Folates in fruits and vegetables: contents, processing, and stability.

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ABSTRACT:

Folates play a key role in human one-carbon metabolism and are provided by food. It is well established that folates are beneficial in the prevention of neural tube defects and cardiovascular and neurodegenerative diseases. Fruits and vegetables, and especially green vegetables, are the main sources of folates. In parallel, fruits and vegetables, with high contents of folates, are mostly consumed after processing, such as, canning, freezing, or home-cooking, which involve folate losses during their preparation. Hence, it is important to know the percentage of folate losses during processing and, moreover, the mechanisms underlying those losses. The current knowledge on folate losses from fruit and vegetables are presented in this review. They depend on the nature of the respective fruit or vegetable and the respective treatment. For example, steaming involves almost no folate losses in contrast to boiling. Two main mechanisms are involved in folate losses: i) leaching into the surrounding liquid and ii) oxidation during heat treatment, the latter of which depending on the nature of the vitamer considered. In this respect, a vitamer stability decreases in the order starting from folic acid followed by 5-HCO-H_4folate, 5-CH_3-H_4folate, and, finally, H_4folate. Further studies are required, especially on the diffusion of the vitamers in real foods and on the determination of folate degradation products.

Keywords: Folate, folic acid, oxidation, heat treatment, degradation,

Abbreviations:

5-CH_3-H_4folate: 5-methyltetrahydrofolate;

5-CH_3-H_2folate: 5-methyldihydrofolate;

5-HCO-H_4folate: 5-formyltetrahydrofolate;

10-HCO-H_4folate: 10-formyltetrahydrofolate

10-HCO-H_2folate: 10-formyldihydrofolate

10-HCO-PteGlu: 10-formylfolic acid;

H_4folate: tetrahydrofolate;

H_2folate: 7,8-dihydrofolate;

5,10-CH^+-H_4folate: 5,10-methenyltetrahydrofolate;

5,10-CH_2-H_4folate: 5,10-methylenetetrahydrofolate.

INTRODUCTION

Our review aims to establish the current knowledge on folate contents in fruits and vegetables, and in particular on their fates from the raw plant to the consumed products. Folate is a complex group of molecules, and, in spite of recent analytical progress, their distribution in food is still poorly known, with many uncertainties as to their degradation mechanisms and kinetics.

Folate is a group of water-soluble vitamers with a three-part structure: a pterin cycle bound to a *para*-aminobenzoic acid via a C-N bond, the latter carrying a polyglutamate chain, in which a varying number of glutamic acids are connected through a γ-peptidic bond. Folate structure varies by the reduction and substitution on the pterin group, and by the length of the glutamate chain (**Figure 1**). Folates play a key role in one-carbon metabolism, that is, key reactions in the synthesis of proteins with the amino acids methionine, histidine, serine, and glycine, but also DNA with purines and thymidilate or vitamin B5 (pantothenate). In humans, its deficiency is primarily linked to defects in the development of the neural system of the foetus, but can also lead to megaloblastic anemia (Steegers-Theunissen 1995). Moreover, poor folate status has been related to increased risks of cardiovascular diseases (Moat and others 2004), colorectal cancers (Jennings and Willis 2015), and Alzheimer's disease (Snowdon and others 2000).

The European Food Safety Authority (EFSA) has established a Population Reference Intake of 330 µg/day (EFSA 2014), with an increase to 600 µg per day during pregnancy or lactation (or for women planning pregnancy). Low folate status has a high prevalence in Europe where no systematic supplementation with folic acid is carried out (in contrast to, for example, the USA) (Dhonukshe-Rutten and others 2009). For example, the INCA-1 and 2 studies indicate a deficit especially before and during pregnancy of women, with average intakes of 268 µg per day (Lafay 2009). Folate daily intakes have been established for the population of 11

Europeans countries, folates intakes varied from 190 µg/ day in the Dutch population to 431 µg/ day in the UK for men and from 190 µg/ day in the Dutch population to 465 µg/ day in the UK for women (Dhonukshe-Rutten and others 2009). It is, therefore, of major importance to optimize the folate supply from foods.

Folates are found in high concentrations in wheat germ, yeasts, innards (especially liver, which is the folate storage organ in mammals), some cereals, as well as in pulses and leafy vegetables. Contribution to dietary intake is a function of both the concentration in a particular food and the consumption of this food in the general population. Although wheat germ, yeasts, and organ meats are high in folates, their consumption is lower than that of fruits and vegetables and, hence, also their contribution to folate supply. Analogously, pulses contain high concentrations of folate, but due to their low consumption levels they only contribute 1.8% of folate intake in the French diet (Lafay 2009). In France, fruits and vegetables (including potatoes and dry fruits) represent >35% of folates' intake within the diet of adults (Lafay 2009), with about 20% for vegetables alone, while breakfast cereals, due to vitamin supplementation, are the main source for children 3 to 17 years old. In fruits and vegetables, folates are mainly present as 5-CH_3-H_4folate, followed by 5-HCO-H_4folate and 10-HCO-PteGlu, with a total folate concentration ranging from 1 - 2 µg/100 g for peach and watermelon to an average 300 µg/100 g for spinach and soybean. All are present mostly as polyglutamate. Low concentrations and marked losses of folates during processing of fruits and vegetables (as well as low consumption of organ meat) explain that folate deficiency can easily occur. It is therefore highly relevant to mitigate these losses, particularly in the processing of vegetables. Other interesting possibilities are selective breeding (though only limited data are available to date on folate genetic variability) and biofortification through plant folate engineering (Scott and others 2000; Hanson and Gregory 2011; Blancquaert and others 2014).

Folates are synthetized in plants and microorganisms using the same basic pathways, and then they undergo interconversion to different forms, which are implicated in specific one-carbon syntheses. Hanson and Gregory (2011) have recently reviewed in detail folate biosynthesis and interconversion in plants. Therefore, only a short overview is given here. In plants, the 2 main subunits of folate, pterin and *p*-aminobenzoic acid (*p*ABA) are synthesized in 2 distinct cell compartments, namely cytosol and plastids. Their assembly, however, only occurs in mitochondria. Pterin as 6–hydroxymethyldihydropterin is activated in the mitochondria by pyrophosphorylation (by 6–hydroxymethyldihydropterin pyrophosphorylase) and coupled by dihydropteroate synthase to *p*ABA by a single, multifunctional protein. The molecule is further coupled to a glutamate to yield dihydrofolate (DHF synthase) and reduced to tetrahydrofolate monoglutamate (DHF reductase), which may undergo polyglutamation under action of folylpolyglutamate synthase. In plants, polyglutamate forms are dominant, though there is considerable variation in the polyglutamate chain length; presence of γ-glutamyl hydrolases is implicated both in the regulation of polyglutamate chain length and in deglutamation that may occur during fruit and vegetable processing (and laboratory analysis). Folates further undergo transport, so that they are present in all plant cell compartments.

Folates are absorbed primarily in the small intestine (duodenum and jejunum); the proton-coupled folate transporter has the primary role in the transport across the gut endothelium (Visentin and others 2014). As the proton-coupled folate transporter is highly specific for the monoglutamate form, folate absorption first requires deglutamation, by intestinal folate hydrolase (glutamate carboxypeptidase II), located in the brush border. There has been concern over the influence of polyglutamation on folate bioavailability (Melse-Boonstra and others 2002), but deglutamation does not appear to be a limiting factor (Konings and others 2001; McKillop and others 2002). The colon may further contribute to absorption of folates,

131 when synthesized by the microbiota (Aufreiter and others 2009). Once folates enter the portal
132 circulation, they are transported into the liver, where they are metabolized to the
133 polyglutamate form, which can be used intracellularly, stored, or released into blood or bile
134 (Bailey and Caudill 2012).
135
136 Though less investigated than vitamin C, there is a substantial body of work on folate stability
137 in model solutions, fewer in foods, but covering some vegetables and fruit juices. Folates are
138 described to be sensitive molecules: they can be degraded by heat and by oxidation, which
139 leads to the cleavage of folate into a pterine and a *p*-aminobenzoic acid linked to a
140 polyglutamate tail, neither of which has biological functions (Scott and others 2000). There is
141 a major impact of pH on folate stabilities, unfortunately with lowest stabilities at the pH
142 conditions commonly encountered in plant foods (pH 4 to 6) (Paine-Wilson and Chen 1979;
143 Mnkeni and Beveridge 1983; Indratawi and others 2004a). Glycation has been shown to occur
144 for folic acid and 5-CH_3-H_4folate in the presence of reducing sugars, and notably fructose
145 (Schneider and others 2002; Verlinde and others 2010), a major constituent in fruit, which
146 will further enhance degradation. However, fruits and vegetables are also rich in antioxidants
147 such as ascorbic acid and polyphenols, which may limit or retard folate oxidation (Indratawi
148 and others 2004a; Ng and others 2008; Rozoy and others 2013). Folates are also light-
149 sensitive (Steindal and others 2006), which has been shown to affect their stability in juices
150 stored in transparent bottles (Iniesta and others 2009; Frommherz and others 2014). Due to
151 their hydrophilic character, they can be easily lost by leaching (Delchier and others 2014).
152 Further, some deglutamation can occur during fruit and vegetable processing, due to presence
153 of endogenous folate hydrolases in plants (Melse-Boonstra and others 2002; Verlinde and
154 others 2008). However, there is no consensus concerning either their degradation mechanisms
155 or products.

156 The most recent review on folates in raw and processed fruits and vegetables is that of Scott
157 and others (2000). Since then, advanced analytical methods, and in particular the wider use of
158 mass spectrometry methods, have allowed more detailed information on folate composition
159 and degradation and, thus, opened new areas for research. This review is structured from
160 methods through quantitative data to mechanisms. Therefore, in the following paragraphs, we
161 summarize recent advances in folate quantification, current knowledge on their contents and
162 profiles in raw and processed plant foods, and on their degradation kinetics and mechanisms.
163 This finally allows formulation of the main areas needing further research. This review
164 specifically does not address difficulties in quantification (Strandler and others 2015) and
165 biological aspects of folate, either in plants (Hanson and Gregory 2011) or in humans.
166
167 ***METHODS FOR FOLATE ANALYSIS***
168 The wide range of folate derivatives, their high sensitivity to light, heat, and oxidation make
169 folate analysis in foodstuffs quite difficult. Two main methods are applied to folate
170 measurement, the first one by microbiological assay (MA) and the second one by HPLC with
171 different detection methods such as fluorimetric, UV-Vis, or mass spectrometric detection. A
172 folate assay is generally divided into 3 steps: the extraction from the matrix, the deconjugation
173 meaning cleavage of the polyglutamate chain by a γ-carboxy-peptidase (also called
174 conjugase) and the purification prior to the quantification (**Figure 2**). Recent MS-based
175 methods allow elucidation of the vitamer or of the glutamation profiles (Strandler and others
176 2015) (**Tables 1** and **2**). Fruit and vegetables present specific analytical challenges in the
177 analysis of folate profile. This is linked to the possibility of enzymic deglutamation and
178 vitamer interconversion from the onset of tissue destructuration. Blanching prior to grinding
179 for extraction may mitigate some of the enzymatic effects, but with risks of chemical
180 degradation.

As mentioned by Arcot and Shrestha (2005), a large number of studies have been performed on the effect of processing on folate contents in foods, but almost no data exist on the effect of extraction temperature on folate content for food extracts. Indeed, folate extraction from food matrixes is carried out with 3 steps: grinding, heating, and centrifuging. Grinding may lead to deglutamation, as evidenced by Munyaka and others (2009). The heating step in the extraction procedure is used for supporting the matrix disruption and the denaturation of folate binding proteins, thus inducing a higher folate release from the matrix. In the same time, heat treatment can involve folate degradation or interconversion. Generally, temperatures for folate extraction range from 70 to 121°C, but extractions are mainly carried out in boiling water (100 °C). In contrast, folate degradation, due to heat treatment, can be limited by the pH of the extraction buffer and the presence of reducing agents such as ascorbic acid (from 0.1 to 2%) and/or β-mercaptoethanol. In this way, folate stability appears higher during heat extraction when ascorbic acid and β-mercaptoethanol are added to the extraction buffer (Vahteristo and others 1996a). Different buffers can be used for folate extraction such as phosphate buffer, acetate buffer, MES buffer, or CHES-HEPES buffer with a pH varying from 4.5 to 7.5.

Apart from being historically the first method for folate measurement, to date the MA is still the official one for folate measurement in food in many countries (AOAC 992.05). It is based on the growth of folate-dependent bacteria in the presence of folates from the sample in culture media, which is then measured by turbidity. The main strain used for folate measurement is *Lactobacillus rhamnosus* (ATCC 7469), which presents the highest response to all folates compared to other strains such as *Streptococcus faecalis* (ATCC 8043), *Pediococcus cerevisiae* (ATCC 8081), *Tetrahymena pyriformis* (ATCC 30008), or *Bacillus coagulans*.

