Journal of Food Composition and Analysis 64 (2017) 223–231

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

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

Original research article

Total folate content in ready-to-eat vegetable meals from the Spanish market

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

Departamento de Ciencias Farmacéuticas y de la Salud, Facultad de Farmacia, Universidad San Pablo-CEU Universities, Urbanización Montepríncipe, 28925 Alcorcón, Madrid, Spain

ARTICLE INFO

Keywords:
Dietary folate
Chilled ready-to-eat products
Mixed dishes
Vegetables
Pulses
CRM 485
Food analysis
Food composition

ABSTRACT

Currently, data concerning the amount of naturally occurring dietary folate in ready-to-eat foods is scarce, in spite of an increasing consumption. Total folate (TF) in 35 chilled ready-to-eat vegetable-based meals, expected to be good natural folate sources, was determined. A validated method that relies on a trienzyme extraction (α-amylase, rat plasma conjugase as a substitute to chicken pancreas conjugase, and protease enzymes), and chloramphenicol-resistant *Lactobacillus casei* subspecies *rhamnosus* folate dependent growth was developed. No previous reports on folate data in refrigerated ready-to-eat foods from the Spanish market have been published. Vegetable burgers, containing pulses and green leafy vegetables, had the highest folate content (103.8 μg TF/100 g fresh weight). Refrigerated recipes including chickpeas, peas, broccoli, or artichokes had an average folate content of 75 μg TF/100 g fresh weight. A typical serving of targeted ready-to-eat meals (175 g) may provide up to 60% of the Dietary Reference Intake for an adult. No differences in TF were found after mild heat treatment, as recommended by the manufacturer before its consumption. Therefore, these products may be used to increase folate intake by choosing naturally rich dietary sources. Data will assist dietary studies in the assessment of population folate dietary intakes.

1. Introduction

Folates are a group of water-soluble B vitamins. They play a major role as coenzymes in the metabolism of one-carbon groups as co-factors in the synthesis of purine and thymidine nucleotides, amino acid biosynthesis like that of methionine from homocisteine, replication, and growth, being essential nutrients for life (Ebara, 2017; Saini et al., 2016). Folates naturally occur in foods (as reduced polyglutamate compounds) whereas folic acid (FA, pteroylmonoglutamic acid), which is the most oxidised and stable form of folate, rarely occurs in foods, but it is the form used in vitamin supplements and fortified food products. Folic acid supplementation has been confirmed to reduce the risk of neural tube defects (Czeizel et al., 2013) and folate deficiency has been associated with the development of certain types of cancer, Alzheimer's disease, dementia, and cardiovascular disease (Ebara, 2017). Accordingly, growing realization of the importance of adequate folate nutrition has led to substantial increases in international intake recommendations for folates (Fajardo et al., 2012; Fajardo and Varela-Moreiras, 2012). European institutions raised Dietary References Intakes (DRI) for folate to levels ranging from 400 to 500 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 (EFSA, 2017; Moreiras et al., 2016). For other population groups, the DRI for folate are established depending on the age and gender of the individuals (children around 150–200 μg/day, and adults and elderly, 300–400 μg/day), but vary considerably among European countries (Krawinkel et al., 2014). To comply with these recommendations, pharmacological supplementation, mandatory or voluntary fortification of staple foods with synthetic FA and the advice to increase the intake of folate rich foods have been introduced in different countries (Arth et al., 2016; Fajardo et al., 2012; Fajardo and Varela-Moreiras, 2012).

The United States of America (USA) and Canada governments were the first to adopt a mandatory fortification policy with synthetic FA in flours and cereal derivatives back in 1998 (Institute of Medicine, 2003). Currently, almost 86 countries worldwide have implemented this strategy (Arth et al., 2016). However, considering the updated DRI, there is some indication that folate deficiency may be a public health problem since epidemiological evidence reveals that suboptimal folate

Abbreviations: AOAC, Association of Official Agricultural Chemists; BEDCA, Base de Datos Española de Composición de Alimentos/Spanish Food Composition Database; CE, Conformité Européenne; CRM, Certified Reference Material; CV, coefficient of variation; DRI, Dietary References Intakes; EuroFIR, European Food Information Resource network of Excellence; FA, folic acid; FCT, Food Composition Tables; HPLC, high performance liquid chromatography; IS, international standard; *L. casei* spp. *rhamnosus*, *Lactobacillus casei subspecies rhamnosus*; MA, microbiological assay; NCIMB, National Collection of Industrial Food and Marine Bacteria; NIBSC, National Institute for Biological Standards and Control; QC, quality control; SD, standard deviation; TF, total folate; UK, United Kingdom; USA, United States of America

* Corresponding author.
E-mail addresses: violeta.fajardomartin@ceu.es (V. Fajardo), eaperte@ceu.es (E. Alonso-Aperte), gvarela@ceu.es (G. Varela-Moreiras).

http://dx.doi.org/10.1016/j.jfca.2017.10.002
Received 26 January 2017; Received in revised form 18 September 2017; Accepted 6 October 2017
Available online 12 October 2017

intake may be widespread in populations, both in developing and developed countries (Novaković et al., 2013). Recently, the Healthy Lifestyle in Europe by Nutrition in Adolescence Study demonstrated that the folate intake was below 55% of recommended (Iglesia et al., 2016). The greatest reported intakes of folate occur in populations with high consumption of leafy vegetables, such as those that follow a Mediterranean dietary pattern (Benhammou et al., 2016; Varela-Moreiras et al., 2010).

Naturally occurring folate is present in a wide range of foods; especially green-leafy vegetables, pulses, some fruits, yeast, eggs and organ meats (Saini et al., 2016). Fortified food products with FA, such as cereals, are also an important dietary source of folate in developing areas (Arth et al., 2016; Fajardo et al., 2015; Fajardo and Varela-Moreiras, 2012). However, different studies suggest that most of the usual daily intakes could exceed their age-specific upper limit by consumption of different sources of FA (natural, fortified, and pharmacological) (Reynolds, 2016; Westenbrink et al., 2012). This evidence raises doubts about the safety of mandatory FA fortification in certain populations of different age and ethnical/genetic background, since some authors hypothesize possible long-term adverse effects of excess FA, such as cancer and tumor promotion, epigenetic hypermethylation, miscarriages, and the well-documented masking of vitamin B_{12} deficiency. Consequently, promoting folate intake from natural food sources is a healthy worldwide strategy to attain safe levels of folate and overall nutritional status, and to avoid potential harm from chronic excessive intakes of FA from fortified food products or supplements in certain population groups (Saini et al., 2016).

