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Analytical Methods

Folate content in fresh-cut vegetable packed products by 96-well microtiter plate microbiological assay

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ABSTRACT

Ready-to-eat foods have nowadays become a significant portion of the diet. Accordingly, nutritional composition of these food categories should be well-known, in particular its folate content. However, there is a broad lack of folate data in food composition tables and databases. A total of 21 fresh-cut vegetable and fruit packed products were analysed for total folate (TF) content using a validated method that relies on the folate-dependent growth of chloramphenicol-resistant *Lactobacillus casei subspecies rhamnosus* (NCIMB 10463). Mean TF content ranged from 10.0 to 140.9 μg/100 g for the different matrices on a fresh weight basis. Higher TF quantity, 140.9–70.1 μg/100 g, was found in spinach, rocket, watercress, chard and broccoli. Significant differences were observed between available data for fresh vegetables and fruits from food composition tables or databases and the analysed results for fresh-cut packed products. Supplied data support the potential of folate-rich fresh-cut ready-to-eat vegetables to increase folate intake significantly.

1. Introduction

Folate is the generic term for the naturally occurring folates and folic acid (FA) is the synthetic form of folate added to foods as a fortifier and used in dietary supplements. Folates are essential micronutrients involved in the prevention of macrocytic anaemia, and act as cofactors in cellular development and homeostasis throughout all life stages. This group of vitamers has been identified as a potential relevant factor in the prevention of: cardiovascular diseases, certain type of cancers, neurocognitive decline in the elderly, and congenital abnormalities in the development of the spinal cord and central nervous system (Amarin & Obeidat, 2010; Lee & Chan, 2011).

Folates are present in a wide range of foods, but especially green-leafy vegetables have been identified as the major source in the human diet (Johansson, Jägerstad, & Frølich, 2007; Shohag et al., 2012). Fortified food products with synthetic FA are also important to dietary folate worldwide (Fajardo & Varela-Moreiras, 2012). However, considering the mean dietary reference intakes, epidemiological evidence suggests that a suboptimal folate intake may be widespread in populations in both developing and developed countries (Herrmann & Obeid, 2011). Nonetheless, possible adverse effects of synthetic FA, such as masking symptoms of vitamin B_{12} deficiency and promoting certain types of cancer, delay and/or block mandatory FA fortification in some countries (Fajardo & Varela-Moreiras, 2012). Taking into account that naturally occurring food folate has been shown to markedly increase plasma folate concentrations (Ashfield-Watt et al., 2003), the promotion of folate intake from natural food sources continues to be a health strategy to reach a safe and adequate nutritional status.

European food consumption and dietary patterns and, therefore, nutrient intakes have markedly changed in the last 40 years, mainly in the youngest populations. This phenomenon together with rapid changes in lifestyle, produce a widespread consumption of ready-to-eat foods, like *fresh-cut packed vegetables*, that partially or completely replace more traditional food products in lunch and dinner. Fresh-cut packed ready-to-eat specialities are defined as any vegetable, fruit, or their combination, that has been somewhat modified from the original form (by trimming, washing, peeling and chopping), but still 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 and/or preservatives (Martín-Belloso & Soliva-Fortuny, 2011). As a response to consumer needs, the ready-to-eat food group, such as fresh-cut packed vegetables, is becoming increasingly popular worldwide. The demand for frozen,

Abbreviations: CV, coefficient of variation; FA, folic acid; FCT, food composition tables; HPLC, high-performance liquid chromatography; MA, microbiological assay; TF, total folate; SD, standard deviation.

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dried or sterilised products is being substituted by the consumption of refrigerated products since they are considered healthier and of higher quality. Particularly, the market of these products has shown substantial growth in Europe over the most recent decades due to: (i) customers claim for fresh, safe and additive-free foods, (ii) a need to facilitate daily fruit and vegetable consumption everywhere, (iii) the integration of women into the workforce, and (iv) commodity for convenience due to lack of time for cooking (Fajardo, Alonso-Aperte, & Varela-Moreiras, 2012). Paradoxically, in spite of the tremendous increase in food market availability and consumption of these food products, there is a lack of information about their nutrient composition, particularly for folate content. Accordingly, the nutritional composition of these food categories should be, firstly, investigated, in order to further estimate their contribution in vitamins and other nutrients and their impact on dietary habits for the European populations (Fajardo et al., 2012; Lee & Chan, 2011). At present, there is a need not only to improve, but also to provide new data on total folate and individual forms of folate in mixed diets which contain novel foods like ready-to-use commercial products to supply data for Food Composition Tables (FCT) or databases and/or to support regulatory purposes (Bouckaert et al., 2011).