As reviewed by Arcot and Shrestha (2005), the advantages of this method are (1) the requirement of low equipment set-up, (2) showing globally a similar response to most folate isomers linked to a polyglutamate tail including 1 to 3 glutamates and (3) a good sensitivity in the measurement. However, as explained in that review, different studies have reported that the responses of *L. rhamnosus* to folic acid, 5-HCO-H_4folate, and 5-CH_3-H_4folate may be similar, but can also be significantly different depending on the assay conditions and also on the source of the folates used as reference. Moreover, the response of *L. rhamnosus* to folates depends on the pH of each medium. The length of the glutamate tail also results in different responses of *L. rhamnosus* to the vitamers. In this way, Tamura and others (1972) found a similar response of folates linked to 1 to 3 glutamates and a lesser response of folates linked to 4 to 8 glutamates, while Goli and Vanderslice (1992) described a response from 90 to 60% of folates linked to 1 to 3 glutamate residues, respectively. Furthermore, the MA is assumed to show limitations due to stimulation or inhibition of the bacterial growth by nonfolate compounds. The use of microorganisms requires proper and sterilized material, appropriate media and sample dilution, the right pH, and absence of ascorbic acid among others. Moreover, the MA needs the use of a chloramphenicol-resistant strain of *L. rhamnosus* in order to avoid a sample sterilization step, which could lead to folate degradation (Arcot and Shrestha 2005).

Automation of the MA has been proposed at the end of the 1980's based on the use of microtiter plates and automated reading. This improvement results in a decrease of the time needed, from 36-48 h for the classical MA to 18 h by using microplate assays. Moreover, this method leads to a 10-fold decrease of the detection limit (Arcot and Shrestha 2005).

Even after the development of automation, the MA for folate measurement is still time-consuming and limited with regard to the response of *L. rhamnosus* to folate derivatives, which is not always identical.

One of the other methods used for folate measurement is the protein binding assay (e.g. radioprotein binding assay). This methodology is based on the specific reaction of folate vitamers with a folate-binding protein (fbp) obtained from milk. This binding can be used in analysis either for assays in a competitive or a "sandwich" design. The designs can further involve radiolabelled or enzyme-labelled folates competing with the folates endogenously present in the samples or enzyme-labelled proteins additionally binding to the fbp-folate complex. The reaction is usually carried out in a microtitration plate format and the quantification is based either on the evolution of the color formed during the involved enzymatic reaction or on measuring the radioactivity of bound radio-labelled folates. This method is easy and rapid to perform and does not require specific equipment. However, there are wide variations between different kits partly due to different affinities of the single folate vitamers. Moreover, the kits are not suitable for measuring folate di and polyglutamate forms. These constraints are one reason why these kits are no longer available from manufacturers (Arcot and Shresta 2005; Shane 2011).

Methods for folate measurement by HPLC have been developed since the beginning of the 1980's. These methods involve separation and purification of the deconjugated extract and the detection and quantification of the eluted monoglutamate forms (Gregory 1989). Purification of the deconjugated extract is a key step for HPLC methods in order to limit matrix interferences. Two main ways are commonly used for folate purification: one is based on ion-exchange chromatography (DEAE-Sephadex anion exchange column or cation-exchange column) and the other one on the biological specificity of folate binding protein (FBP). The main problem by using FBP columns is their low affinity for 5-HCO-H_4folate, which needs to be previously converted, for example, to 10-HCO-PteGlu. In this way, Ndaw and others (2001) developed a rapid and efficient method for folate measurements by conversion of all derivatives into 5-CH_3-H_4folate. Moreover, those authors compared the tri-enzyme treatment

255 (amylase, protease, and conjugase) with the single use of chicken pancreas for deconjugation
256 and folate release from the matrix. They showed that the use of α-amylase and protease for
257 folate release from proteins and polysaccharides was not necessary as previously reported by
258 Lim and others (1998) and Shrestha and others (2000). This was later confirmed by Iwatani
259 and others (2003), who reported no significant differences for folate contents in spinach after
260 a tri-enzyme extraction compared to a single-enzyme extraction. The latter authors mentioned
261 that tri-enzyme extraction is not necessary, especially when fruits or vegetables contain low
262 amounts of starch and protein. In the same time, Hyun and others (2005) reviewed the
263 trienzyme extraction for food folate assays and recommended the incubation of food
264 homogenates with protease, amylase and conjugase, even though the treatment can differ
265 among food and became potentially time consuming and intensive. Utility of the trienzyme
266 extraction depends on the nature of the foodstuff, and it may not be necessary, especially
267 when fruits or vegetables contain low amounts of starch and protein. One of the key steps for
268 folate measurement is cleavage of the glutamate tail by a conjugase, due to the limitations in
269 detecting the longer-chain glutamate derivatives, both for *L. rhamnosus* during MA and for
270 detection by HPLC (Arcot and Shresta 2005). Currently, only few methods have been
271 reported that involve the direct detection of polyglutamic folates (Garratt and others 2005;
272 Verlinde and others 2008; Munyaka and others 2010). Most current methods are based on
273 detection of the monoglutamates after deconjugation of polyglutamates. This deconjugation is
274 often carried out with chicken pancreas powder, which contains an exopeptidase with an
275 optimum pH of 7.8 and produces diglutamate residues. Further added human plasma, rat
276 serum, or hog kidney produce monoglutamate derivatives and, thus, likewise are suitable for
277 folate deconjugation. However, these conjugases differ by their optimum pH, the presence of
278 endogenous folate, and their susceptibility to inhibition by food components. Their efficiency
279 is difficult to compare due to the scarcity of reports in the literature, but most commonly

chicken pancreas and rat serum are used for HPLC methods (Arcot and Shresta 2005), often in combination for straightforward formation of monoglutamates. For MA, chicken pancreas treatment is often sufficient as the microorganism involved can utilize di and triglutamic forms to the same extent as monoglutamates as mentioned above. However, as deconjugation is often critical and depends on the type of food, stable-isotope-labelled polyglutamates have been synthesized for tracing deconjugation efficiency in LC-MS/MS apart from labelled monoglutamates used as internal standards for quantitation (Mönch and Rychlik, 2012).

Moreover, HPLC with fluorimetric or DAD detection shows limitations, especially for some derivatives due to their low spectral response and their similar chemical properties. One of the most specific, accurate, and sensitive methods for measurement of the vitamers uses stable isotopic dilution with quantification by mass spectrometry. This so-called stable isotope dilution assay (SIDA) is based on the addition of isotopically labelled compounds to the sample. The labeled compounds present the same chemical properties as the analytes and, therefore, behave identically during sample preparation. The analytes and the labeled standards are measured simultaneously at the same retention time by HPLC. However, detection by mass spectrometry allows to discriminate the analyte and the labeled standard due to their mass difference. Several SIDAs for folates in foods have been published, with the first one for folic acid in fortified foods being mentioned by Pawloski and Flannagan (2001). Thereafter, the first SIDA for quantification of endogenous food folates, by using fourfold deuterated folic acid, along with the most abundant folate monoglutamates, was reported by Freisleben and others (2003), and further developments involved the use of advanced LC-MS/MS for enhancing specificity and sensitivity (Rychlik and others 2007) and 4-morpholineethanesulfonic acid (MES) buffer for improved deconjugation (Mönch and Rychlik 2011). The most recent progress allows quantification of 7 folate derivatives (Ringling and Rychlik 2014). With the commercial availability of [^{13}C]-labeled folate

isotopologues several research groups are currently applying SIDA in food folate analysis (Rychlik 2012).

Folate analysis in food samples remains quite difficult due to their presence as trace compounds and the variability in the amounts between the single derivatives. Folate extraction and purification appears to be a key step for folate measurement due to their instability against oxygen and heat (Strandler and others 2015). Further analytical development is still needed, particularly for differentiating folate derivatives with variable lengths of the polyglutamate tail.

FOLATE CONTENT IN RAW AND PROCESSED FRUITS AND VEGETABLES.

Although the biosynthesis pathways for folate in plants have been elucidated (Hanson and Gregory 2011) and, in spite of interest in biofortification (Scott and others 2000; Blancquaert and others 2014), there is little available information on the variability of folate concentration as a function of variety or physiological status in plants. Iniesta and others (2009) have reported, for ripe tomatoes of different varieties, concentrations between about 30 and 5 µg/100 g (more than a 5-fold difference). They did not detect a consistent trend in folate concentration with ripening. Van Daele and others (2014) investigated folate profiles in potato tubers of the variety 'Désirée' at different stages of maturation, with little variation in concentration, vitamer profile, or polyglutamation between mature and immature potatoes, but a sharp decline of concentration upon sprouting. They also evidenced variation in concentrations with a decrease from the peel to the center, again with a stable vitamer profile.

Total folate concentration

Folate content in raw fruits and vegetables is presented in **Figure 3**, which regroups concentrations determined by different methods (MA or HPLC with fluorimetric or mass

330 spectroscopy detection or SIDA combined with LC-MS detection), differentiated by the
331 symbols used. Overall, folates are higher in vegetables than in fruits. Among fruits,
332 muskmelon, strawberries, and kiwi present higher folate amounts (on average 115, 47, and 42
333 µg/100 g, respectively), whereas peach, watermelon, and apple contain low folate amounts
334 (on average 2, 6, and 4 µg/100 g, respectively). Pulses, and especially chickpeas, beans,
335 lentils, and peas, present high folate contents (on average 275, 169, 147, and 142 µg/100 g,
336 respectively). However, the values reported for pulses correspond to their raw, dry form,
337 whereas they are consumed after soaking and cooking in water, which involves a decrease of
338 their folate content due to dilution. High amounts have also been reported for the "fresh"
339 vegetables from the Fabaceae family, with an average of 52 µg/100 g in green beans and 44
340 µg/100 g for green peas. Vegetables with high folate contents are spinach, turnip, and cabbage
341 (on average 165, 124, and 66 µg/100 g, respectively). Moreover, even in a same class of
342 vegetables, a large variability in folate content has been observed. In particular the folate
343 content of cabbages ranges from 17 µg/100 g for cauliflower to 114 µg/100 g for Chinese
344 cabbage.
345 There is also a significant variability in reported concentrations for individual fruits and
346 vegetables: values range from 45.7 to 110 µg/100 g for peas and from 12.6 to 117 µg/100 g
347 for carrots. For green beans, the values range from 11 to 78 µg/100 g; 17 to 122 µg/100 g for
348 cauliflowers; 29.4 to 207 µg/100 g for beetroots, and 5 to 30 µg/100 g for potatoes. For
349 broccoli, the values range from 19 to 299 µg/100 g, and for spinach, from 27 to 261 µg/100 g.
350 In both these vegetables, for which more data are available, the impact of measurement
351 method can be examined. Though the ranges of concentrations overlap, higher average
352 concentrations are obtained by the microbiological method (MA), followed by HPLC after
353 derivatization and by SIDA. This is probably linked, at least in part, to increased selectivity,
354 and lack of a complete set of standards for SIDA. High variability of concentrations is

observed for folate contents in raw fruits or vegetables, this can be assigned, first, to the variability in the raw material, linked to variety or physiological state at harvest of fruits and vegetables and, second to the accuracy of the measurement methods used.

Individual folate vitamers

Some studies have reported certain folate derivatives in raw and processed fruits and vegetables (**Table 1**). However, as this analysis requires availability of standards, of which only very few are commercially available, not all derivatives have been systematically investigated. This might explain part of the discrepancies observed between authors. Main derivatives quantified in fruits and vegetables were 5-CH3-H_4folate (methylated on the nitrogen 5 and fully reduced); the 5-HCO-H_4folate (formylated on nitrogen N-5 and fully reduced); the 10-HCO-PteGlu (formylated on nitrogen N-10 and fully reduced); 5,10-CH+-H_4folate (corresponding to the cyclysation between nitrogens N-5 and N-10 and fully reduced), and the 5,10-CH_2-H_4folate (corresponding to a cyclysation between the nitrogens N-5 and N-10 with a carbocation form on nitrogen N-5 and fully reduced) (Bailey and Caudill 2012). The most complete characterizations are reported by Ringling and Rychlik (2013), Delchier and others (2013), and Wang and others (2013). Particular attention has been paid to spinach, cabbages, and the various Fabaceae vegetables, either fresh or dried pulses (beans, peas, broad beans, chickpeas, and so on), as these are known to have high folate contents. The main derivative found in fruits and vegetables is 5-CH_3-H_4folate. 5-HCO-H_4folate and H_4folate are also found in high concentrations. 10-HCO-PteGlu, when analyzed, can also be highly abundant, notably in cabbages and spinach. 5,10-CH^+-H_4folate and 5,10-CH_2-H_4folate are found in low concentrations and have only been reported in a few studies, mainly due to lack of analytical capability. Therefore, it is difficult at this stage to evaluate the variations in compositions between authors. However, it can be stated that the proportions of the various

derivatives appear quite variable between samples. Folic acid has been quantified in some vegetables such as spinach, chickpeas, tomatoes, green beans, cabbages among others. However, folic acid generally does not occur naturally in foodstuffs. Folic acid presence in plant matrixes and foodstuff might be due to the degradation of folate vitamers during analytical procedures or in food matrixes prior to analysis. However, literature still remains rather scarce in this respect.