European food consumption and dietary patterns and, therefore, nutrient intakes have markedly changed in the last 40 years in Mediterranean countries, like Spain, mainly in people aged 30 y or younger (Benhammou et al., 2016; Varela-Moreiras et al., 2010). Customers claim for fresh, safe and additive-free foods. They demand recipes which facilitate daily vegetable consumption everywhere, and commodity for convenience due to lack of time for cooking. It is therefore very common to display convenience products which tend to have a “healthier” look and are more flavorsome than comparable products from the frozen area. Technological advances and cultural shifts have transformed the chilled prepared foods sector into one of the most dynamic departments in the food market (Fajardo et al., 2015). Accordingly, as a response to consumer demands, the so-called ready-to-eat food group is becoming increasingly popular between European consumers in the past few years, bringing total consumption of chilled ready-to-eat meals to 12.9 kg per capita in 2015 in Spain (MAGRAMA, 2016). *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).

Despite the increasing consumption of ready-to-eat food products and the importance of folate in human nutrition, there is a lack of information about the final nutrient composition of these specialties, particularly folate content (Fajardo et al., 2012). At present, most of the folate data recorded in Food Composition Tables (FCT) and databases is scarce or incomplete, especially for novel products such as precooked or ready-to-eat meals (Bouckaert et al., 2011; Nicolas et al., 2016).

Folate analysis in foods is a difficult task, due to the multiple chemical forms, their low stability, and low concentrations in biological systems, therefore complex extraction and detection techniques are required (Arcot and Shrestha, 2005). Although high performance liquid chromatography (HPLC) and more recently, liquid chromatography–tandem mass spectrometry and biospecific procedures have been actively used for folate analysis, the microbiological assay (MA) using *Lactobacillus casei* is still considered the preferred method for food composition purposes, despite being tedious and time consuming (Chew et al., 2012; Devi et al., 2008; Hefni et al., 2010). The Association of Official Analytical Chemists (AOAC) has approved this method for total folate analysis in foods as a standardized technique, after validated inter-laboratory studies (AOAC, 2006), with a previous enzyme extraction, based on the use of amylase, protease and/or folate conjugase to release the carbohydrate and protein-bound folates from food matrices. The optimum pH, order of enzyme addition, incubation time, and other conditions of trienzyme extraction have been investigated for food folate determination in different matrices (Arcot and Shrestha, 2005).

The aim of the present study was to analyze the current availability of chilled ready-to-eat meals from the Spanish market and provide new data on its folate content by a validated microbiological assay.

2. Materials and methods

2.1. Selection of ready-to-eat food products

A total of 35 chilled ready-to-eat meals were purchased at five different local supermarkets and retail stores from Madrid (Spain), selected in terms of highest market share (Kantar Worldpanel, 2016). Food items expected to be good folate sources (mainly vegetable-based foods), and commonly offered and/or consumed according to consumption data, were selected (MAGRAMA, 2016). These included ready-to-eat recipes of green leafy vegetables, pulses, broccoli and other vegetables, from 19 different own-label and commercial brands. Whole packages of two different commercial batches, from three random most highly consumed commercial brands of each product, were grounded in a Thermomix® TM31 (Vowerk, Wupperpal, Germany) for 40 min at refrigeration temperature. After homogenization, food mixtures are immediately sampled and processed under subdued light, minimizing contact with air in duplicate. Food products were assayed as presented in the container and after mild heating, as indicated by the manufacturer before its consumption (3 min 100W in microwave; 5 min frying without oil or butter; no heat treatment or any preparation). All assays were completed within a week of the product’s acquisition using analytical grade reagents.

2.2. Reagents and standards section

Analytical grade chemicals were used throughout the experiments and purchased from authorized manufacturers and suppliers. Certified Reference Material (CRM 485, Institute for Reference Materials and Measurements, Geel, Belgium) and an International Standard IS 03/178 (WHO 1 st Serum folate International Standard) from the National Institute for Biological Standards and Control (NIBSC, Hertfordshire, UK) were used. Internal quality controls from pooled trienzymatic extracts of chilled ready-to-eat products were also prepared.

2.3. Folate trienzyme extraction

Extraction and enzyme treatments were carried out according to previously described trienzyme extraction method (Martin et al., 1990) with modifications (Fajardo et al., 2015). Briefly, after homogenization of the selected ready-to-eat product, 0.5–1 g of the stock sample was suspended in 10 vols of a 0.026 M Tris–HCl extraction buffer (pH 7.4) containing sodium ascorbate 11% (w/v) and 0.02 mCi/L [3′,5′,7′,9-^{3}H] folic acid (FA) diammonium salt tracer (69 Ci/mmol; Movereck Biochemicals, Brea, CA, USA), 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 Chemical Co., St Louis, MO, USA). Then, samples were treated with rat plasma conjugase (Charles River, UK) for 4 h, instead of conjugase from chicken pancreas, applied in the standard method (Martin et al., 1990). Next, 2 mg/mL protease solution (type XIV, *Streptomyces griseus*, Sigma) was set 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 x *g* at 4 °C.

Finally, supernatants were filtered through sterile syringe filters (Millex-AA, 0.8 mm, Milipore, Ireland). In order to calculate folate recovery after the extraction and enzyme treatment, radioactivity was measured in an aliquot of neutralized eluate of each analyzed food product by quantification and comparison of radioactivity in the starting mixture. FA diammonium salt tracer recovery was within the range 85–110%. Folate extracts were stored at − 20 °C until further analysis.

2.4. Moisture determination

Duplicate samples were separately analyzed for moisture content immediately according to the Association of Official Agricultural Chemists (AOAC) method 950.46 (AOAC, 1990).