The two general approaches currently used for analysis of food folate are either a microbiological assay and/or high-performance liquid chromatography (HPLC) (Arcot & Shrestha, 2005; Fajardo et al., 2012). The microbiological assay involves quantifying the growth response of a specific microorganism to the mixture of folate that is present in the sample (Koontz et al., 2005; Samaniego-Vaesken, Alonso-Aperte, & Varela-Moreiras, 2010). Although there are other organisms suitable for the quantification of total folate content, *Lactobacillus casei subespecies rhamnosus* responds to most metabolic forms of folate, including 5-methyltetrahydrofolate and the formyl derivatives (Arcot & Shrestha, 2005). Microbiological assay has been applied to the determination of total naturally occurring folates in foods (Bassett & Sammán, 2010) and was validated for FA by fortified foods (Samaniego-Vaesken et al., 2010). The accuracy of the results depends on the preparative steps, such as previous trienzyme extraction using amylase and protease, which are necessary to degrade the matrix of carbohydrates and proteins, and/or folate conjugase, for the deconjugation of the terminal glutamyl peptides of folate to monoglutamyl or diglutamyl derivatives (Arcot & Shrestha, 2005).

Taking into account all the considerations exposed, the purpose of this study was to analyse the folate content in different highly consumed commercial fresh-cut vegetable packed products by a validated microbiological assay.

2. Materials and methods

2.1. Selection of fresh-cut vegetable packed products and folate trienzyme extraction

A total of twenty-one fresh-cut refrigerated products, including vegetables and fruits, were purchased at local supermarkets and retail stores from the Region of Madrid (Spain). These included fourteen monoingredient and seven multiingredient fresh-cut items from 19 different own-label and popular commercial brands. Two different batches from three diverse random most highly consumed commercial brands of each product were independently grounded, processed and analysed in duplicate under subdued light, minimising contact with air. All assays were completed within a week of the product's acquisition using analytical grade reagents.

Extraction and enzyme treatments were carried out according to a previously described trienzyme extraction method (Martin, Landen, Soliman, & Eitenmiller, 1990) with some modifications (Póo-Prieto et al., 2006). Briefly, after homogenisation of the selected fresh-cut product, 0.5–1 g of the stock sample was suspended in 10 volumes 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), and chicken pancreas conjugase (Difco, Detroit, MI, USA) for 4 h, followed by addition of 2 mg/mL protease solution (type XIV, *Streptomyces griseus*, Sigma) for 1 h. Enzyme activity was stopped in a boiling water bath for 5 min. Homogenates were cooled on ice and centrifuged for 20 min at 36,000*g* and 4 °C. Finally, supernatants were filtered through sterile syringe filters (Millex-AA, 0.8 mm, Milipore, Ireland). In order to calculate the folate recovery of the extraction and enzyme process, radioactivity in an aliquot of neutralised eluate of each analysed food product was measured by quantification and compared with radioactivity in the starting mixture. FA diammonium salt tracer recovery had to be at least 85%. Folate extracts were stored at −20 °C until further analysis.

2.2. Moisture determination

Duplicate samples were separately analysed for moisture content immediately following homogenisation using a vacuum oven at 90 °C overnight, according to the AOAC method 964.22 (AOAC, 1990).

2.3. Microbiological assay

TF was determined in each extracted sample by a microbiological method on sterile 96-well microtitre plates (Costar 3596, Corning Inc., Tewksbury, MA, USA), using chloramphenicol-resistant cryoprotected *L. casei ssp. rhamnosus* (NCIMB 10463) as the growth organism (O'Broin and Kelleher, 1992).