Polyglutamate chain

Data on the length of polyglutamate chains of folates in fruits and vegetables are still scarce (**Table 2**). Globally, the main polyglutamatic forms found in vegetables are mono, di, penta, hexa, and heptaglutamates, though some authors report triglutamate as the major compound (Verlinde and others 2008). However, the polyglutamate patterns appear very variable within the same plant material: the only example with many data reported is broccoli (florets), for which the most abundant polyglutamate chain is either diglutamate, octoglutamate, or triglutamate, but with relatively high amounts of di, tetra, heptaglutamate, and triglutamate according to Zheng and others (1992), Wang and others (2010), Munyaka and oters (2010), and to Verlinde and others (2008), respectively. This is probably due to factors such as variety, plant organ, and maturity, or maybe even work-up for analysis. The effect of maturity is clearly illustrated by Wawire and others (2012) for cowpea, harvested at 4 to 8 weeks (**Table 2**), with a shift to longer polyglutamates after 8 weeks. The impact of sample preparation is shown by Munyaka and others (2009), also for broccoli (**Table 2**), where crushing at pH 4.5 leads to a marked shift of the polyglutamate distribution to shorter chains, compared to crushing at pH 6.5, probably related to endogenous conjugase activity. As polyglutamate length and the possible presence of inhibitors in foodstuff are known to affect bioaccessibility (Finglas and Wright 2002; Melse-Boonstra and others 2004), clearly this

405 needs further research, in particular with a view to documenting and hierarchizing the factors
406 that have an impact on polyglutamate chain length.
407 Though some information is available both on the vitamers present and the length of folate
408 derivatives, there is no information on the distribution of the polyglutamate length for
409 individual vitamers, again a factor that needs further investigation.
410
411 ***IMPACT OF FRUIT AND VEGETABLE PROCESSING ON FOLATE CONTENTS***
412 Studies on folate losses during food processing have been limited by analytical difficulties,
413 and they concentrate on products originally rich in folates. Most of them report on the impact
414 of single operations, without systematic exploration of individual parameters such as
415 temperature or duration, so that only general trends can be discerned at this point. Main
416 advances since the review of Scott and others (2000) include a confirmation of the impact of
417 leaching, the impact of novel processing methods, primarily high-pressure processing (HPP),
418 and degradation during frozen storage of raw fruits and vegetables, different stabilities of the
419 various vitamers, and evidence for deglutamylation in ground raw fruits and vegetables.
420
421 ***Folates in heat-treated fruits and vegetables***
422 Most fruits and vegetables are consumed as processed products such as industrial or home-
423 cooked, which both involve folate losses. Folate contents in fruits and vegetables after various
424 treatments are presented in **Figure 4**. Main processes studied are cooking in boiling water
425 (boiling), blanching, steaming, freezing, canning, and juicing. Highest folate losses are
426 observed after canning, where folate concentrations have been shown to decrease by 65% for
427 spinach to 77% for chickpeas. Boiling and blanching also strongly affect folate
428 concentrations. Decrease in concentration during boiling varies from 25% for green peas to
429 70% for chickpeas, but we can suppose that for chickpeas some of this may be due to dilution

caused by water intake. Maximum losses during blanching are observed for spinach, with an average decrease in concentration of 50% or 95% for hashed spinach, whereas steaming and microwave heating involve no folate losses (Bureau and others 2015).

Petersen (1993), comparing steaming and sous vide, found 89% retention of folates in sous vide versus 59% for steaming and 25% for boiling (all for 40 min, 100 °C) of broccoli florets. For blanching, Melse-Boonstra and others (2002) found a limited impact on folate contents in leeks, cauliflower, and green beans with losses of 28%, 10% and 21%, respectively (200 g of vegetables in 10 L water, blanching of 5 min (leeks), 8 min (cauliflower), and 6 min (green beans)). In pickled sliced beetroots (Jastrebova and others 2003), about half of the folates were lost (beetroot/acidified water: 2/1 ratio, 40 min, 90 °C). Indratawi and others (2004a) compared 5-CH_3-H_4folate contents in orange juice (pH 3.75), kiwi puree (pH 3.41), carrot juice (pH 6.52), and asparagus (pH 5.60) after 30 min of heating between 70 °C and 110 °C. Only limited differences were noted for the different temperatures: for orange and kiwi, decrease was slight, whereas for carrot and asparagus a significant degradation (40-70%) was noted. This was in contradiction to a better stability observed in model solutions at higher pH values, and was ascribed by Indratawi and others (2004a) to the presence of ascorbic acid in orange and kiwi. When comparing various cooking procedures for peas, broccoli, and potatoes, Stea and others (2006) reported a significant folate reduction only for blanching of peas (98 °C, 2 min), boiling of potatoes (11 min, vegetable/water: 1/10 ratio) and oven-baking of unpeeled potatoes (225 °C, 80 min). No significant reduction was observed for steaming or microwaving of peas, and neither for boiling nor sous vide processing of broccoli. Holasova and others (2008) investigated the retention of 5-CH_3-H_4folate in boiled (2-12 min, vegetable/water: 1/3 ratio) Brussels sprouts, cauliflower, broccoli, spinach, savoy cabbage, and carrots and reported high retentions (> 75% after 8 min) for Brussels sprouts, cauliflower, and broccoli, and lower (52-37%) for the other 3 vegetables. The graph was remarkable for

455 spinach with a marked slope (loss of 70% of initial content in 8 min) as a function of time, in
456 contrast to most other vegetables. High temperature, short time (HTST) (90 °C, 4 min,
457 vacuum-packed) treatment allowed better folate retention in broccoli than low temperature,
458 long time (LTLT) (40 °C, 40 min, 71-87% versus 63-81%, respectively), while acidification
459 (pH 6.5 to 4.3) increased the loss (retention 63-76% versus 76 to 87%, respectively)
460 (Munyaka and others 2009). Treatment at 100 °C, 10 min, in hermetically sealed pouches
461 with crushed broccoli, carrots, and tomatoes (Munyaka and others 2010) did not result in
462 marked folate losses, and the amounts even increased for broccoli (also for HTST treatment
463 90 °C, 4 min, (Munyaka and others 2009), which might be related to an increase in folate
464 extraction. Increase in detected folate after a short heat treatment is also reported for tomatoes
465 (98 °C, 40 s) by Iniesta and others (2009).
466 Recently, Bureau and others (2015) reported folate losses from 30% to 95% after boiling of 9
467 and 15 min and from 10 to 62% after a pressure-cooker treatment of 3 and 6 min for green
468 bean and hashed spinach, respectively. Boiling and pressure-cooker treatments involved 60%
469 and 54% of folate losses; 37% and 29% for broccoli and cauliflower. At the same time,
470 microwave heating and steaming caused no folate losses for green beans and broccoli, 14 and
471 12% for hashed spinach and 21 and 25% for cauliflower, respectively.
472 Folate vitamer compositions after processing have been compared mostly for pulses, apart
473 from some comparisons for beans and one for spinach (**Table 1**). Delchier and others (2014a)
474 found that the relative proportions of the vitamers were strongly modified after thermal
475 treatment (**Table 1**), with increased proportions of the formyl derivatives. This was
476 confirmed by degradation under 40 kPa oxygen (Delchier and others 2014b), where 5-CH_3-
477 H_4folate followed a first-order kinetic degradation, whereas the formyl derivatives were
478 stable.

Another mechanism of folate loss is leaching. This behavior was described by Dang and others (2000) in legumes, with higher retention for pressure-cooking than for boiling for chickpeas and field peas, and high concentrations of folates found in the cooking liquids. This was confirmed by Hefni and others (2014) (**Table 1**) for broad beans (faba bean), and chickpeas. Delchier and others (2012, 2013, 2014a) also clearly identified the loss by leaching as a major mechanism besides chemical degradation for folates in spinach and green beans. During industrial (Delchier and others 2013) or home-processing (Delchier and others 2012), major losses corresponded to steps with high vegetable / water ratios. There was, for example, very little loss after steaming compared to boiling. Leaching was then modeled (Delchier and others 2014a) postulating that diffusion of the folates inside the vegetable was the limiting step, and diffusivity constants were calculated using Fick's second law. The diffusivity constants were little influenced by temperature and their average values (between 25 and 45 °C) were calculated as $7.45\ 10^{-12}$ m^2/s and $5.86\ 10^{-10}$ m^2/s in spinach and green beans, respectively.

Stea and others (2006) found limited loss of total folates upon reheating or storage at warm temperature of cooked peas (steam, 3 min), broccoli (steam, 5 min and held at 60 °C for 2h) and potatoes (steam, 15 min). However, in another report, gentle reheating (according to the producers' guidelines) of various ready-to-eat vegetable-based foods (Fajardo-Martin and others 2012) led to a significant total folate loss, though with different impacts depending on the foods. As the reheating conditions were not specified, it is not possible to identify which food or duration of reheating has the most impact on this loss.

Overall, there is a significant effect of heat-processing, especially when using water, on folate concentrations in fruits and vegetables and, therefore, there is still room for improvement.

High-pressure processing

504 Remarkably, folates are sensitive to degradation during high-pressure processing (HPP).
505 Melse-Boonstra and others (2002) reported marked losses for folates during HPP at 200 MPa
506 starting from raw leeks, cauliflower, or green beans (81%, 43% and 47%, respectively). This
507 was at least partly due to enzymatic activity, and when HPP was performed after blanching,
508 losses were only 38%, 23% and 24%, respectively.
509 However, synergism between temperature and pressure was also found in model solutions.
510 Nguyen and others (2003) compared degradation of folic acid and 5-CH_3-H_4folate at pH 7 and
511 up to 800 MPa. Whereas folic acid was stable, degradation of 5-CH_3-H_4folate, while still
512 following first-order kinetics, was enhanced by increased pressure as revealed by an increase
513 of k (**Table 3**), and with limited impact on its activation energy (as deduced from
514 temperature-dependence). Nguyen and others (2006) and Oey and others (2006) found
515 accelerated 5-HCO-H_4folate and 5-CH_3-H_4folate degradation, respectively, at high pressures
516 (100 to 800 MPa), with little impact on activation energies. Butz and others (2004) reported a
517 synergistic impact of high pressure and high temperature (600 MPa, 80 °C) on the
518 degradation of the natural folates in orange juice (5-HCO-H_4folate, 5-CH_3-H_4folate, and
519 H_4folate). Good retention was observed at 25 °C and 600 MPa for all folates (with the
520 exception of losses up to 20% for H_4folate after 5 min). In the model juice and freshly
521 squeezed orange juice, 5-HCO-H_4folate was less stable than 5-CH_3-H_4folate, with formation
522 of 5,10-CH_2-H_4folate. At 80 °C and 600 MPa, stability was lowest in the model juice, good in
523 freshly squeezed juice, and best in phosphate buffer. HPP also resulted in significant folate
524 losses from vegetables: Verlinde and others (2008) reported 48-78% losses from broccoli,
525 even at low temperatures (HPP: 100 to 600 MPa, temperature: 25 °C - 45 °C). Interestingly,
526 HPP could induce deglutamylation in plant tissues (Verlinde and others 2008), which might
527 favor bioaccessability.

Though HPP is generally considered to lead to better preservation of vitamins than heat treatment, this obviously is not the case for folates.

Freezing

Czarnowska and Gujska (2012) reported folate losses during industrial freezing (including a blanching step) of 20% for peas and 26% for spinach, and no significant losses for yellow or green beans, cauliflowers, or broccoli. More recently, Delchier and others (2013) found a decrease of total folate concentration of 39% (on a wet basis) for industrial freezing of spinach (again including a blanching step), but no significant difference for concentrations expressed on a dry basis. This discrepancy might be due to water uptake or small solute diffusion during processing.