2.5. Microbiological assay

TF was determined in each extracted sample by a microbiological method on sterile 96-well microtiter plates (Costar 3596, Corning Inc., Tewksbury, MA, USA), using chloramphenicol-resistant cryoprotected *Lactobacillus casei* ssp. *rhamnosus* (NCIMB 10463, National Collection of Industrial Food and Marine Bacteria Ltd., Scotland, UK) as the growth organism (Fajardo et al., 2015; O'Broin and Kelleher, 1992).

Cryoprotected cultures were prepared from lyophilized *L. casei* spp. *rhamnosus* chloramphenicol-resistant (NCIMB 10463) and used as an assay organism following the procedure described by O'Broin and Kelleher (1992).

The absorbance values obtained after incubation of the samples at 37 °C for 42 h in 96-well sterile plates (Costar 3596, Corning Inc., Tewksbury, MA, USA) with the Folic Acid Medium Casei (FACM) (Difco, Becton Dickinson and Co., Sparks, MD, USA) were determined by an automatic microplate reader fixed at $\lambda = 620$ nm (DigiScan Reader, Asys Hitech, Austria). Standard stock solutions were prepared by dissolving FA (Sigma) in 0.01 mol/L NaOH (20 mmol/L) and concentrations were determined in pH 7.0 buffered solutions, using UV absorption at $\lambda = 282$ 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 (0–30 pg/100 μL) and were included in each assay with the target samples.

Samples dilutions to folate concentrations of 5–11 pg/100 μL, within the range of the calibration curve, were prepared with ascorbic acid. Additionally, to test reliability of the developed method, for each duplicate of two different comercial batches from three random mostly highly consumed comercial brands, folate content was also measured by absorbance from two different volumes (4 wells with 100 μL of the diluted sample and 4 wells with 50 μL of the diluted sample plus 50 μL of ascorbic acid). Second wells have absorption values which are half of the first wells.

Folate contents are given on a fresh food product weight basis. Enzyme blanks were assayed to account for potential endogenous folate contribution.

Method performance was considered acceptable according to the AOAC criteria, using linearity, reproducibility, accuracy and repeatability parameters (AOAC, 1998), as detailed in Fajardo et al. (2015).

2.6. Quality control

A Certified Reference Material, provided as a lyophilized mixture of vegetables, containing canned chopped tomatoes, frozen carrots and sweet corn (CRM 485, Institute for Reference Materials and Measurements, Geel, Belgium), was used as an external quality control for intra- and inter-assay reproducibility. The CRM 485 contains a certified value of 315 ± 28 μg TF/100 g dry matter, being 5-methyltetrahydrofolate the main natural form of the vitamin (Ollilainen et al., 2001). Intra-assay results for CRM 485 were expressed as the coefficient of variation (CV) of the TF concentrations in five samples extracted in parallel with ready-to-eat food products and run separately on the same day. Inter-assay reproducibility was determined by extraction and analysis of CRM 485 samples extracted over five consecutive days.

The international standard IS 03/178 (WHO 1 st Serum folate International Standard) from the National Institute for Biological Standards and Control (NIBSC, Hertfordshire, UK), with a certified value of 5.3 ng/mL of TF in lyophilized human serum, was also employed as an external quality control to display the accuracy of the developed microbiological method. It is not tested with the trienzymatic extraction since total folate in this external quality control is given by the average value of the sum of individual folates vitamers, which are quantified by liquid chromatography–mass spectrometry as analyzed in a collaborative study among 24 laboratories from 7 different countries (Thorpe et al., 2007).

Internal quality controls from pooled trienzymatic extracts from a number of chilled ready-to-eat products were prepared. It was necessary to select those trienzymatic extracts from food samples within the range of the TF concentration in all selected food products (see Results). Consequently, dilutions of internal quality controls were adapted at three levels: low, medium and high (≈20, 70 and 100 μg TF/100 g food product, respectively). Quality controls were stored at −20 °C and assayed in parallel to ready-to-eat food samples on a weekly basis to account for repeatability.

2.7. Method recovery

Recovery from food folate extraction was evaluated by addition of 0.02 mCi/L [3′,5′,7′,9-^{3}H] FA diammonium salt tracer (69 Ci/mmol, Movereck Biochemicals, Brea, CA, USA) to each analyzed food sample, including CRM 485 and internal quality controls, as described above in the trienzyme protocol. The assays with a percentage recovery of added [^{3}H] FA tracer outside the range of 85–110% were not accepted and the values were discarded.

2.8. Statistical analysis

Data are represented as mean ± standard deviation. Statistical analysis was carried out to perform a descriptive analysis of the samples and its main quantitative variables, expressed through centralization and dispersion parameters. The analyses were performed using the SPSS v.24.0 program (IBM Corp., Armonk, NY, USA).

3. Results

3.1. Precision, accuracy and repeatability of the developed trienzyme-microbiological method

Linearity for FA standard (0–30 pg/100 μL) was corroborated each day of analysis with a regression coefficient $r^2 = 0.999$.

Method reproducibility was determined by intra- and inter-assay coefficients of variation (CV) for the Certified Reference Material (CRM 485). Intra-assay results for CRM 485 provided average values ± standard deviation of 310.9 ± 23.9 μg/100 g dry matter with a CV of 7.6% (n = 5). Inter- assay reproducibility of CRM 485 samples resulted in values of 313.8 ± 26.3 μg/100 g dry matter with a CV of 12.7% (n = 20). These results were within the range for TF content of the CRM 485 (315 ± 28 μg/100 g dry matter).