Cryoprotected cultures were prepared from lyophilised *L. casei spp. rhamnosus* chloramphenicol-resistant (NCIMB 10463), purchased from the National Collection of Industrial and Marine Bacteria Ltd. (Scotland, UK) 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. Folate contents are given on a fresh weight basis. Enzyme blanks were assayed to account for potential endogenous folate contribution.

Method performance was considered acceptable according to the AOAC criteria including linearity, reproducibility, accuracy and repeatability parameters (AOAC, 1998).

2.4. Quality controls

A Certified Reference Material, provided as a lyophilised mixed of vegetables (CRM 485, Institute for Reference Materials and

Measurements, Geel, Belgium), was used as an external quality control for intra- and inter-assay reproducibility (Johansson et al., 2007; Koontz et al., 2005). 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 fresh-cut 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 CRM 485 (315 ± 28 μg TF/100 g dry matter) is a preparation which contains canned chopped tomatoes, frozen carrots and sweet corn.

An international standard (IS 03/178 NIBSC) from the National Institute for Biological Standards and Control (Hertfordshire, UK), with a certified value of 5.3 ng/mL of TF in serum, was also employed as an external quality control to display the accuracy of the developed method.

Internal quality control food samples from pooled trienzymatic extracts at three levels (≈30, 90 and 140 μg TF/100 g food products) were prepared, stored at −20 °C and assayed in parallel to fresh-cut food samples on a weekly basis to account for repeatability. It was necessary to adjust sample dilution within the range of the TF concentrations of these internal controls.

2.5. 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 food samples as it was 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.

2.6. Statistical analysis

Statistical analysis was performed using Statistical Package for Social Science (SPSS) version 17.0 (IBM, NY, USA). Data were presented as means ± SD based on fresh weight. For each sample, two different batches were analysed and TF values obtained in each were compared with Student's *t*-test for independent samples, with a significance level set at $p < 0.05$.

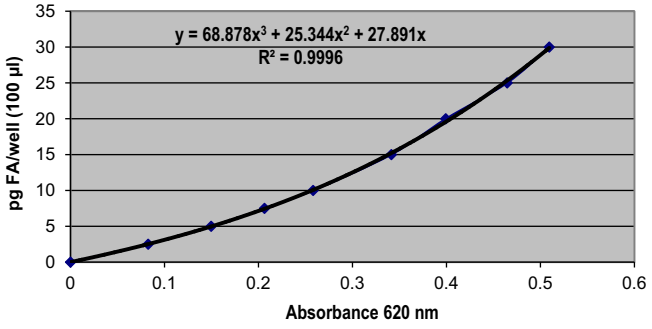

Fig. 1. Linearity test of the microbiological method with *L. casei spp. rhamnosus* chloramphenicol-resistant (NCIMB 10463).

3. Results

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

Linearity for folic acid (FA) standard (0–30 pg/100 μL) was corrobated each day of analysis with a regression coefficient $r^2 = 0.99$ (Fig. 1).

Method reproducibility was determined by intra- and inter-assay coefficients of variation (CV) for the Certified Reference Material (CRM 485) (Table 1). Intra-assay results for CRM 485 provided average values ± standard deviation of 311.9 ± 24.7 μg TF/100 g dry matter. Inter- assay reproducibility of CRM 485 samples resulted in values of 315.1 ± 27.6 μg TF/100 g. These results were within the range for TF content in the CRM 485 (315 ± 28 μg/100 g dry matter), being 5-methyltetrahydrofolate the main natural form of this vitamin (Johansson et al., 2007).

Results obtained after analysing the external quality control IS 03/178 NIBSC (5.2 ± 0.3 ng TF/mL and 5.1 ± 0.4 ng TF/mL for intra- and inter-assays analysis, respectively) were considered adequate versus the certified value (5.3 ng TF/mL) (Table 1).

Repeatability of the assay was assured on a weekly basis testing internal quality control food samples from pooled trienzymatic extracts at three levels (≈30, 90 and 140 μg TF/100 g food products) (results not shown).