Storage

Vahteristo and others (1998) did not observe folate losses in frozen strawberries over 6 months at -20 °C. Puupponen-Pimiä and others (2003) also found no evolution of folates' content during frozen storage (up to 18 months) in peas, cauliflower, broccoli, cabbages, or spinach. Accordingly, Phillips and others (2005) found no degradation of 5-CH_3-H_4folate in frozen vegetables (storage at -60 °C, which is not representative for food usages). In contrast to this, Czarnowska and Gujska (2012) reported high losses for frozen storage of various vegetables. Upon freeze-thawing of leeks, cauliflower, and green beans (not blanched prior to freezing) Melse-Boonstra and others (2002) reported marked losses of folates (85%, 65% and 79%, respectively) with folate deglutamylation, while blanched vegetables retained most of their folates (losses of 28%, 16% and 35%, respectively), indicating that enzymatic mechanisms were involved.

552 During cold storage, good stability was observed for 5-CH_3-H_4folate in seabuckthorn berry
553 juice (Gutzeit and others 2008) and orange juice (Öhrvik and Witthöft 2008), and for folic
554 acid (supplemented) in orange juice (Öhrvik and Witthöft 2008). However, low folate loss
555 was found upon storage of tomato juice (Iniesta and others 2009), with no differences
556 between 8 °C and 37 °C, but with higher loss in glass bottles (circa 74-78% in 12 months)
557 than in Tetrapaks (42-40%). Folate losses from pickled beetroots were about 25% over 15
558 months of storage at 8 °C (Jastrebova and others 2003).
559
560 ***Other processing operations***
561 Gutzeit and others (2008) studied folate concentrations during seabuckthorn berry juice
562 production: 5-CH_3-H_4folate was the main vitamer in the fruits and was little affected by juice
563 production and concentration, in contrast to H_4folate or 5-HCO-H_4folate, which disappeared
564 during juice production. Losses during juicing varied from 10% for carrots and oranges to
565 50% for tomatoes (**Figure 4**). Wang and others (2013) observed limited loss of total folates
566 during juicing of various vegetables, except carrot greens (loss of about 30%) but with
567 significant deconjugation and some interconversion.
568 Soaking of pulses (Dang and others 2000; Xue and others 2011) can lead to losses of folates,
569 probably by leaching to the soaking water.
570 Some studies have focused on deglutamylation (or deconjugation), as monoglutamate forms
571 may be more bioavailable than polyglutamates (**Table 2**). Crushing of raw broccoli or carrot
572 (but not tomato) resulted in significant deglutamylation of folates (Munyaka and others 2010).
573 Deglutamylation was also higher during LTLT (low-temperature, long-time) treatment than
574 HTST (high-temperature, short-time) during blanching of broccoli florets, probably due to
575 enzyme persistence under LTLT conditions (Munyaka and others 2009). Crushing and
576 acidification, combined with HTST blanching, allowed enhanced stability of folates in

broccoli (Munyaka and others 2009). Wang and others (2013) also reported deglutamylation of folates during vegetable juice processing. This phenomenon is clearly linked to exposure of folates to the native plant enzymes due to tissue disruption during processing.

Lactic fermentation may increase folate contents in vegetables, depending on the strain used (Jägerstad and others 2004); however this is a complex issue depending on the lactic bacteria and their metabolism, as described in a recent review (LeBlanc and others, 2011), with very little published data on fruit and vegetables fermentation.

In spite of their reputation as a fragile vitamin, folates appear to be quite stable during heat treatments applied to fruits and vegetables. Their stability during HPP is comparable to that observed during heat treatments. Leaching actually appears to be similar to or more relevant than degradation, but data on this phenomenon are still scarce.

KINETICS OF FOLATE DEGRADATION

Folates are described as sensitive to heat, light, and oxidation. Kinetics data for folates' degradation in model solutions or in food matrixes are summarized in **Table 3**. Degradation of the various folate vitamers is generally found to follow an (apparent) first-order reaction. Due to commercial availability of the vitamers, most studies have used either 5-CH_3-H_4folate, the major folate in fruit and vegetables, folic acid, or H_4folate, with marked differences between these vitamers. Only very few kinetic studies have been carried out in fruit and vegetable matrixes, due to their complexity, with the presence of different vitamers, and the difficulty in controlling the initial folate concentrations. Mnkeni and Beveridge (1983) found faster degradation rates in fruit matrixes (apple and tomato juice), and especially in apple juice, than in citrate buffer at similar pH. Reaction kinetics of folate degradation were investigated by Delchier and others (2014a) in spinach and green beans between 45 °C and 85

602 °C, under air; folate degradation in vegetables did not follow a simple first-order kinetic as a
603 plateau was reached after an initial fast degradation. This appeared to be a consequence of the
604 presence of different vitamers: whereas 5-CH_3-H_4folate rapidly decreased following an
605 apparent first-order reaction, the minor vitamers were stable. This was in accordance with the
606 report of Petersen (1993), who also showed degradation with a plateau for retention of folate
607 over time in boiled broccoli florets. Indratawi and others (2004a) reported the degradation
608 over time at 120 °C in asparagus; although this was not kinetically exploited, the same trend
609 for total folates was as visible as for the other vegetables. Antioxidants present in fruits and
610 vegetables did not appear to be sufficient to impair their degradation.
611
612 Data on folate degradation in buffers and model systems are, however, relatively abundant,
613 and that has enabled the investigation of various extrinsic factors, notably the presence of
614 oxygen, light, pH, and reducing agents.
615
616 ***Influence of oxygen***
617 Degradation of folates is generally assumed to be an oxidation (see degradation mechanisms).
618 Though degradation is faster in the presence of oxygen, reliable evidence for biphasic
619 behavior (aerobic and anaerobic) when oxygen concentration becomes limiting is very
620 difficult to obtain because oxygen concentrations in aqueous liquids are in the milli-
621 micromolar range (saturation level for dioxygen in water at 25 °C is 0.25 mmol/L; Winkler
622 1889), thus considerably higher than folate concentrations, which are commonly in the
623 nanomolar range. Chen and Cooper (1979) were the first to study the stability of H_4folate and
624 5-CH_3-H_4folate in water, under air or with nitrogen. They reported a first-order reaction
625 kinetics for 5-CH_3-H_4folate from 65 °C to 100 °C (**Table 3**), and higher stability of 5-CH_3-
626 H_4folate than of H_4folate. Flushing with nitrogen significantly decreased the reaction rate.

627 Ruddick and others (1980) studied 5-CH_3-H_4folate degradation in phosphate buffer at pH 7.3,
628 between 40 °C and 100 °C, and with an initial oxygen concentration between 6.3 and 8 mg/L.
629 Under nonlimiting oxygen conditions, they also reported a first-order reaction rate. Day and
630 Gregory (1983) studied the degradation of 5-CH_3-H_4folate and folic acid, at pH 7, in a system
631 mimicking infant formula (milk-based) in hermetically closed bags. They also investigated
632 the impact of iron and ascorbic acid and measured the oxygen concentration in their systems,
633 which was strongly affected by the medium composition. Whereas most studies in the
634 literature indicate a catalytic effect of Fe^{2+} or Cu^{2+} on folate degradation (Hawkes and Villota
635 1989), Day and Gregory (1983) reported a better stability in the presence of iron (but there
636 was also a marked decrease in dissolved oxygen, from 7.8 to 1.4 mg/L) and second-order
637 kinetics between 100 °C and 140 °C. Generally, the degradation levels measured by Day and
638 Gregory (1983) were low, which might be due to oxygen limitation. Mnkeni and Beveridge
639 (1983) also observed a decrease in 5-CH_3-H_4folate degradation when the initial oxygen
640 concentration was decreased from 8 mg/L to 5.3 mg/L (by nitrogen-flushing), between 100 °C
641 and 140 °C. Barrett and Lund (1989) also found a faster degradation of 5-CH_3-H_4folate in the
642 presence of oxygen between 40 °C and 92 °C when comparing the data obtained under
643 oxygen saturation or in an oxygen-free system. Reaction rates and activation energies are
644 reported in **Table 3**: the reaction was only slightly faster in the presence of oxygen at 92 °C
645 ($t_{1/2}$ of 4.5 min versus 7.4 min without oxygen), but much faster at 40 °C ($t_{1/2}$ about 150 min
646 versus 1200 min) in connection to a higher activation energy. Viberg and others (1997) also
647 studied the impact of oxygen on 5-CH_3-H_4folate at high temperatures (110 °C to 150 °C),
648 with 2 initial concentrations of dissolved oxygen (6.3 to 0.3 mg/L). Delchier and others
649 (2014b) compared folate degradation in ground spinach and green beans under strictly
650 controlled anaerobiosis and with elevated oxygen (40 kPa). They confirmed the absence of
651 folate degradation in the absence of oxygen. Under 40 kPa oxygen, marked degradation was

only observed for 5-CH_3-H_4folate, and it followed first-order kinetics (**Table 3**). No degradations were found for 5-HCO-H_4folate or 10-HCO-PteGlu, the other two main folates in these vegetables.

Overall, all studies have shown an impact of oxygen on folate degradation, especially for 5-CH_3-H_4folate, H_4folate, and folic acid. Most authors reported (apparent) first-order reaction kinetics, a few noted a second-order reaction: indeed the discrepancy in concentration between oxygen and folates means that oxygen will almost always be in large excess, leading to apparent first-order kinetics, and it is not possible to quantify reliably oxygen consumption in the reaction or ascertain its role and stoichiometry.

Effect of pH

Paine-Wilson and Chen (1979) investigated in detail the impact of pH on stability of 5-CH_3-H_4folate, 5-HCO-H_4folate, folic acid, and H_4folate at 100 °C. All 4 folates followed first-order kinetics, with better stability of folic acid and 5-HCO-H_4folate (pH 4-10), while 5-CH_3-H_4folate was more stable at pH 7, and H_4folate, always the least stable, was more stable under acidic conditions (**Table 3**). However, the latter authors also noted marked differences between buffer systems, with much lower stability in universal buffer (probably citric acid, potassium dihydrogen phosphate, boric acid, and diethyl barbituric acid, but not specified in the study) than in citrate-phosphate buffer. Mnkeni and Beveridge (1983) also noted slower degradation kinetics for 5-CH_3-H_4folate from 100 °C to 140 °C, as pH increased from 3 to 6 in citrate buffer (**Table 3**). Indratawi and others (2004a) also reported both a decreased degradation of 5-CH_3-H_4folate from acidic to neutral pH (and an increase in alkaline medium) and a marked impact of the precise buffer composition (**Table 3**). Ng and others (2008) also found a better stability of 5-CH_3-H_4folate in phosphate buffers at pH 7 than at 3.5. However,

677 Liu and others (2012) reported the opposite behavior, with better stability at pH 4 (acetate)
678 than at pH 6.8 (phosphate); this is most likely to be linked to the low oxygen contents (but
679 these were not measured) in their systems, plus the nature of the buffers. They also reported
680 much better stability in skimmed milk and soy-based food matrices.
681 De Brouwer and others (2007) compared the stability of 9 folate vitamers as a function of pH
682 in the conditions used for extraction and analysis. No marked losses were observed for 5-CH_3-
683 H_4folate, folic acid, and 10-HCO-PteGlu at 100 °C (10 min) between pH 2 and 10. At 37 °C
684 (2h) they reported that most folates are stable between pH 4 and 8 except H_4folate, and
685 H_2folate, which are degraded in acidic conditions. 5-HCO-H_4folate and 5,10-CH_2-H_4folate
686 can interconvert in the presence of formate and as a function of pH: in acidic conditions (used
687 for analysis), they form 5,10-CH_2-H_4folate and H_4folate.
688 Delchier and others (2014b) compared the stability of folic acid and 5-CH_3-H_4folate at 85 °C
689 and pH 5 and 7, under anaerobic conditions and with an oxygen partial pressure of 40 kPa.
690 Folic acid was stable in all conditions, while 5-CH_3-H_4folate was only stable at pH 5 and in
691 the absence of oxygen. Marked degradation was observed in the presence of 40 kPa of oxygen
692 and was higher at pH 7 than at pH 5 under the same oxygen conditions. Thus, there might be
693 interactions between pH and oxygen.
694
695 Nguyen and others (2006) studied the degradation of 5-HCO-H_4folate as a function of pH.
696 Like Paine-Wilson and Chen (1979), they found some lability in acidic conditions and better
697 stability in a broad zone between pH 5 and 8. However, the reaction rates were very different,
698 probably linked to the experimental systems: Nguyen and others (2006) obtained a $t_{1/2}$ of 11
699 min at 100 °C, pH 3.4, whereas Paine-Wilson and Chen (1979) reported much slower loss:
700 their $t_{1/2}$ values at pH 3 (13.3 h), though dependant on the buffer used, were of a few hours,
701 with no quantifiable degradation above pH 5 at 100 °C. In contrast to this, losses of about

10% after 2 h and 5 h were reported by Paine-Wilson and Chen (1979) and by Nguyen and others (2006) at pH 7 and 9.2, respectively. The buffers, the analytical methods and the vessel sizes (and hence probably oxygen availability) were all different between the 2 studies.