Results obtained after analyzing the other external quality control, IS 03/178 NIBSC, were 5.2 ± 0.1 ng TF/mL (n = 5) and 5.0 ± 0.3 ng TF/mL (n = 20) for intra- and inter-assays analysis, respectively, and both were considered adequate as compared to the certified value (5.33 ng TF/mL). CVs for this external quality control were 6.1% (n = 5) and 7.5% (n = 20) in both intra- and inter-assays, respectively. Repeatability of the assay was assured on a weekly basis, by testing internal quality control food samples from pooled trienzymatic extracts

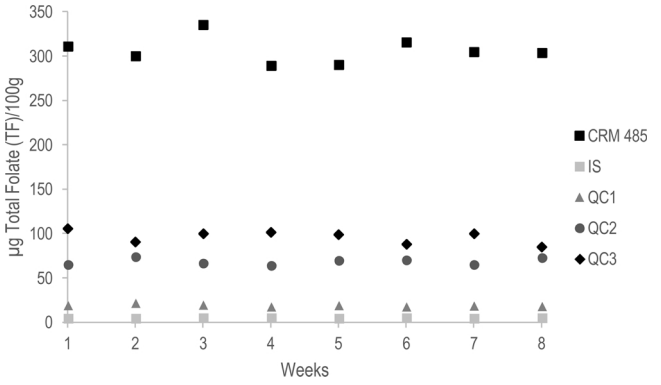

Fig. 1. Example of the reproducibility and repeatability of assayed values for quality controls developed in the validated microbiological method for total folate content in chilled ready-to-eat food products. External controls: CRM 485 (Certified Reference Material 485, lyophilized mixture of vegetables of 315 ± 28 µg TF/100 g dry matter); IS (International standard 03/178, certified value of 5.3 ng TF/mL in serum). Internal controls: QC1 (low TF concentrations trienzymatic extracts, ≈20 µg TF/100 g soy and carrot burger); QC2 (medium TF concentrations trienzymatic extracts, ≈70 µg TF/100 g hummus); QC3 (large TF concentrations trienzymatic extracts, ≈100 µg TF/100 g seitan, tofu and vegetable bar). TF: total folate.

at three levels: low, medium and large (≈20, 70 and 100 µg TF/100 g food product, respectively). Assayed values for these quality controls were reproducible for all in each level, with CVs lower than 5%.

Intra and inter-assays CVs higher than 10 and 15%, respectively, are not aceptable (Reed et al., 2002).

Fig. 1 represents an example of the reproducibility and repeatability of assayed values for the different external and internal controls. It should be noted that highly reproducible results were obtained every week for each of the selected quality control. Recovery values for the [^{3}H] FA tracer in the trienzyme extraction method resulted within the range of 85–110% (AOAC, AOAC, 1998). In addition, enzyme blank preparations were assayed for folate determination at similar concentrations to that used in the deconjugation of the ready-to-eat food samples. No measurable folate response was observed for the enzyme blank preparation.

3.2. Folate content in chilled ready-to-eat meals

Table 1 shows food classification and ingredients of each of the analyzed chilled ready-to-eat products, in alphabetical order. LanguaL™ Thesaurus EuroFIR was adopted for food group and subgroup classification and codification system (Møller and Ireland, 2009). European food composition databases have been successfully integrated, harmonized and managed by the European Food Information Resource network of Excellence (EuroFIR) project, from which Spain was part with the development of the Spanish Food Composition Database network (BEDCA). Ingredients were also listed as declared in the label of each product. Unfortunately, in most cases, labels provided poor or null information (i.e. vegetable pie).

Mean total folate (TF) content in the 35 commercial chilled ready-to-eat meals, containing mainly vegetable ingredients, ranges widely from 4.6 to 103.8 µg/100 g fresh weight (Table 2). The highest folate content (103.8 µg TF/100 g) was found in vegetable burgers, followed by recipes including chickpeas (i.e. tripe, 85 µg TF/100 g, or hummus, 72.9 µg TF/100 g), broccoli (i.e. broccoli pie, 56.8 µg TF/100 g), peas (i.e. peas with ham, 50.6 µg TF/100 g) or artichokes (i.e. artichokes with ham, 49.8 µg TF/100 g). Other vegetable ready-to-eat products such as vegetable bars or burgers, made with tofu, spinach or/and broccoli, had lower folate contents (between 30 and 15 µg TF/100 g), whereas lentil stew and tabbouleh had a markedly lower content (< 15 µg TF/100 g). Finally, pumpkin cream showed the lowest amount (< 5.0 µg TF/100 g).

The coefficient of variation (CV) for duplicates analysis of two different dilutions (100 µL of the diluted sample and 50 µL of the diluted sample plus 50 µL of ascorbic acid) of two different commercial batches, from three randomly selected most highly consumed commercial brands of the same product, was less than 15% (Reed et al., 2002). However, CVs rose to 15 and 35% after analysis of miscellaneous chilled products like “vegetable burgers and pies”, since the composition of these multiingredient prepared food product is not specified in quantities in the label, and varied significantly depending on the marketable trademark, even when under the same commercial name (Table 2). In addition, selected chilled ready-to-eat vegetable meals were also analyzed after a mild heating, as specified by the manufacturer before consumption (3 min 100W in microwave; 5 min frying without oil or butter; no heat treatment or any preparation) (Table 2). No changes were found in folate content after minimal processing. After heating of the tested food products, mean folate content was 97.1 ± 12.5% and the CV was 12.8% (results do not shown).

4. Discussion

The promotion of folate intake from natural food sources continues to be a potential health strategy to acquire an adequate and safe nutritional status (Ebara, 2017; Fajardo et al., 2015). 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 emphasized by several authors (Bouckaert et al., 2011; Elmadfa and Meyer, 2010). The demand for frozen, dried or sterilized products is being partially substituted by the consumption of precooked refrigerated products since they are considered healthier and of higher quality, but there is a lack of information about their final nutrient composition, particularly for folate and folic acid content (Fajardo et al., 2015; Nicolas et al., 2016).

The present study reports novel data on total folate (TF) content of commonly consumed chilled ready-to-eat vegetable foods found in the Spanish market by a validated microbiological assay. These new and useful data could be used to complete Food Composition Tables (FCT) or databases, but also to support regulatory purposes. Vast majority of methodologies published to date for folate content quantification are focused on fresh (Chew et al., 2012; Devi et al., 2008; Hefni et al., 2010), processed (Bassett and Sammán, 2010; Hefni and Witthoft, 2014) or cooked/fermented (Park et al., 2015; Vishnumohan et al., 2009) foods and meals. However, most of these studies aimed their research at the analysis of the variability in folate values according to the extraction method applied (conjugase, enzyme treatment, incubation periods) (Devi et al., 2008; Park et al., 2015), the folate quantification technique used (Hefni et al., 2010; Chew et al., 2012) or to test folate retention after food processing (Delchier et al., 2013; Hefni and Witthoft, 2014).