Recovery values for [^{3}H] FA tracer in the trienzyme extraction method were within the range of 85–110%, and were considered acceptable according to AOAC (1998) (Table 1). In addition, enzyme blank preparations were assayed for folate determination at similar concentrations to that used in the deconjugation of fresh-cut vegetable samples. No measurable folate response was observed for the enzyme blank preparation. Therefore, its addition in the trienzyme extraction protocol does not provide endogenous folate.

3.2. Folate content in fresh-cut vegetable packed products

Mean total folate (TF) content in the selected 21 fresh-cut refrigerated products, including vegetables and fruits, ranges widely from 10.0 to 140.9 μg/100 g on the basis of fresh weight. These included 14 monoingredient (Table 2) and 7 multiingredient (Table 3) food items from different own-label and popular commercial brands.

Among the analysed green-leafy vegetables (Table 2), spinach (*Spinacia oleracea)* showed the highest folate content (140.9 μg TF/100 g), followed by rocket (*Eruca sativa*) (139.2 TF μg/100 g), watercress (*Nasturtium officinale*) (127.9 μg TF/100 g) and chard (*Beta vulgaris* subsp. *cicla*) (95.3 μg TF/100 g). Other vegetables such as broccoli (*Brassica oleracea* L. var. *italica*), corn salad (*Valerianella locusta*) or escarole (*Cichorium endivia* L. var. *latifolium*) had

Table 1
Reproducibility and recovery of the microbiological method (*L. casei spp. rhamnosus*, NCIMB 10463).

	Intra-assay (n = 5)	CV (%)	RE (%)	Inter-assay (n = 20)	CV (%)	RE (%)
CRM 485	311.9 ± 24.7 μg TF/100 g	7.9	0.98	315.1 ± 27.6 μg/100 g TF	8.7	0.03
IS	5.2 ± 0.3 ng TF/mL	5.7	1.5	5.1 ± 0.4 ng TF/mL	7.5	3.9
[^{3}H] FA tracer	96.5 ± 3.2%	–	–	97.8 ± 5.5%	–	–

Results are expressed as average ± standard deviation (SD) of n assays.
TF: total folate; CV: coefficient of variation; RE: relative error.
CRM 485: Certified Reference Material (Institute for Reference Material and Measurement, Geel, Belgium), mixed vegetables, 315 ± 28 μg TF/100 g.
IS: International standard 03/178 NIBSC (National Institute for Biological Standards and Control), certified value of 5.3 ng TF/mL in serum.
[^{3}H] FA tracer: folic acid (FA) diammonium salt tracer (Movereck Biochemicals, Brea, CA, USA).

Table 2
Total folate content in fresh-cut vegetable packed products (monoingredient) on a fresh weight basis.

Fresh-cut product	Latin name	Moisture (g/100 g)[a]	Folate content (μg/100 g)[a]	Folate content (μg/portion)[b]	Reference data (μg/100 g)		
					FCT[c]	USDA[d]	BEDCA[e]
Spinach	*Spinacia oleracea*	91.5 ± 1.9	140.9 ± 21.3	422.6 ± 64.0	140	194	143.2
Rocket	*Eruca sativa*	90.2 ± 2.3	139.2 ± 15.4	139.2 ± 15.4	97	97	–
Watercress	*Nasturtium officinale*	91.4 ± 1.8	127.9 ± 10.8	159.9 ± 16.2	214	9	–
Chard	*Beta vulgaris* subsp. *cicla*	92.3 ± 1.2	95.3 ± 13.0	286.0 ± 39.2	140	14	–
Broccoli	*Brassica oleracea* L. var. *italica*	88.0 ± 5.6	70.1 ± 11.6	210.0 ± 34.9	90	63	110
Corn salad	*Valerianella locusta*	92.6 ± 1.0	64.8 ± 12.0	81.0 ± 15.0	–	14	–
Escarole	*Cichorium endivia* L. var. *latifolium*	95.0 ± 0.5	47.7 ± 16.1	81.1 ± 27.4	267	–	–
Iceberg lettuce	*Lactuca sativa*	95.9 ± 0.5	34.8 ± 10.4	69.6 ± 20.7	53	29	–
Beetroot	*Beta vulgaris*	87.7 ± 0.5	29.4 ± 06.7	132.5 ± 30.2	90	109	–
Carrot	*Daucus carota* L. var. *sativus*	90.5 ± 1.9	18.6 ± 03.5	27.8 ± 05.9	10	19	–
Corn cob	*Zea mays*	76.0 ± 0.9	14.9 ± 04.4	59.6 ± 17.5	20	42	–
Potato	*Solanum tuberosum*	81.5 ± 0.3	12.3 ± 05.5	55.3 ± 24.8	25	30	–
Pineapple	*Ananas comosus*	85.3 ± 1.0	10.5 ± 01.2	42.0 ± 05.3	10	18	–
Coconut	*Cocos nucifera*	49.0 ± 3.1	10.0 ± 04.0	10.0 ± 04.0	26	26	–

