food Composition and Analysis 24, 494–505.

Uysal, U.D., Oncu-Kaya, E.M., Tuncel, M., 2010. Determination of folic acid by CE in various cultivated variety of lentils. Chromatographia 71, 653–658.

Varela-Moreiras, G., Ávila, J.M., Cuadrado, C., Del Pozo, S., Ruiz, E., Moreiras, O., 2010. Evaluation of food consumption and dietary patterns in Spain by the Food Consumption Survey: updated information. European Journal of Clinical Nutrition 64, S37–S43.

Vishnumohan, S., Arcot, J., Pickford, R., 2011. Naturally-occurring folates in foods: method development and analysis using liquid chromatography–tandem mass spectrometry (LC–MS/MS). Food Chemistry 125, 736–742.

Vishnumohan, S., Arcot, J., Sini, S., Uthira, L., Ramachandran, S., 2009. Determination of folate contents in selected Indian foods using the tri-enzyme extraction and estimated folate intakes of the population based on 24-h recall. International Journal of Food Sciences and Nutrition 60, 170–180.

Wang, C., Riedl, K.M., Schwartz, S.J., 2010. A liquid chromatography–tandem mass spectrometric method for quantitative determination of native 5-methyltetrahydrofolate and its polyglutamyl derivatives in raw vegetables. Journal of Chromatography B 878, 2949–2958.

WHO, 2004. The Global Burden of Disease. 2004 update.

Xue, S., Ye, X., Shi, J., Jiang, Y., Liu, D., Chen, J., Shi, A., Kakuda, Y., 2011. Degradation kinetics of folate (5-methyltetrahydrofolate) in navy beans under various processing conditions. LWT—Food Science and Technology 44, 231–238.

Yang, Q., Cogswell, M.E., Hamner, H.C., Carriquiry, A., Bailey, L.B., Pfeiffer, C.M., Berry, R.J., 2010. Folic acid source, usual intake, and folate and vitamin B-12 status in US adults: National Health and Nutrition Examination Survey (NHANES) 2003–2006. American Journal of Clinical Nutrition 91, 64–72.

Yeung, L.F., Cogswell, M.E., Carriquiry, A.L., Bailey, L.B., Pfeiffer, C.M., Berry, R.J., 2011. Contributions of enriched cereal-grain products, ready-to-eat cereals, and supplements to folic acid and vitamin B-12 usual intake and folate and vitamin B-12 status in US children: National Health and Nutrition Examination Survey, 2003–2006. American Journal of Clinical Nutrition 93, 172–183.

Yon, M., Hyun, T.H., 2003. Folate content of foods commonly consumed in Korea measured after trienzyme extraction. Nutrition Research 23, 735–746.

Zhang, X., 2007. New approaches on improving the quality and safety of fresh cut fruits and vegetables. Acta Horticulturae 746, 97–102.