Impact of co-solutes

Presence of antioxidants such as ascorbic acid, dithiothreitol (DTT), β-mercaptoethanol, or 2,3-dimercaptoethanol (Lucock and others 1993; Jastrebova and others 2013) allows better preservation of folates; this is used in analytical procedures in order to stabilize folates during extraction (Strandler and others 2015). Fruits and vegetables naturally contain high concentrations of antioxidants, notably ascorbic acid but also polyphenols. Efficiency of ascorbic acid on stability has been verified in food systems for 5-CH_3-H_4folate by Day and Gregory (1983), Indratawi and others (2004a), Oey and others (2006), and Liu and others (2012), and for folic acid by Day and Gregory (1983). It has also been shown in model systems for 5-CH_3-H_4folate by Chen and Cooper (1979), Indratawi and others (2004b), Ng and others (2008). Lucock and others (1993) stated a pH-dependence of the effect of DTT as an antioxidant to protect 5-CH_3-H_4folate. Ascorbic acid diminishes the degradation of folates in neutral as well as in acidic media with a higher effect in acidic conditions (Ng and others 2008). The pH dependence of the effect of these 2 antioxidants could be a hint for different protection mechanisms. Catechins, which are polyphenols of the flavan-3-ol class, common in many fruit species, also preserve folates (Rozoy and others 2013).

In contrast, reactions with carbohydrates also may affect folates in foods, especially with reducing sugars such as fructose or glucose. Reducing sugars lead to glycation by a Maillard-like mechanism reacting with the primary amine of the pterin cycle (Schneider and others

726 2002; Rychlik and Mayr 2005; Verlinde and others 2010). As fructose has been shown to be
727 highly reactive, this is particularly relevant for heat-treated fruit (Verlinde and others 2010).
728
729 The impact of the nature of buffers has also been noted by Paine-Wilson and Chen (1979) and
730 Indratawi and others (2004a). However, most studies on folate degradation have been carried
731 out using phosphate buffer. This might also explain some of the discrepancies in the studies
732 using "real" foods.
733 Hawkes and Villota (1989) further reported an impact of the moisture content in
734 microcrystalline cellulose:glycerol systems on the degradation of 5-CH_3-H_4folate and
735 H_4folate, but they found only a limited impact on the degradation of folic acid. Water activity
736 might also be a factor in folate degradation. They also reported better stability by addition of
737 glycerol or sucrose (a non-reducing disaccharide) to aqueous systems.
738
739 ***Photo-degradation: the light-oxygen synergy***
740 While impact of light is highly relevant for folate analysis, its impact on folate stability in
741 fruits and vegetables is limited to processed items in clear packaging. Photo-degradation has
742 indeed been detected in fruit juices stored in clear bottles, either for folic acid used for
743 fortification (Frommherz and others 2014) or for naturally present folates (Iniesta and others
744 2009). Raw fruits and vegetables are exposed to light, but they regulate folate contents
745 through their metabolism, while canned or frozen fruits and vegetables are usually in opaque
746 packaging. However, research on photo-degradation revealed interesting conjunctions of
747 light-triggered degradation, with oxygen helping to understand its impact on the degradation
748 mechanism of folates.
749 Photo-degradation has mostly been studied by exposure of folic acid to the fraction of day-
750 light rich in energy (UV radiation). In agreement with thermal treatment studies, pH

751 influences also the photo-degradation of folates. In an early study, Scheindlin and others
752 (1952) reported better stability of folic acid at pH 6.5 in comparison with pH 4.0 after
753 exposure to light. The pH-dependence of photo-degradation has been assessed also by Akhtar
754 and others (2003). Folic acid was irradiated by UV light at pH 2-10 under aerobic conditions,
755 and faster degradation was observed in acid medium. Interestingly, a synergy between light
756 exposure and the presence of oxygen has been established by several groups. Scheindlin and
757 others (1952) reported on decreasing degradation rate when oxygen is replaced by nitrogen.
758 Thomas and others (2000) observed even a surprising stability when folic acid was irradiated
759 by light at a wavelength of 350 nm in the absence of oxygen. The photo-stability under
760 anaerobic conditions was corroborated by the work of Dántola and others (2010) who used
761 UV-A radiation.
762 Scheindlin and others (1952) also investigated the accelerating impact of riboflavin on the
763 photo-degradation of folic acid. Riboflavin is known as a sensitizer producing singlet oxygen.
764 Given the fact that light impacts the degradation only when oxygen is present and the
765 influence of riboflavin as an oxygen sensitizer on the degradation rate, it seems fairly
766 probable that light interacts in the photo-degradation of folates by activating oxygen and not
767 directly with folates. Furthermore, this hypothesis is reinforced by the fact that triplet oxygen
768 cannot react with nonradicals, which implies activation, for example, by light. This
769 prerequisite for oxidation reactions could explain the acceleration of folates' degradation by
770 energy-rich UV light in the presence of oxygen.
771 However, it remains still unclear which form of activated oxygen is involved in the photo-
772 degradation of folates.
773 Nevertheless, an involvement of the reactive oxygen species (ROS) also in photo-oxidation of
774 folates seems to be quite clear. Besides UV light, heat could also be an activator (Devlin and
775 Harris 1984). The latter authors supposed that ROS such as oxygen radicals, hydrogen

776 peroxide (H_2O_2), or ozone may be formed in aqueous solutions by catalysis at high
777 temperatures.
778
779 ***Activation energies for chemical degradations***
780 Most data for activations energies of folate degradation concern 5-CH_3-H_4folate (**Table 3**);
781 they vary globally from 40 to 90 kJ/mol, depending on the medium and oxygen availability.
782 Influence of pH on activation energy for 5-CH_3-H_4folate degradation appears limited (Mnkeni
783 and Beveridge 1983; Indratawi and others 2004a), though lower activation energies are found
784 in food media (Mnkeny and Beveridge 1983) or in the presence of ascorbic acid (Indratawi
785 and others 2004a). Under air (or with an initial oxygen concentration of 8 mg/L,
786 corresponding to saturation of water by oxygen in ambient conditions), similar activation
787 energies have been reported for 5-CH_3-H_4folate degradation by Mnkeni and Beveridge
788 (1983), Barrett and Lund (1989), Viberg and others (1997) and Indratawi and others (2004a),
789 while much lower activation energies were reported by Chen and Cooper (1979) and Ruddick
790 and others (1980). This was attributed by Barrett and Lund (1989) and by Viberg and others
791 (1997) to a limitation by oxygen diffusion in some of the experimental systems. Data on
792 impact of oxygen itself are contradictory: slightly higher activation energies are reported for
793 5-CH_3-H_4folate degradation after degassing by Barrett and Lund (1989) and Mnkeki and
794 Beveridge (1983) in apple juice, but lower ones at 5.3 mg/L than at 8 mg/L initial oxygen in
795 citrate buffer by Mnkeni and Beveridge (1983), while Viberg and others (1997) determined
796 activation energies as 107 kJ/mol for the aerobic degradation, and 62 kJ/mol for anaerobic
797 degradation. Activation energies for folate degradation are higher than those reported for
798 vitamin C losses (about 30 to 40 kJ/mol) and lower than those for carotenoid degradation (>
799 100 kJ/mol) (Renard and Maingonnat 2012).
800

Deconjugation

Deconjugation, which is degradation of the polyglutamate fraction of folate, is an enzymic phenomenon, which does not entail loss of folate activity. Very little systematic data are available, due to difficulties in the quantification of polyglutamate length plus the presence in raw fruit and vegetables of active γ-glutamyl hydrolase. Deconjugation occurs upon grinding of raw fruits and vegetables and leads to false analytical results unless precautions are taken. It can also occur during juice production, in some heat treatments when enzyme inactivation is not immediate, as in LTLT (Munyaka and others 2009), and in freeze-thaw cycles of unblanched fruit and vegetables (Melse-Boonstra and others 2002). Wang and others (2013) thus found that 5-CH_3-H_4-folate, initially present mostly as polyglutamates with chain lengths of 4-6 in various vegetables, were converted during juicing mostly to triglutamates in turnip, turnip greens, and broccoli. Conversion of folates to triglutamates in *Brassicacea* had already been observed by Munyaka and others (2009) and by Wang and others (2010). However, the glutamate profiles of beet, beet greens, and Romaine lettuce were hardly affected by juicing, while juicing of carrot or carrot greens led to conversion to monoglutamate, with persistence of polyglutamates.

CHEMICAL MECHANISMS IN FOLATE DEGRADATION

Knowing the factors impacting on folate stability, degradation mechanisms may be proposed. However, the literature data on folate degradation mechanisms are scarce. Only a few studies have been carried out, and exclusively with 5-CH_3-H_4folate and H_4folate. Two degradation mechanisms have been demonstrated: oxidation and glycation. It is generally accepted that folate oxidation during processing results in cleavage of the C(9)-N(10) bond leading to liberation of the pterine ring from para-aminobenzoyl-glutamate. This oxidative process results in a loss of the vitamin activity of folates. A radical initiation of the degradation

826 mechanism has been proposed by Verlinde and others (2009) and for (photo)oxidation by
827 Akhtar and others (2003). Patro and others (2005) generated hydroxy radicals by a Fenton
828 system (Fe^{2+}-EDTA-H_2O_2) and inferred from the results that an involvement of hydroxy
829 radicals in the oxidative degradation of folic acid is possible. Glycation is of particular interest
830 in fruits, as they may contain high concentrations of fructose, which efficiently promotes this
831 reaction as indicated by Verlinde and others (2010).
832
833 ***Degradation of folates by oxidation reactions***
834 Most studies have identified some of the degradation products, but the mechanisms proposed
835 still remain hypothetical, with insufficient quantitative data (conversion yields). Moreover,
836 studies were carried out in buffer systems or in water, and only for 5-CH_3-H_4folate and
837 H_4folate. Furthermore, the conditions applied in the degradation studies were quite
838 heterogeneous, which may lead to observations of different mechanisms and, therefore, add
839 unwarranted complexity to the proposed reactions.
840 Degradation studies for H_4folate were carried out in buffers at pH 3 to 10 and between 25 °C
841 and 30 °C under air or oxygen (Blair and Pearson 1974; Reed and Archer 1980). For 5-CH_3-
842 H_4folate the degradation studies were carried out in buffers (pH 7 and 13) or in water, under
843 air or oxygen (Blair and others 1975). Other studies were carried out with the initial oxygen
844 concentration varying from 228 to 258 µmol, at different temperature (25 °C to 90 °C) and
845 pressure (0.1 to 800 MPa) (Verlinde and others 2009).
846 Identification of the degradation products (**Figure 5**) was based on: (i) thin layer
847 chromatography (TLC) (Blair and Pearson 1974); (ii) TLC and UV spectroscopy (Blair and
848 others 1975); (iii) chromatographic methods with UV detection (Reed and Archer 1980;
849 Verlinde and others 2009); and (iv) mass spectrometry and NMR methods (Verlinde and
850 others 2009). Only 4 degradation products were unambiguously identified both for 5-CH_3-

851 H_4folate (compounds A to D) (Verlinde and others 2009) and for H_4folate (compound E to H)
852 (Blair and Pearson 1975; Reed and Archer 1980). In the same studies, 8 products remained
853 hypothetical, 4 for the degradation of 5-CH_3-H_4folate (compound I to L) (Verlinde and others
854 2009) and 4 for the degradation of the H_4folate (compound M to P) (Reed and Archer 1980).
855 Verlinde and others (2009) proposed their reaction scheme according to previous studies of
856 Ehrenberg and others (1970), Gapski and others (1971), and Blair and others (1975). They
857 assumed that 5-CH_3-H_4folate is degraded into 2-amino-8-methyl-4,9-dioxo-7-methyl-p-
858 aminobenzoylglutamate-6,7,8,9-tetrahydro-pyrazino(1,2-a)-s-triazine (compound C)
859 following 2 potential pathways, the first one involving the formation of a radical (compound
860 I), a quinoid (compound J), an isocyanate (compound K), and a hydroperoxide (compound L),
861 while the second way involves the formation of the 5-methyldihydrofolate. Finally, compound
862 C is degraded into *para*-aminobenzoyl glutamic acid and the related pteridin oxidative
863 products.
864 Reed and Archer (1980) proposed that 4-hydroxy-5-CH_3-H_4folate (compound A) is a
865 degradation product of 5-CH_3-H_4folate through the oxidation of either the 5-CH_3-H_4folate or
866 the quinoid dihydrofolate (compound J), or the 5-CH_3-H_2folate (compound B), in the presence
867 of water.
868 An oxidative mechanism for H_4folate was described by Reed and Archer (1980), based on the
869 studies of Archer and Scrimgeour (1970), Stuart and others 1966), Whiteley and others
870 (1968), Sletzinger and others (1955), and Waller and others (1950), but only few modern
871 analytical data are available to corroborate this scheme. Accordingly, H_4folate is degraded
872 into 6-formyltetrahydropterin (compound O) or 6-xanthopterin (compound H). The
873 degradation into 6-formyltetrahydropterin involves the formation of a Schiff base, which is
874 then hydrolyzed. The degradation into 6-xanthopterin can take place through 2 pathways. The
875 first one is through the oxidation into dihydrofolic acid (compound F) and then into

dihydroxanthopterin (compound G). The second way for the formation of 6-xanthopterin from H_4folate is through the formation of a quinoid (compound M), a dihydropterin (compound E), and the 6-hydroxytetrahydropterin (compound P) (Reed and Archer 1980).