[a]Results are expressed as mean ± standard deviation (SD) from duplicates from two batches of 3 random selected brands of the same product.
[b]Portion = package weight.
[c]Spanish Food Composition Tables (Moreiras et al., 2013).
[d]USDA Nutrient Database (2013).
[e]BEDCA Spanish Food Composition Database (2013).
[c,d,e]Reference data: data on fresh vegetables and fruits (not fresh-cut packed ready-to-eat products).

Table 3
Total folate content in fresh-cut vegetable packed products (multiingredient) on a fresh weight basis.

Fresh-cut product (commercial name)	Main ingredient/s	Moisture (g/100 g)[a]	Folate content (μg/100 g)[a]	Folate content (μg/portion)[b]
"Sprouts Salad"	Sprouts of: escarole, lollo rosso, lollo bionda and batavia lettuces	94.6 ± 1.1	82.2 ± 65.1[*]	82.23 ± 65.1
"Mezclum Salad"	Escarole, red radicchio, mizuna, spinach and rocket	93.9 ± 1.5	73.2 ± 59.0[*]	109.8 ± 97.6
"Julienne soup"	Chard, cabbage, carrot, turnip, onion and leek	92.7 ± 0.2	42.4 ± 04.3	169.6 ± 17.1
"Gourmet Salad"	Escarole, radicchio and canon	94.3 ± 0.2	34.2 ± 03.8	59.9 ± 06.6
"Four seasons Salad"	Iceberg lettuce, carrots, red cabbage and carrot	95.0 ± 0.7	33.6 ± 03.7	83.9 ± 09.2
"Caesar Salad"	Romaine lettuce, Caesar dressing and croutons	80.0 ± 3.9	17.4 ± 02.7	36.6 ± 05.7
Tofu	Water, soybean and nigari coagulant	74.3 ± 0.2	12.0 ± 04.4	30.0 ± 10.9

[a] Results are expressed as mean ± standard deviation (SD) from duplicates from two batches of 3 random selected brands of the same product.
[b] Portion = package weight.
[*] CV > 15%.

lower folate contents (between 70 and 40 μg TF/100 g), whereas carrot (*Daucus carota* L. var. *sativus*), corn cob (*Zea mays*) and potato (*Solanum tuberosum*) had a markedly lower content (<20 μg TF/100 g). Coconut (*Cocos nucifera*) and pineapple (*Ananas comosus*) presented the lowest amount (<10.0 μg TF/100 g).

Folate content in the targeted multiingredient fresh-cut products is shown in Table 3. The values range from the lowest for *tofu* (12.0 μg TF/100 g) to the highest for the so-called "Mezclum Salad" (73.2 μg TF/100 g) and "Sprouts Salad" (82.2 μg TF/100 g).

The coefficient of variation (CV) for duplicates of two batches from three different commercial brands of the same product was less than 15% for monoingredient fresh-cut items. However, CVs rose to 70% and 80% after analysis of miscellaneous salads like "sprouts and mezclum salad", respectively, since the composition of these multiingredient fresh-cut prepared salads is not specified in quantities in the label, and varied significantly depending on the marketable trademark, even though under the same commercial name.