According to previous findings, the introduction of a trienzyme treatment, with the inclusion of α-amylase and protease enzymes, is often proposed by many researchers as the method of choice for folate measurement in foods, rather than conjugase alone, to enhance the release of the folate that is bound or trapped in the food matrix of proteins and oligosaccharides (Devi et al., 2008; Park et al., 2015). Among folate analysis procedures, microbiological assay is the method of choice for the quantification of TF content in foods if data on individual folates is not deemed necessary; it is therefore suitable for food composition data and nutritional labelling purposes (AOAC, 2006 Fajardo et al., 2012).

Besides, it is well known that degradation of vitamins depends on specific conditions during culinary process (i.e. temperature, presence of oxygen, light, moisture, pH, and length of heat treatment) and type of food (McKillop et al., 2002). Particularly, losses of folates in cooking and soaking waters were considerable in vegetable based foods, mainly due to leaching into cooking water (40% retention) (Bassett and Sammán, 2010). Consequently, boiling is a good choice of cooking vegetables for folate intake when cooking water is consumed together

with these vegetables (Maharaj et al., 2015; McKillop et al., 2002). Accordingly, steaming of vegetables resulted in no significant decrease in folate content, even for long steaming periods (McKillop et al., 2002). In this sense, meals with ingredients suggesting high concentrations of antioxidants were less prone to loss folate when heated, since antioxidants probably protected folates. Cooking methods that minimize direct contact of food with the cooking water, such as in pressure-cooking, microwave cooking, or stir-frying have been to be preferable to boiling for folate retention (Maharaj et al., 2015). Regardless of all these studies, there is a lack of data on micronutrient content and retention for convenience products which results in difficulties to accurately estimate real intakes (Ahlgren et al., 2004; Fajardo et al., 2012). Therefore, the growth 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 (Fajardo et al., 2012).

Determination of folate forms in different food samples is a challenge because folate data in different food database are widely variable and inaccurate (Bouckaert et al., 2011; Fajardo et al., 2012; Westenbrink et al., 2012). Most of the differences found between compiled and analyzed data could be due to the fact that the information and values showed in FCT and databases are mostly estimated from relatively previous and obsolete data (Bouckaert et al., 2011). Moreover, 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, food composition and analytical procedures (Elmadfa and Meyer, 2010; Nicolas et al., 2016). Fortunately, the European Food Information Resource network of Excellence (EuroFIR) project (2005–2010) aimed at harmonization and standardization of food composition data in Europe, creating comparable databases for future pan-European food consumption research. Targeting folate values, attention should be paid to the analytical method used, as well as to the correct food identification and component descriptions (i.e. TF can be the sum of food folate and FA or the factored summation that gives folate equivalents). Before copying or extrapolating values from different databases, careful scrutinizing is needed to see if values are fit for purpose, preferably using more detailed food and value description systems, such as LanguaL and EuroFIR thesaurus (Nicolas et al., 2016; Westenbrink et al., 2016).

We show that chilled ready-to-eat meals may provide a significant amount of the vitamin needs. In Spain, Dietary References Intakes (DRI) for folate are 100 to 300 μg/day for boys and girls (under 12 y), 400 μg/

Table 1
Food classification and ingredients of each of the selected chilled ready-to-eat products[a].

Prepared food product (A0861)		Ingredients (information giving in the label)
Pasta dish (A1204)	Cantonese fried rice	Brown rice, peas, bacon, cooked chicken, cooked shrimp peeled, onion, carrot, green pepper, soy sauce, sugar, vinegar, olive oil, salt
	Lasagna with vegetables	Vegetables (55%) (tomato, onion, carrot, zucchini, mushroom, eggplant, olive oil (1,7%), white wine, sunflower oil, salt, thickener (starch modified from tapioca), pesto sauce, garlic); bechamel (27%) (water, cream, thickener (starch modified from potato, corn and wheat flour), additive (E407), prepared milk, nutmeg, paprika; pasta (14.3%) (wheat semolina), egg; cheese (3.7%) (cheese, butter, vegetable fat, potato starch, thickener, salt, colorant)
	Seitan	Seitan (83%): water, wheat gluten, wheat flour, kombu seaweed broth (water and kombu kelp (16%)), soy sauce (water, soy (33–46%), salt, koji), salt, garlic, ginger. Cooking broth: water, soy sauce (water, soy (33–46%), salt, koji), garlic, ginger, bay
Pies (A1296)	Broccoli pie	Dough: water, tapioca starch, brown rice and-chickpea flour, olive oil, additive (E415), salt. Filling: Broccoli (32%), red pepper, onion, leek, tomato, sunflower oil, garlic, salt, fructose, tapioca starch, additive (E407)
	Vegetable pie	Dough: brown wheat germinated, water, olive oil, brown spelt flour, salt; Stuffing: broccoli (10.9%), red pepper, onion, olive oil, sunflower oil, tomato, leek, garlic, salt, fructose, algae
Pulse dish (A0832)	Chickpeas cooked (*cocido*)	Chickpeas, (51%), broth (water, chicken, leek, olive oil, salt) (30%), beef meat (6%), blood sausage (4%), chorizo (5%), bacon (3%), carrot (2%)
	Chickpeas with spinach	Chickpeas (58%), spinach (18%), carrot, onion, olive oil, Iberian pork ham (Iberian pork ham and salt), salt, sweet paprika
	Chickpeas with tripe (*callos*)	Beef tripe (35%), vegetable broth, beef legs, chickpeas, pork mouth, pork ham, chorizo (pork meat, peppers, salt, garlic), olive oil, flavor (E621), gelatin, salt, paprika, garlic, spices, additives (E451; E452, E407, E250, A331)
	Hummus	Chickpeas (87%), vegetable fat, sesame pasta, lemon juice (2%), olive oil (2%), garlic, salt, additives (E330, E270, E202, E211).
	Lentil stew	Water, lentils (70%), fried tomato (tomato, sunflower oil, sugar, salt, cornstarch, garlic, onion, citric acid), onion, carrot, green pepper, leek, sunflower oil, smoked bacon (51%) (bacon, water, salt, starch, soy protein, additives (E450, E407, E410, E415, E331, E316, E250), smoked flavor, chorizo (5,8%) (pork meat, paprika, salt, garlic), salt, sweet paprika, meat broth (salt, wheat starch, animal hydrogenated fat, monosodic glutamate, extracts protein from corn and sunflowers, lactose, candy, flavor, onion, spices, additives (E220), thickener (starch modified, dextrin, lactose, vegetable fat, salt, flavor)
	Lentils with chorizo	Water, lentils (16%), fried tomato (concentrated tomato, vegetable fat, sugar, salt, onion, garlic), chorizo (9%) (pork meat, paprika, salt, garlic), carrot, onion, olive oil, vegetable broth
	Peas with ham	Peas (82%), cooked ham (6%) (pork ham, water, salt, additives (E407, E451, E452, dextrose, natural flavor, sodium ascorbate, E250), onion, sunflower oil, maltodextrin, salt, corn flour, yeast extract, potato starch, flavor, tomato powder, spices, parsley
Salads (A0866)	Couscous salad with chicken	Wheat semolina rehydrated (49%), vegetables (peppers, tomato, onion), cooked chicken (10%) (chicken 88%, water, salt, spices), corn, concentrated lemon juice, olive oil, vegetable fat, spices, salt, flavor, additive (E202)
	Tabbouleh	Wheat semolina rehydrated (62%), vegetables (peppers, tomato, onion), olive oil, vegetable fat, concentrated lemon juice, raisins, mint, spices, vinager, salt, preservative (E202)
Soups (A0865)	Pumpkin	Pumpkin (48%), leeks (21%), olive oil (4.1%), cilantro (0.3%), carrot, water, salt, garlic
	Vegetables mix	Vegetables (71%) (zucchini, carrot, green beans, spinach, leek, onion and garlic), olive oil (5%), water, salt
	Zucchini	Zucchini (47.1%), leek (19.4%), olive oil (3.9%), garlic, celery (3%), water, salt