Folate interconversion

Interconversion of folate is essential in physiological conditions, where different vitamers, interconverted by various enzymes, are involved in the different 1-C reactions. Wang and others (2013) observed such enzymatic interconversions in juiced vegetables left for 1 h at room temperature, with an increase of H_4folate and concomitant decrease of 5-CH_3H_4folate and 10-HCO species. However, interconversion may also occur spontaneously, either in the foods or during analysis.

De Brouwer and others (2007) determined folate interconversion as a function of pH and heat treatment by analyzing the evolution of folate standards by LC-MS/MS (**Figure 6**). They described that 5,10-CH_2-H_4folate can be converted to H_4folate at low pH, which can be oxidised to H_2folate (under low pH conditions and/or heating). H_2folate can be converted to folic acid (under low pH conditions and/or heating) being susceptible to further degradation. 5-HCO-H_4folate can be converted either to 5,10-CH^+-H_4folate or to 10-HCO-H_4folate, with the reverse reactions being possible. Finally, 10-HCO-H_4folate can be oxidized to 10-HCO-PteGlu.

Photo-degradation

In terms of products formed by photo-degradation, the reports are quite coherent. Akhatar and others (2003) identified 2 molecules, 6-carboxy pterine and p-aminobenzoyl-L-glutamic acid, and postulated a reaction mechanism for degradation under acid and alkaline conditions. They suggested that folic acid is excited by UV light and oxidised, that is, dehydrogenated in

position C(9) and N(10), which entails an enamine being more prone to hydrolysis in acid than in alkaline medium. The cleavage of the C(9)-N(10) bond is also known to occur during thermal degradation of folates (Verlinde and others 2009).

Thomas and others (2000) determined the same decomposition end products as Akhtar and others (2003), but additionally identified the intermediate 6-formylpterine, which is converted to 6-carboxy pterine during the course of the reaction. Off and others (2005) confirmed the formation of para-aminobenzoyl-L-glutamic acid and 6-formylpterine as intermediates, the latter of which is converted into 6-carboxy pterine acid after UV exposure of folic acid.

Akhtar and others (1997) examined photodegradation products in the presence of riboflavin. Besides the already mentioned degradation products para-aminobenzoyl-L-glutamic acid and 6-carboxy pterine they identified para-aminobenzoic acid and pteroic acid.

Glycation

As mentioned before, glycation of the exocyclic group of folates in the presence of reducing sugars has been observed and might hence also play a role in processed fruits and vegetables. Important work has been carried out to elucidate the molecular mechanism and its influencing factors. Schneider and others (2002) worked with folic acid in model solution and detected the derivative N^2-[1-(carboxyethyl)]folic acid (CEF) after heating a model solution at 100 °C. Up to 50% of folic acid was converted under their conditions to CEF. Rychlik and Mayr (2005) quantified CEF in food products by a stable isotope dilution assay (SIDA). In fortified, baked cookies yields up to 28% were detected, and the CEF formation depended on the sugar used, with fructose > glucose > lactose and sucrose. More relevant for fruit and vegetables, Verlinde and others (2010) assessed 5-CH_3-H_4folate stability in the presence of fructose and glucose in model solutions between 25 and 90 °C. Fructose significantly accelerated degradation of 5-CH_3-H_4folate, but glucose did not. Irrespective of the added fructose

926 concentration, 1.6 mmol/L and 1.5 mol/L respectively, the acceleration rate remained
927 constant. Addition of ascorbic acid prevented depletion at 100 °C during 45 min. A
928 degradation mechanism, including the formation of the carboxyethyl derivative, has been
929 postulated by Verlinde and others (2010) applying multiresponse modeling (**Figure 7**).

930 The importance of folate glycation in fruit and vegetables remains, however, unclear given the
931 fact that antioxidants are inherently present. In addition, ascorbic acid is often added to
932 processed products, which might protect folate from glycation. The added amount of ascorbic
933 acid, and its own degradation rate, might therefore determine whether and when glycation of
934 folates can take place.

935

936 Obviously, folate degradation mechanisms have been proposed essentially for H_4folate and 5-
937 CH_3-H_4folate. Some degradation products still need to be identified and the degradation
938 pathway of formyl derivatives also needs to be elucidated. Moreover, rate constants are
939 scarcely described in the literature and, particularly, the topic of interconversions and
940 degradation in food matrixes requires further studies.

941

942 ***CONCLUSIONS***

943 In summary, retention of folates after processing can be good in absence of prolonged contact
944 with water. Considering chemical degradation, folates are clearly more stable than vitamin C.
945 Degradation of folates is faster, especially at lower temperatures, in the presence of oxygen,
946 and they might be protected by antioxidants. The different folate vitamers have markedly
947 different stabilities, with the following order of decreasing stability: folic acid > 5-HCO-
948 H_4folate > 5-CH_3-H_4folate > H_4folate. This stability is further influenced by pH, with
949 generally better stability at neutral or near-neutral pH. Leaching is another major mechanism

involved in folate losses, especially when fruits and vegetables are processed in contact with water.

Very few data are available, however, on the kinetics of folate losses in fruits and vegetable matrixes, as most work has been performed on model solutions. Because fruits and vegetable-based foods are likely to have low pH (typically pH from 3 to 6), there may be significant degradation. There are clear indications of protective interactions with some of the food components, notably ascorbic acid, but also other antioxidants; however, they appear unable to prevent folate degradation in many processing operations. Other components, such as fructose, might increase reaction rates, but there are no data regarding fruits and vegetables on the balance between protection by antioxidants and acceleration by sugars. Therefore, understanding and modeling the degradation of folates is highly relevant for preventing folate losses during processing.

Priority needs for further research are therefore:

- Further analytical development, notably for quantification of the polyglutamic forms;
- Systematic investigation of the repartition of the individual vitamers in the different fruits and vegetables;
- Better identification of oxidative and non-oxidative degradation cascades, including the role of ROS, identification of reaction products in foods, and interactions with the food matrix composition, notably antioxidants and sugars;
- Quantification of losses by leaching, and establishing the relative part of leaching and degradation in losses as a function of the processing steps and conditions.

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978

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1299

1300 ***TABLES***

1301 Table 1: Concentrations (µg/100 g or % of identified vitamers) of specific folate vitamers in fresh and processed fruits and vegetables.

	5-CH_3-H_4folate	5-HCO-H_4folate	10-HCO PteGlu	H_4folate	Folic acid	5,10CH^+-H_4folate	10-HCO-H_2folate	Reference
Vegetables								
Beet[$]	19 ± 5							Konings and others 2001
Beet	84%	7%	2%	4%		Tr.	3%	Wang and others 2013
Beets (red, pickled)	37							Vahteristo and others 1996b
Beet fresh cooked	24 ± 12							Konings and others 2001
Cabbages (various types of Brassica oleracea)								
Broccoli	98 ± 10.1		1	18 ± 1.9				Vahteristo and others 1996b
Broccoli	94 ± 3.5		3.3	11 ± 0.6				Vahteristo and others 1996b
Broccoli	24.6 - 35.7	nd - 8.1						Freisleben and others 2003
Broccoli	67%	11%	1%	16%		4%	1%	Wang and others 2013
Broccoli fresh cooked	49 ± 11	6 ± 9						Konings and others 2001
Brussels sprouts	88			9				Vahteristo and others 1996b
Brussels sprouts fresh cooked	76 ± 28	14 ± 6						Konings and others 2001
Cabbage (white)	27			4				Vahteristo and others 1996b

Cabbage (red)	25 ± 3							Konings and others 2001
Cabbage (red, fresh cooked)	22 ± 3							Konings and others 2001
Cabbage (red, preserved cooked)	14 ± 0							Konings and others 2001
Cabbage (Savoy)	99.8	25.3	12.2	16.3	0.6	1.7	6.7	Ringling and Rychlik 2013
Cauliflower	80			9				Vahteristo and others 1996b
Cauliflower	18		12					Konings and others 2001
Cauliflower	82.9	11.9	3.4	1.2		0.6	1.2	Ringling and Rychlik 2013
Cauliflower fresh cooked	57 ± 2							Konings and others 2001
Kale frozen cooked	27	9	3					Konings and others 2001
Kale fresh cooked	49 ± 6	36 ± 9	3 ± 3					Konings and others 2001
Sauerkraut cooked	7 ± 1							Konings and others 2001
Carrot	16			1				Vahteristo and others 1996b
Carrot	11 ± 3		1 ± 1	1 ± 0				Konings and others 2001
Carrot	8.1 - 9.4			4.9 - 6.7				Freisleben and others 2003
Carrot	20			1				Hefni and others 2010
Carrot	81%	14%	2%	10%		1%	2%	Wang and others 2013
Carrot boiled	10	3		1				Vahteristo and

								others 1996b
Carrot fresh cooked	16 ± 7							Konings and others 2001
Cucumber	9			1				Vahteristo and others 1996b
Cucumber fresh	3 ± 1			2 ± 0				Konings and others 2001
Cucumber	13		1	1				Hefni and others 2010
Leek fresh cooked	61 ± 30							Konings and others 2001
Lettuce	44			9				Vahteristo and others 1996b
Lettuce fresh	34 ± 11		8 ± 2	2 ± 1				Konings and others 2001
Iceberg lettuce fresh	38 ± 2		1 ± 1	4 ± 1				Konings and others 2001
Lettuce	51		8	6				Hefni and others 2010
Romaine lettuce	41%	6%	13%	3%		4%	34%	Wang and others 2013
Potato	21			3				Vahteristo and others 1996b
Potato	10			1				Hefni and others 2010
Potato boiled with skin	11		0.5					Vahteristo and others 1996b
Potatoes boiled	10 ± 2							Konings and others 2001
French fries frozen	15			1				Vahteristo and

								others 1996b
Potatoes French fries cooked	14 ± 3		1 ± 1		1 ± 1			Konings and others 2001
Potatoes fried	11 ± 3							Konings and others 2001
Spinach	46		11					Konings and others 2001
Spinach	72.8 - 140.0	4.8 - 54.7		nd - 18.7				Freisleben and others 2003
Spinach	77.9 ± 4.3	30.4 ± 0.5	3.3 ± 0.5	44.2 ± 5.6				Zhang and others 2005
Spinach	72.7 ± 2.9	29.6 ± 0.7	2.6 ± 0.5	51.6 ± 4.9				Zhang and others 2005
Spinach	90.3 ± 4.8	30.4 ± 5.2	3.5 ± 0.3	40.4 ± 0.2				Zhang and others 2005
Spinach	96.1 ± 3.3	37.9 ± 5.4	3.1 ± 0.2	41.5 ± 1.3				Zhang and others 2005
Spinach	79		24	31				Hefni and others 2010
Spinach	119.8 ± 5.2	88.3 ± 20.6	31.6 ± 29.2	6.8 ± 2.2	13.1 ± 3.9	4.5 ± 2.4	21.6 ± 20.2	Delchier and others 2013.
Spinach	43.5	9.9	21.6	0.5	0.8	0.4	2.0	Ringling and Rychlik 2013
Spinach	46.5	23.9	132.1	2.7	1.9	0.4	19.0	Ringling and Rychlik 2013
Spinach	159.1 ± 30.8	108.5 ± 20.7				19.2 ± 4.05		Tyagi and others 2015
Spinach minced	85.9	10.9	14.8	0.6		0.5	4.1	Ringling and Rychlik 2013
Spinach fresh cooked	45 ± 14		4 ± 3					Konings and others 2001
Spinach frozen	50							Vahteristo and others 1996b