4. Discussion

The increasing significance of folate in health, due to its potential role in disease prevention, and the fact that the average daily intake of folate is somewhat below the recommendations for most groups (children, adolescent, fertile women, elderly) (Fajardo & Varela-Moreiras, 2012; Pozo et al., 2012), emphasise the need for an accurate and critical evaluation of all types of dietary folate sources (Dhonukshe-Rutten et al., 2009; Fajardo et al., 2012). However, there is only very limited information on food folate and folic acid (FA) content (Bouckaert et al., 2011), in some food groups with an increasing consumption rate during the last years, such as the ready-to-eat specialities. These include a potential source of natural folate: *fresh-cut packed vegetables* (Fajardo et al., 2012).

Pandrangi and LaBorde (2004) determined folate retention, carotenoids, and other quality characteristics in commercially packaged fresh spinach. Nevertheless, there are no other published studies on folate content in other fresh-cut vegetarian products. Therefore, total folate (TF) data reported in the present study (Tables 2 and 3) are new and very useful to complete Food Composition Tables (FCT) or databases, but also to support regulatory purposes.

Compared to data reported in diverse studies for fresh vegetables or fruits, folate content of most of the analysed fresh-cut vegetable products were within the same range (Johansson et al., 2007; Phillips, Rasor, Ruggio, & Amanna, 2008; Shohag et al., 2012). Lower losses of folate have been observed comparing to others (Pandrangi & LaBorde, 2004) using similarly stored fresh vegetables (i.e. spinach). Moreover, data obtained were in accordance with the quantity expressed in the nutritional label of the few fresh-cut products which had a declared folate amount (i.e. pineapple, labelled as 14 μg TF/100 g) (Table 2). On the contrary, as it is shown in Table 2, significant differences were observed between

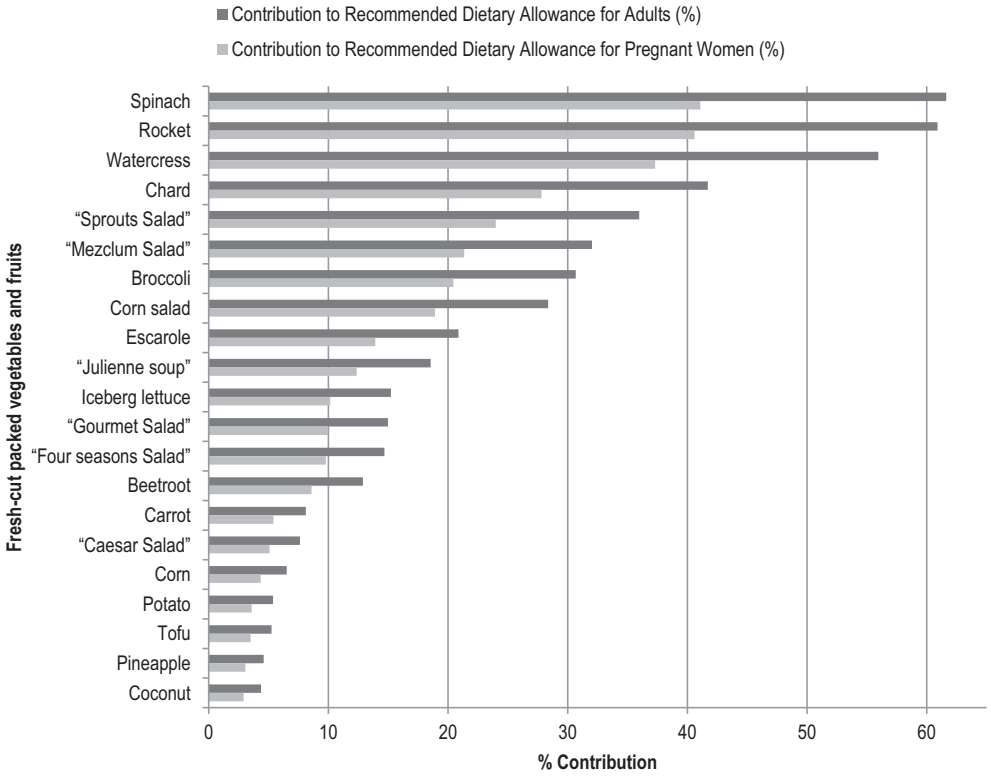

Fig. 2. Daily contribution of the analysed fresh-cut vegetable packed products to recommended dietary allowances (400 μg/day for adult and 600 μg/day for pregnant women) for Spanish population.