(*continued on next page*)

Table 1 (*continued*)

Prepared food product (A0861)			Ingredients (information giving in the label)
Vegetable dish (A0828)	Artichokes sauteed		Artichokes, sunflower oil, Iberian pork ham (Iberian pork ham and salt), bacon (bacon, salt, starch, soy protein, additives (E450, E451, E407, E410, E415, E331, E301), natural flavors, additives (E250, E120), salt, garlic
	Artichokes with ham		Cooked artichokes (82.5%) (artichokes, water, salt), olive oil (10%), ham (7%) (pork ham, salt, sugar, additives (E252, E250, E331, E301) garlic
	Green beans sauteed		Green beans, sunflower oil, Iberian pork ham (Iberian pork ham and salt), garlic, salt
	Omelette with spinach		Potatoes (41%), egg, spinach (19%), fried onion (onion, olive oil, salt, lemon juice), sunflower oil, olive oil, salt, additives (E407, E415, E410, E330, E202, E211)
	Ratatouille		Zucchini (36%), onion (27%), tomato (22%), green pepper (9%), sunflower oil, salt, sugar, thickener (starch modified from potatoes and corn, dextrin, lactose), vegetable fat, salt, flavor
	Vegetables sauteed		Onion, carrot, zucchini, leek, green bean, peas, sunflower oil, Brussel sprouts, bacon (bacon, salt, starch, soy protein, additives (E450, E451, E407, E410, E415, E331, E301), natural flavors, additives (E250, E120), Iberian pork ham (Iberian pork ham and salt), garlic, salt
	Mushroom dish (A1335)	Mushrooms with garlic	Mushroom (90%), olive oil (4%), garlic (2.5%), white wine, salt, wheat flour, parsley
	Burgers/Bars	Algae and eggplant	Oat flakes, eggplant, spaghetti seaweed, olive oil, wheat gluten, egg albumin, onion, salt, spices (pepper, leaf celery, parsley, mace, turmeric, laurel, yeast extract, onion, sugar)
		Broccoli	Fresh tofu (water, soy, nigari), broccoli (23%), textured soy protein, cooked rice, carrot, onion, olive oil, tapioca starch, soy sauce (water, soy (33–46%), salt, koji), gelling agent, sesame, salt, garlic, paprika, sweet and black pepper
		Cheese and broccoli	Oat flakes, borage, broccoli (9.1%), olive oil, wheat gluten, gouda cheese (6.1%), gorgonzola cheese (4.1%), egg albumin, onion, salt, garlic
		Seitan, tofu and vegetables	Seitan (gluten, wheat flour, water), fresh tofu (water, soy, nigari), brown rice, red and green pepper, olive oil, carrot, onion, algae extract, salt, garlic, flax
		Soy and carrot	Water, carrots, soy drink 21% (water, soy (10%)), soy protein (19%), sunflower oil, tomato concentrate, concentrated juice of vegetables (carrot, leek), salt, wheat fibers, sugar, gelling agent)
		Soy and vegetables	Water, vegetables 21% (carrots, peas, corn), soy drink 21% (water, soy beans 10%), soy protein 19%, sunflower oil, tomato concentrated, vegetable juice concentrated (carrot, leek), salt, gluten fiber, sugar, gelling agent
		Tofu and algae	Tofu (39%) (water, soy (25%), nigari), sunflower oil, water, emmental cheese (6%), egg albumin, parmesano cheese (2.4%), onion, salt, yeast extract, rice vinegar, agave syrup, pepper, nutmeg, gelling agent
		Tofu, cheese and spinach	Tofu (59.9%) (water, soy (25.1%), nigari), brown rice, spinach (11.4%), smoked tofu (8.6%) (water, soy (25.1%), nigari, salt), brown wheat flour, cheese (5.7%), oat starch, sunflower oil, shoyu (soy (3.7%), wheat, salt, water), garlic, salt
		Tofu and mushrooms	tofu (water, soy, nigari), mushrooms (20.6%), textured soy protein, brown rice, carrot (water, soy (33–46%), salt, koji), tapioca starch, gelling agent, olive oil, onion, soy green germinated (water, soy), gelling agent, garlic, salt, raw cane sugar, natural mushroom flavor, other natural flavors, white and black pepper
		Tofu and spinach	Tofu (36%) (water, soy, nigari), spinach (14%), brown rice, onion, smoked tofu (8%) (water, soy, coagulant (water, soy, wheat, salt, koji, garlic, salt
		Tofu, spinach and cheese	Tofu (37.1%) (water, soy (25.1%), nigari), brown rice, spinach (11.4%), smoked tofu (8.6%) (water, soy (25.1%), nigari, salt), brown wheat flour, cheese (5.7%), oat starch, sunflower oil, shoyu (soy (3.7%), wheat, salt, water), garlic, salt