Spinach chopped frozen cooked	52	2	6					Konings and others 2001
Spinach after industrial freezing	105.6 ± 6.6	23.9 ± 0.8	8.8 ± 2.0	12.6 ± 1.4	22.3 ± 4.4	2.5 ± 0.5	1.4 ± 0.1	Delchier and others 2013.
Sweet potato	21			2				Hefni and others 2010
Tomato	11			1				Vahteristo and others 1996b
Tomato	6 ± 3			2 ± 0				Konings and others 2001
Tomato	10			1				Hefni and others 2010
Tomato	10.6 ± 1.4	5.9 ± 0.6				1.5 ± 0.2		Tyagi and others 2015
Crushed tomato canned	12			3				Vahteristo and others 1996b
Tomato juice	14 ±1	1 ± 1			3 ± 1			Konings and others 2001
Tomato puree	34 ± 0							Konings and others 2001
Tomato sauce	13 ± 1				2 ± 0			Konings and others 2001
Turnip	73%	6%	1%	15%		2%	2%	Wang and others 2013
Fabaceae (incl. pulses)								
Phaseolus vulgaris L.								
Traditionally cooked beans	4.7 ± 0.23	7.5 ± 0.7	1.4 ± 0.14	2.5 ± 0.6	2.4 ± 0.19			Ruggeri and others 1999
Haricot beans canned reheated	15 ± 0		2 ± 0					Konings and others 2001
Kidney beans canned	13 ± 0		3 ± 0					Konings and

reheated								others 2001
Kidney beans dried	75			24				Hefni and others 2010
Baked beans canned reheated	17 ± 1		2 ± 0		2 ± 0			Konings and others 2001
Green beans fresh cooked	22 ± 2		1 ± 0					Konings and others 2001
Green beans raw	12	1	1	23	1			Rychlik and others 2007
Green beans fresh cooked	20	14	1	3	1			Rychlik and others 2007
Green beans raw	44.41 ± 3.12	5.93 ± 0.9	10.91 ± 0.9	2.12 ± 0.23	0.85 ± 0.12	0.63 ± 0.07	0.36 ± 0.18	Delchier and others 2013.
Green beans after industrial sterilization	33.25 ± 2.89	2.89 ± 0.44	2.93 ± 0.68	0.68 ± 0.10	0.12 ± 0.08	0.08 ± 0.03	0.03 ± 0.01	Delchier and others 2013.
Snap beans fresh cooked	27 ± 5	4 ± 6	1 ± 0					Konings and others 2001
Snap beans frozen cooked	31	3						Konings and others 2001
Vicia faba L.								
Broad beans fresh cooked	120 ± 2	13 ± 7	1 ± 0					Konings and others 2001
Faba beans dried	73		10	16				Hefni and others 2010
Faba beans canned	16		2	1				Hefni and others 2010
Faba beans raw	36*		34*	48*				Hefni and others 2014
Faba beans soaked	127*		24*	28*				Hefni and others 2014
Faba beans blanched	117*		25*	20*				Hefni and others 2014
Faba beans autoclaved (121 °C)	84*		27*	19*				Hefni and others 2014

Faba beans autoclaved (128 °C)	88*		31*	18*				Hefni and others 2014
Faba beans autoclaved (121 °C) – canning medium	20*		8*	nd				Hefni and others 2014
Faba beans autoclaved (128 °C) – canning medium	16*		6*	nd				Hefni and others 2014
Lens culinaris								
Lentils dried	71	49	6.3	43	14			Rychlik and others 2007
Lentils red dried	56			22				Hefni and others 2010
Cicer arietinum L.								
Traditionally cooked chickpeas	9.1 ± 0.57	6.1 ± 0.59	2.3 ± 0.12		1.0 ± 0.08		15.7 ± 0.62	Ruggeri and others 1999
Chickpeas dried	120		16	17				Hefni and others 2010
Chickpeas dried	106.4	118.4	15.3	27.2	10.7	1.5	2.9	Ringling and Rychlik 2013
Chickpeas canned	51.9	7.8	2.0	1.5		0.2		Ringling and Rychlik 2013
Chickpeas raw	195*		14*	26*				Hefni and others 2014
Chickpeas soaked	330*			25*				Hefni and others 2014
Chickpeas blanched	266*		3*	13*				Hefni and others 2014
Chickpeas autoclaved (121 °C)	187*		4*	12*				Hefni and others 2014
Chickpeas autoclaved (128 °C)	142*		6*	12*				Hefni and others 2014
Chickpeas autoclaved (121 °C) – canning	68*		4*	nd				Hefni and others 2014

medium								
Chickpeas autoclaved (128 °C) – canning medium	42*		4*	nd				Hefni and others 2014
Pisum sativum L.								
Green peas	54			2				Hefni and others 2010
Green peas	156.2 ± 7.6	27.9 ± 2.7				4.3 ± 0.8		Tyagi and others 2015
Peas green frozen	51			10				Vahteristo and others 1996b
Peas green frozen	79	29	3	19	2			Rychlik and others 2007
Peas	71.7	9.6	1.7	10.4	1.2	0.4	0.6	Ringling and Rychlik 2013
Vigna unguiculata								
Black-eyed peas dried	58	29.5	42	28	28			Rychlik and others 2007
Cow peas dried	51		40	16				Hefni and others 2010
Soy beans dried	84	17	28	155	37			Rychlik and others 2007
Mungo beans dried	264	0	18	32	5.2			Rychlik and others 2007
Peanuts	6.5	67.5	6.5	4.5	6			Rychlik and others 2007
Fruit								
Apple peeled and cored	3							Vahteristo and others 1996b
Apple juice	3 ± 0		2 ± 0					Konings and others 2001
Banana	12			1				Vahteristo and others 1996b

Banana	14 ± 5		1 ± 0	2 ± 0				Konings and others 2001
Banana	7		1	1				Hefni and others 2010
Bilberry	12							Vahteristo and others 1996b
Black currant	8							Vahteristo and others 1996b
Kiwi	23 ± 4			1 ± 0				Konings and others 2001
Pineapple canned	9							Vahteristo and others 1996b
Strawberry	36			1				Vahteristo and others 1996b
Strawberry	49 ± 4	14 ± 10		4 ± 2				Konings and others 2001
Strawberry	79			3				Hefni and others 2010
Citrus fruit								
Orange	27							Vahteristo and others 1996b
Orange juice	16							Vahteristo and others 1996b
Orange	18 ± 1			1 ± 1				Konings and others 2001
Orange juice	18 ±1				2 ± 1			Konings and others 2001
Grapefruit	15 ± 6			1 ± 0				Konings and others 2001
Tangerine	13 ± 1			1 ± 1				Konings and others 2001

Results are expressed in µg/100 g of fresh weight. Results with * corresponded to the folate content expressed in µg/100 g of dry matter. Canning medium is expressed in µg/100 g of fresh weight.

[$]: unless otherwise mentioned, data are reported for raw fruit and vegetable.

nd: not detected; empty cells: not reported in the publications, probably not analysed.

Empty boxes corresponded to folate form not considered or analysed.

Derivatives were determined by HPLC-UV-FD (Vahteristo and others 1996; Konings and others 2001; Hefni and others 2010 - 2014), by LC-MS/MS (Zhang and others 2005; Wang and others 2013, Tyagi and others 2015), and by LC-MS SIDA (Freisleben and others 2003; Delchier and others 2013; Ringling and Rychlik 2013). Total folate content was determined by microbiological assay (Ruggerri and others 1999).

1311 Table 2: Distribution of polyglutamate length of folates in fruit and vegetables. Data are presented as molar % of the identified polyglutamate for
1312 all folate vitamers.

Vegetables	Glu 1	Glu 2	Glu 3	Glu 4	Glu 5	Glu 6	Glu 7	Glu 8	Total folates	Reference
Spinach	15	Tr.	2	1	78	5	Tr.	Tr.	6.27*	Wang and others 2010
Spinach	25	NR	NR	3	60	15	NR	NR	2.2*	Ndaw and others 2000
Broccoli	3	0	3	2	3	18	16	56	2.19*	Wang and others 2010
Broccoli	4	11	32	13	5	13	22	NA	6.0*	Munyaka and others 2010
Broccoli florets	ND	48	20	NR	4	7	13	8	1.37*	Zheng and others 1992
Broccoli florets	ND	41	23	5	5	3	10	12	1.00*	Zheng and others 1992
Broccoli florets	ND	ND	1	4	15	12	30	39	1.20*	Zheng and others 1992
Broccoli var. Milady	1	5	55	11	10	5	12	NA	3.2*	Verlinde and others 2008
Broccoli	4	7	58	7	9	7	8	NA	2.4*	Verlinde and others 2008
Broccoli florets raw, crushed pH 6.5	4	10	46	15	12	5	5	NA	62.6#	Munyaka and others 2009
Broccoli florets raw, crushed pH	14	24	31	15	6	4	5	NA	57.2#	Munyaka and others 2009

4.3										
Broccoli florets LTLT pH 4.3	15	27	18	22	12	5	6	NA	42.1[#]	Munyaka and others 2009
Broccoli florets HTST pH 4.3	2	6	6	8	9	30	40	NA	39.4[#]	Munyaka and others 2009
Cauliflower florets	ND	23	8	0	5	22	36	7	0.81*	Zheng and others 1992
Cauliflower	6	0	3	1	3	60	32	0	2.00*	Wang and others 2010
Brussels sprouts	6	0	4	3	20	46	18	3	2.78*	Wang and others 2010
Cabbage leaves	ND	50	ND	23	27	ND	ND	ND	0.26*	Zheng and others 1992
Cabbage leaves	ND	46	ND	25	30	ND	ND	ND	0.21*	Zheng and others 1992
Collard greens	7	Tr.	1	1	12	42	29	8	1.59*	Wang and others 2010
Kale	3	0	2	1	4	36	36	19	1.18*	Wang and others 2010
Turnips green	9	1	2	1	7	22	32	27	2.53*	Wang and others 2010
Romain lettuce	40	1	Tr.	1	41	16	1	ND	2.13*	Wang and others 2010
Lettuce leaves	ND	19	27	17	37	ND	ND	ND	1.87*	Zheng and others 1992
Lettuce leaves	ND	28	ND	ND	72	ND	ND	ND	1.50*	Zheng and others 1992
Carrots	ND	69	19	ND	12	ND	ND	ND	0.22*	Zheng and others 1992
Peas	25	ND	ND	ND	75	ND	ND	ND	1.4*	Ndaw and others 2000

Raw cowpea 4 weeks	61	39	ND	ND	ND	ND	ND	ND	3.7#	Wawire and others 2012
Raw cowpea 6 weeks	83	8	ND	ND	ND	ND	10	ND	2.9#	Wawire and others 2012
Raw cowpea 8 weeks	47	25	16	ND	8	4	ND	ND	7.0#	Wawire and others 2012
Apple	31	ND	ND	ND	ND	19	27	23	0.078*	Ndaw and others 2000

1313

1314 Tr.: detected but below 0.5%; NR: not reported; NA: not analyzed; ND: not detected

1315 Total folates reported as: *: nmol/g fresh weight; #: nmol/g dry weight.

1316 LTLT: low-temperature, long-time blanching; HTST: high-temperature, short-time blanching.

Table 3: Kinetics constants for degradation of folate vitamers under various conditions