available data for fresh vegetables from FCT or databases (BEDCA Spanish Food Composition Database 2013; Moreiras, Carbajal, Cabrera, & Cuadrado, 2013; USDA Nutrient Database, 2013) and the results achieved after analysis of the targeted fresh-cut products. Moreiras et al. (2013) and the USDA Nutrient Database report that rocket contains 97 μg TF/100 g, whereas the achieved folate content in the tested fresh-cut samples was approximately 47 μg TF/100 g. No comparison of folate data from FCT or databases is possible for multiingredient fresh-cut vegetable packed products, because there is no available data to compare with and their composition is not specified in quantities in the label varying significantly depending on the marketable trademark, even though under the same denomination.

Most of the differences found between compiled and analysed 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 (Bassett & Sammán, 2010; Fajardo et al., 2012). To avoid these limitations, systematic studies for the compilation of compositional food data are required to: (i) elaborate complete and updated FCT, 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 & Meyer, 2010).

Despite the lack of accurate and updated data on folate content, fresh-cut vegetables and fruits may provide a significant amount of the vitamin needs. In Spain, the recommended dietary allowances (RDA) for folate, as expressed in dietary folate equivalents (micrograms of DFE = micrograms of folate from food), are 100–300 μg/day for boys and girls (under 12 years), 400 μg/day for teenegars and adults, and 600 μg/day for pregnant women (IOM, 2000; Moreiras et al., 2013). TF content in the analysed fresh-cut vegetable packed products was found to vary from 10.00 to 140.9 μg/100 g on a fresh weight basis (Table 2). Spinach had the highest amount of folate (140.9 μg/100 g), and it is a good source of natural folate. However, the popular iceberg lettuce, which dominates the production of minimally processed vegetables, contained only 35 μg TF/100 g. The average folate content considering the five highest fresh-cut mono and multiingredient products analysed in this study was ≈109 μg TF/100 g on wet weight basis. Thus, a typical serving of different vegetables (150–200 g) (Moreiras et al., 2013), provides 4.4–61.6% of the RDA for an adult and ranges from 2.9% to 41.1% of the RDA for pregnant women. A typical serving of fresh-cut prepared salad like "sprouts salad" may provide about 20.6% of the RDA for an adult and 13.7% of the RDA for pregnant women. Fig. 2 illustrates how targeted fresh-cut packed vegetables may contribute to the recommended dietary intake for folate in different groups of Spanish population.

Lin and Lin (1999) reported that 60–100% folate is retained in vegetables after cooking ("stir frying"), but the available folate could be higher in sprouts and salads than in other vegetables since those are most often consumed in fresh. Thus, the considerable amount of folate in some of the analysed fresh-cut vegetable products demonstrates their potential to increase folate intake substantially. Minimally processed vegetables like fresh-cut vegetables, attractive to consumers, because of their convenience and nutritional value, could be an extraordinary and fast complimentary item to cope the World Health Organization (WHO) recommendations of at least five servings of vegetables and/or fruits per day.

5. Conclusions

During the past few years, as a response to consumer demands, ready-to-eat foods, fresh-cut vegetable packed products among

them, are becoming increasingly popular worldwide. Consequently, the nutritional composition of these food categories should be investigated in order to estimate its contribution to total energy, vitamin and nutrient intakes, in particular its folate content, since these are potentially good sources. The broad lack of folate data in Food Composition Tables and databases justifies this approach. New data on folate content for twenty-one fresh-cut vegetable and fruit packed products are available. The considerable amount of folate content in some of the analysed fresh-cut products indicates their potential to increase folate intake significantly. In this sense, obtained data should be incorporated into the national food databases and will assist dietary studies needed to estimate and evaluate the adequacy of population folate intakes.

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