[a]According LanguaL™ Thesaurus European Food Information Resource network of Excellence (EuroFIR) classification and codification system (Møller and Ireland, 2009). Ingredients declared on the label.

day for teenagers and adults, and 600 μg/day for pregnant women (Moreiras et al., 2016). TF content in the analyzed chilled ready-to-eat products was found to vary from 4.6 to 103.8 μg/100 g fresh weight (Table 2). Vegetable burgers had the highest folate content since its main ingredients, soybeans, peas and green leafy vegetables, are a good source of natural folate. Other good sources of folate are recipes that include chickpeas and peas, vegetables like broccoli, artichokes or spinach, in accordance with raw ingredients data analysis (BEDCA, 2017; Moreiras et al., 2016). However, the ingredients and the amount of each them used in ready-to-eat meals, vary considerably according to the recipe, manufacturer or commercial brand, providing, therefore, a great difference in the concentrations of folate. The average folate in the five ready-to-eat meals with a higher folate content was ≈ 75 μg TF/100 g fresh weight. Thus, a typical serving of the different tested ready-to-eat meals (from 100 to 250 g according to the label of each food product) (Moreiras et al., 2016), provides 3.0% to 56.2% of the DRI for an adult and ranges from 2.0% to 37.5% of the DRI for pregnant women. A typical serving of chilled ready-to-eat vegetable food products like “peas with ham” (175 g) may provide about 24.1% of the DRI for an adult and 16.1% of the DRI for pregnant women. So, this food product, as well as most of the foodstuffs tested in the present work, could be labelled as “source of folates” or “high in folates” since they provide more than 15 and 30% of the recommended daily amount (CDR) (Regulation (CE) N° 1924/2006Regulation, 2006Regulation (CE) N° 1924/2006). Fig. 2 illustrates how targeted ready-to-eat vegetable meals may contribute to the DRI for folate in different age groups of the Spanish population. Folate content of each ready-to-eat product is expressed as TF in the standard serving suggested on the package label, and after manufactureŕs indications as to how the meal should be consumed (3 min 100W in microwave; 5 min frying without oil or butter; no heat treatment or any preparation before its consumption).

The highest reported intakes for folate occur in populations which follow a Mediterranean dietary pattern like Spain. Given the impact of good dietary habits on the prevention of chronic non-transmittable diseases, health policies should focus on adherence to a healthy diet supporting traditional dietary patterns, such as Mediterranean, in an era where intense commercial pressures promote the consumption of ready-to-eat food products because of their convenience (Benhammou et al., 2016). Within this context, although the considerable amount of folate in some of the analyzed ready-to-eat vegetable meals demonstrates their potential to increase folate intake substantially, it should be pointed out that many of these products could be highly processed

Table 2
Total folate content in ready-to-eat vegetable products on a fresh weight basis.

Prepared food product			Total folate (μg/100 g)[a]	Standard serving (g)[b]	Total folate (μg/standard serving)[c]	Samples food product preparation
Pasta dish	Cantonese fried rice		15.8 ± 0.1	175	22.2 ± 1.9	5 min frying without oil or butter
	Lasagna with vegetables		8.7 ± 0.6	200	18.2 ± 0.8	idem
	Seitan		12.0 ± 0.0	150	14.4 ± 0.9	3 min 100W in microwave
Pies	Broccoli pie		56.8 ± 8.5[A]	200	101.0 ± 8.5	5 min frying without oil or butter
	Vegetable pie		22.2 ± 7.6[B]	200	44.4 ± 7.6[D]	no heat treatment
Pulse dish	Chickpeas cooked (*cocido*)		30.1 ± 1.0	250	85.5 ± 2.7	5 min frying without oil or butter
	Chickpeas with spinach		29.3 ± 0.9	250	68.0 ± 0.6	idem
	Chickpeas with tripe (*callos*)		85.0 ± 3.9	250	224.7 ± 6.1	3 min 100W in microwave
	Hummus		72.9 ± 3.5	100	72.9 ± 3.5	no heat treatment
	Lentil stew		13.3 ± 0.1	250	45.1 ± 0.8	5 min frying without oil or butter
	Lentils with chorizo		10.7 ± 0.6	250	42.3 ± 0.8	idem
	Peas with ham		50.6 ± 1.1	175	96.6 ± 2.5	idem
Salads	Couscous salad with chicken		23.0 ± 0.2	175	40.2 ± 0.2	no heat treatment
	Tabbouleh		11.1 ± 0.8	175	19.4 ± 0.8	idem
Soups	Pumpkin		4.6 ± 0.1	250	12.0 ± 0.1	5 min frying without oil or butter
	Vegetables mix		34.1 ± 2.8	250	87.5 ± 0.5	idem
	Zucchini		36.9 ± 2.1	250	92.7 ± 0.4	idem
Vegetable dish	Artichokes sauteed		20.9 ± 1.3	225	42.7 ± 2.7	idem
	Artichokes with ham		49.8 ± 0.6	225	90.9 ± 2.1	idem
	Green beans sauteed		14.1 ± 1.4	250	33.2 ± 0.8	idem
	Omelette with spinach		47.3 ± 1.2	175	92.4 ± 0.1	no heat treatment
	Ratatouille		14.6 ± 1.1	250	41.7 ± 1.9	5 min frying without oil or butter
	Vegetables sauteed		17.5 ± 0.1	250	57.5 ± 0.2	idem
	Mushroom dish	Mushrooms with garlic	21.9 ± 0.1	215	46.2 ± 0.1	idem
	Burgers/Bars	Algae and eggplant	13.3 ± 0.5	150	23.6 ± 0.3	3 min 100W in microwave
		Broccoli	16.6 ± 0.8	150	28.3 ± 0.1	idem
		Cheese and broccoli	47.9 ± 1.2	150	64.6 ± 2.8	idem
		Seitan, tofu and vegetables	103.8 ± 21.2[C]	150	123.2 ± 21.2[D]	idem
		Soy and carrot	26.1 ± 2.1	150	37.9 ± 2.1	idem
		Soy and vegetables mix	12.6 ± 0.8	150	20.2 ± 0.2	idem
		Tofu and algae	10.4 ± 0.1	150	15.9 ± 0.7	idem
		Tofu, cheese and spinach	19.3 ± 1.3	150	18.9 ± 0.1	idem
		Tofu and mushrooms	17.0 ± 0.0	150	32.4 ± 0.0	idem
		Tofu and spinach	27.5 ± 0.2	150	52.5 ± 0.8	idem
		Tofu, spinach and cheese	18.2 ± 1.9	150	25.8 ± 1.4	idem

[A,B,C,D]Samples with coefficient of variation higher than 15% (15, 34, 20, and 17%, respectively).

[a] Results are expressed as mean ± standard deviation from duplicates analysis of two different dilutions (100 μL of the diluted sample and 50 μL of the diluted sample plus 50 μL of ascorbic acid) of two different commercial batches, from three random most highly consumed commercial brands, of each ready-to-eat product as packaged (Table 1).

[b] Standard serving means standard portion size (according package label).

[c] Results are expressed as mean ± standard deviation from duplicates analysis of two different dilutions (100 μL of the diluted sample and 50 μL of the diluted sample plus 50 μL of ascorbic acid) of two different commercial batches, from three random most highly consumed commercial brands, of each ready-to-eat product as manufacturer's indicated in the standard serving suggested on the package label.

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 (Celnik et al., 2012). Minimally processed foods could be good items to cope with folate intake recommendations in the context of a diverse and healthy diet (Fajardo et al., 2012).

5. Conclusions

The expanding role of folate nutrition has major health implications. Precise and accurate data on food folate content are essential in food categories increasingly demanded by consumers, such as ready-to-eat meals. This context warrants the need for providing new data on total folate and individual forms of folate in ready-to-eat vegetable food products, either to complete Food Composition Tables or databases and/or achieve regulatory objectives, or to assess population dietary intakes. The present study provided valuable information on folate content in commonly consumed chilled ready-to-eat vegetable meals in Spain, and showed that their contribution to the Dietary References Intakes might be considered as a good natural folate source. Cooperation between chemical analysts and compilers of Food Composition Databases and consensus on analytical methods that are fit for purpose needs to be an ongoing issue to produce high quality analytical values for food composition databases.

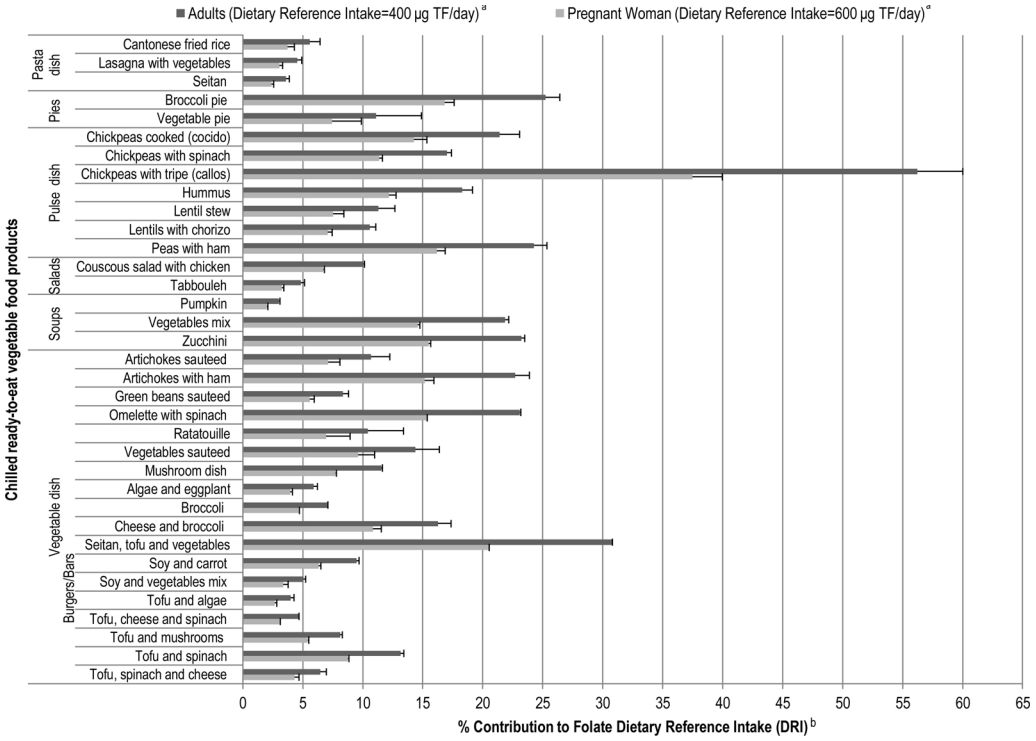

Fig. 2. Folate daily contribution of standard servings of the assayed chilled ready-to-eat vegetable meals to Dietary References Intakes (DRI, 400 µg/day for adult and 600 µg/day for pregnant women) for Spanish population.

Acknowledgements

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

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