Vitamin form	Conditions	Results	Reference
5-CH_3-H_4folate	49-100°C, water 100°C, no ascorbate, under air 49-100°C	First order k = 0.0323 min^{-1} , $t_{1/2}$ = 21 min E_a = 40 kJ/mol	Chen and Cooper 1979
5-CH_3-H_4folate	100°C, under air HCl-KCl: pH 2 pH 3 citrate buffer pH 3 "Universal buffer" pH 3 "Universal buffer" pH 4 "Universal buffer" pH 5 "Universal buffer" pH 6 "Universal buffer" pH 7	First order k = 0.205 h^{-1}; $t_{1/2}$ = 3.4h k = 0.053 h^{-1}; $t_{1/2}$ = 13.1h k = 0.083 h^{-1}; $t_{1/2}$ = 8.4h k = 0.254 h^{-1}; $t_{1/2}$ = 2.7h k = 0.207 h^{-1}; $t_{1/2}$ = 3.4h k = 0.110 h^{-1}; $t_{1/2}$ = 6.3h k = 0.103 h^{-1}; $t_{1/2}$ = 6.7h k = 0.0079 h^{-1}; $t_{1/2}$ = 8.8h	Paine-Wilson and Chen 1979
5-CH_3-H_4folate	Citrate buffer – 8 mg/l initial oxygen content – 100 to 140°C pH 3 pH 4 pH 5 pH 6	 $k_{100°C}$ =0.243 min^{-1} ; Ea = 79 kJ/mol $k_{100°C}$ =0.192 min^{-1} ; Ea = 71 kJ/mol $k_{100°C}$ =0.110 min^{-1} ; Ea = 82 kJ/mol $k_{100°C}$ =0.104 min^{-1} ; Ea = 83 kJ/mol	Mnkeni and Beveridge 1983
5-CH_3-H_4folate	Citrate buffer – 5.3 mg/L initial oxygen content – 100 to 140°C pH 3 pH 4 pH 5 pH 6	 $k_{100°C}$ =0.106 min^{-1} ; Ea = 76 kJ/mol $k_{100°C}$ =0.092 min^{-1} ; Ea = 49 kJ/mol $k_{100°C}$ =0.072 min^{-1} ; Ea = 55 kJ/mol $k_{100°C}$ =0.059 min^{-1} ; Ea = 56 kJ/mol	Mnkeni and Beveridge 1983
5-CH_3-H_4folate	Tomato juice (pH 4.3), 100 to 140°C 8 mg/L initial oxygen content Limited initial oxygen	 $k_{100°C}$ =0.374 min^{-1} ; Ea = 44 kJ/mol $k_{100°C}$ =0.160 min^{-1} ; Ea = 45 kJ/mol	Mnkeni and Beveridge 1983
5-CH_3-H_4folate	apple juice (pH 3.4), 50 to 70°C 8 mg/L initial oxygen content Limited initial oxygen	 $k_{50°C}$ =0.123 min^{-1} ; Ea = 33 kJ/mol $k_{50°C}$ =0.089 min^{-1} ; Ea = 40 kJ/mol	Mnkeni and Beveridge 1983
5-CH_3-H_4folate	Buffer pH 7, 40-92°C Initial saturation of oxygen No oxygen	 $k_{92°C}$ =0.155 min^{-1} ; Ea = 68 kJ/mol $k_{92°C}$ =0.094 min^{-1} ; Ea = 97 kJ/mol	Barrett and Lund 1989
5-CH_3-H_4folate	Phosphate buffer pH 7, 110 to 150°C		Viberg and others 1997

	Initial oxygen 6.8 mg/L Initial oxygen 0.3 mg/L	$k_{110°C}$ =0.300 min^{-1} $k_{110°C}$ =0.242 min^{-1}	
5-CH_3-H_4folate	In ground vegetables, under 40 kPa oxygen Spinach, 45°C Spinach, 65°C Green beans, 45°C Green beans, 65°C	First order k = 0.025 min^{-1} k = 0.070 min^{-1} k = 0.020 min^{-1} k = 0.080 min^{-1}	Delchier and others 2014b
5-CH_3-H_4folate	Citrate-phosphate buffer pH 4, in capillary tubes , 40°C 100 MPa 300 Mpa 700 Mpa	First order $k_{40°C}$ = 0.0054 min^{-1} $k_{40°C}$ = 0.0084 min^{-1} $k_{40°C}$ = 0.0048 min^{-1}	Nguyen and others 2003
5-CH_3-H_4folate	Phosphate buffer pH 7, in capillary tubes, 65-90°C, 0.1 MPa 100 MPa 200 Mpa 400 Mpa 600 Mpa 800 Mpa	First order $k_{90°C}$ = 0.068 min^{-1} ; Ea = 80 kJ/mol $k_{60°C}$ = 0.024 min^{-1} ; Ea = 79 kJ/mol $k_{60°C}$ = 0.035 min^{-1} ; Ea = 72 kJ/mol $k_{60°C}$ = 0.070 min^{-1} ; Ea = 81 kJ/mol $k_{60°C}$ = 0.106 min^{-1} ; Ea = 100 kJ/mol $k_{60°C}$ = 0.150 min^{-1} ; Ea = 90 kJ/mol	Nguyen and others 2003
5-CH_3-H_4folate	Phosphate buffer pH 7, in capillary tubes, 100 to 800 Mpa 30°C, 40°C 50°C 60°C 65°C	First order $k_{600\ mPa}$ = 0.0045 min^{-1} ; Va = -5.8 cm^3/mol $k_{600\ mPa}$ = 0.018 min^{-1} ; Va = -5.2 cm^3/mol $k_{600\ mPa}$ = 0.026 min^{-1} ; Va = -7.1 cm^3/mol $k_{600\ mPa}$ = 0.106 min^{-1} ; Va = -7.2 cm^3/mol $k_{400\ mPa}$ = 0.079 min^{-1} ; Va = -14 cm^3/mol	Nguyen and others 2003
5-CH_3-H_4folate	Various buffers, in capillary tubes, atmospheric pressure Citrate-phosphate, pH 3 Acetate, pH 3 Citrate-phosphate, pH 4 Idem, plus ascorbic acid Acetic acid pH 5 Phosphate pH 7 Citrate-phosphate pH 7	First order $K_{90°C}$ = 0.125 min^{-1} ; Ea = 144 kJ/mol $k_{90°C}$ = 0.165 min^{-1} ; Ea = 114 kJ/mol $k_{90°C}$ = 0.115 min^{-1} ; Ea = 89 kJ/mol $k_{120°C}$ = 0.0022 min^{-1} ; Ea = 26 kJ/mol $k_{90°C}$ = 0.106 min^{-1} ; Ea = 81 kJ/mol $k_{90°C}$ = 0.068 min^{-1} ; Ea = 80 kJ/mol $k_{90°C}$ = 0.014 min^{-1} ; Ea = 96 kJ/mol	Indratawi and others 2004a
5-CH_3-H_4folate	Anaerobic: degassed buffers under nitrogen	First order	Liu and others 2012

	atmosphere in sealed tubes Acetate pH 4 Phosphate pH 6.8	 $k_{75°C}$ = 0.0225 min^{-1} ; Ea = 96 kJ/mol $k_{75°C}$ = 0.211 min^{-1} ; Ea = 72 kJ/mol	
H_4folate	100°C, water, under air	$t_{1/2}$ = 2.25 min	Chen and Cooper 1979
H_4folate	100°C, HCl-KCl: pH 2 "Universal buffer" pH 4 "Universal buffer" pH 6	First order k = 0.113 min^{-1}; $t_{1/2}$ = 6 min k = 0.567 min^{-1}; $t_{1/2}$ = 1.22 min k = 0.207 min^{-1}; $t_{1/2}$ = 3.35 min	Paine-Wilson and Chen 1979
Folic acid	100°C, HCl-KCl: pH 2 pH 3 citrate buffer pH 3 "Universal buffer" pH 3 "Universal buffer" pH 4 "Universal buffer" pH 5 "Universal buffer" pH 6 "Universal buffer" pH 7	First order k = 0.397 h^{-1}; $t_{1/2}$ = 1.7h k = 0.079 h^{-1}; $t_{1/2}$ = 8.8h k = 0.071 h^{-1}; $t_{1/2}$ = 9.8h k = 0.095 h^{-1}; $t_{1/2}$ = 7.3h k = 0.031 h^{-1}; $t_{1/2}$ = 22.4h k = 0.010 h^{-1}; $t_{1/2}$ = 69h3h k = 0.006 h^{-1}; $t_{1/2}$ = 115.5 No quantifiable degradation	Paine-Wilson and Chen 1979
Folic acid	Phosphate buffer pH 7, in capillary tubes, 120-160°C	 $k_{120°C}$ = 0.00104 min^{-1} ; Ea = 52kJ/mol	Nguyen and others 2003
5-CHO-H_4folate	100°C, HCl-KCl: pH 2 pH 3 citrate buffer pH 3 "Universal buffer" pH 3 "Universal buffer" pH 4 to 8	First order k = 0.199 h^{-1}; $t_{1/2}$ = 3.5h k = 0.052 h^{-1}; $t_{1/2}$ = 13.3h k = 0.033 h^{-1}; $t_{1/2}$ = 21h k = 0.034 h^{-1}; $t_{1/2}$ = 20.4h No quantifiable degradation	Paine-Wilson and Chen 1979
5-CHO-H_4folate	80-110°C, in capillary tubes Acetate buffer pH 3.4 pH 5 phosphate buffer pH 7 borax pH 9.2	First order $k_{100°C}$ = 0.061 min^{-1} ; Ea = 64 kJ/mol $k_{100°C}$ = 0.0058 min^{-1} ; Ea = 63 kJ/mol $k_{100°C}$ = 0.0015 min^{-1} ; Ea = 72 kJ/mol $k_{100°C}$ = 0.0003 min^{-1} ; Ea = 47 kJ/mol	Nguyen and others 2006

k corresponds to the degradation rate constant; $t_{1/2}$ corresponds to the half life time (time for reaching one half of its steady state concentration); *Ea* corresponds to the activation energy calculated as ln k= ln A – Ea/RT where k is the degradation rate constant, A a constant, R the universal gas constant and T the temperature in Kelvin.

CAPTIONS TO FIGURES

Figure 1: Chemical structure of folate and derivatives with associated names

R1 and R2 correspond to the atom substitution presented in the associated table.

"n" refers to the number of glutamate residues linked, which may range between 1 and 8.

Figure 2: Steps for folate measurement by microbiological assay, HPLC with fluorimetric detection, or SIDA with LC-MS detection.

Figure 3: Folate content in some raw fruits and vegetables in µg/100 g of fresh weight.

Full circles represent folate content obtained by microbiological assay; empty circles represent folate content obtained by HPLC with fluorimetric or MS-MS detection; triangles represent folate content obtained by SIDA with LC-MS/MS detection; squares represent folate content obtained by radio protein binding assay.

Data were extracted from: De Souza and others 1986; Dang 2000; Ndaw and others 2001; McKillop and others 2002; Melse Boonstra and others 2002; Stralsjo and others 2002; Freisleben and others 2003; Iwatani and others 2003; Jastrebova and others 2003; Puupponen-Pimiä and others 2003; Stralsjo and others 2003; Yon and others 2003; Jagerstad and others 2004; Phillips and others 2005; Stea and others 2006; Rychlik and others 2007; Devi and others 2008; Holasova and others 2008; Iniesta and others 2009; Munyaka and others 2010; Delchier and others 2012; Delchier and others 2013; Moreiras and others 2013; Ringling and Rychlik 2013; Wang and others 2013; Bureau and others 2015; Fajardo and others 2015; Tyagi and others 2015; USDA Food Nutrient Database, and our own results.

Figure 4: Folate content in processed fruits and vegetables.

Full circles represent folate content obtained by microbiological assay; empty circles represent folate content obtained by HPLC with fluorimetric or MS-MS detection; triangles represent folate content obtained by SIDA with LC-MS/MS detection; squares represent folate content obtained by radio protein binding assay. Lozenges represent folate content obtained by LC coupled to electrochemical detection.

Data are extracted from: Klein 1979; De Souza and others 1986; White 1990; Dang 2000; McKillop and others 2002; Jastrebova and others 2003; Puupponen-Pimiä and others 2003; Stralsjo and others 2003; Yon and others 2003; Jagerstad and others 2004; Phillips and others 2005; Stea and others 2006; Rychlik and others 2007; Devi and others 2008; Munyaka and others 2010; Delchier and others 2012; Delchier and others 2013; Wang and others 2013; Bureau and others 2015; USDA Food Nutrient Database, and our own results.

Data are expressed in µg/100 g of fresh weight, except for data with § which are expressed as dry matter.

BB: black beans; *KB*: kidney beans; *WB*: white beans, *CLF:* cauliflower; *CB*: cabbages; *SK*: sauerkraut; *LT*: lentils; *BEP*: blackeyed peas; *TP*: turnip; *STR*: strawberries.

CO: cooked; B: boiled; BW: boiling water; BL: blanched; BLW: blanching water; ST: steamed; STBC: steamed blanched condensate; MW: microwave; PC: pressure-cooked; SVP: sous-vide process; S: stored; F: frozen; FB: frozen boiled; FBW: frozen boiled water; T:

thawed; C: canned; CCL: canned covering liquid; OB: oven-backed, G: grated; GB: grated blanched, P: pickled; J: juice; JA: jam; STE: stewed.

Figure 5: Folate degradation pathway

The degradation pathway of 5-methyltetrahydrofolate as proposed by Verlinde and others (2009) is presented by ⇢. The degradation of 5-methyltetrahydrofolate leading to 4-hydroxy-5-methyltetrahydrofolate as proposed by Blair and others (1975) and represented by ⇢. The degradation pathway of tetrahydrofolate as proposed by Reed and Archer (1980) and presented by ⟶. The degradation of tetrahydrofolate to folic acid as proposed by Blair and Pearson (1973) and represented by ⟶.

Names of compounds in **bold** type correspond to actual identified compounds, names in normal type correspond to hypothetical structures.

Figure 6: Chemical interconversions of folate derivatives

Figure adapted from de Brouwer and others (2007). All compounds were identified according to LC-MS analysis.

Figure 7: Glycation mechanism of 5-CH_3-H_4folate

Figure adapted from Verlinde and others (2010). All compounds were identified according to NMR and mass spectrometry experiments.

1389 **Figure 1.**

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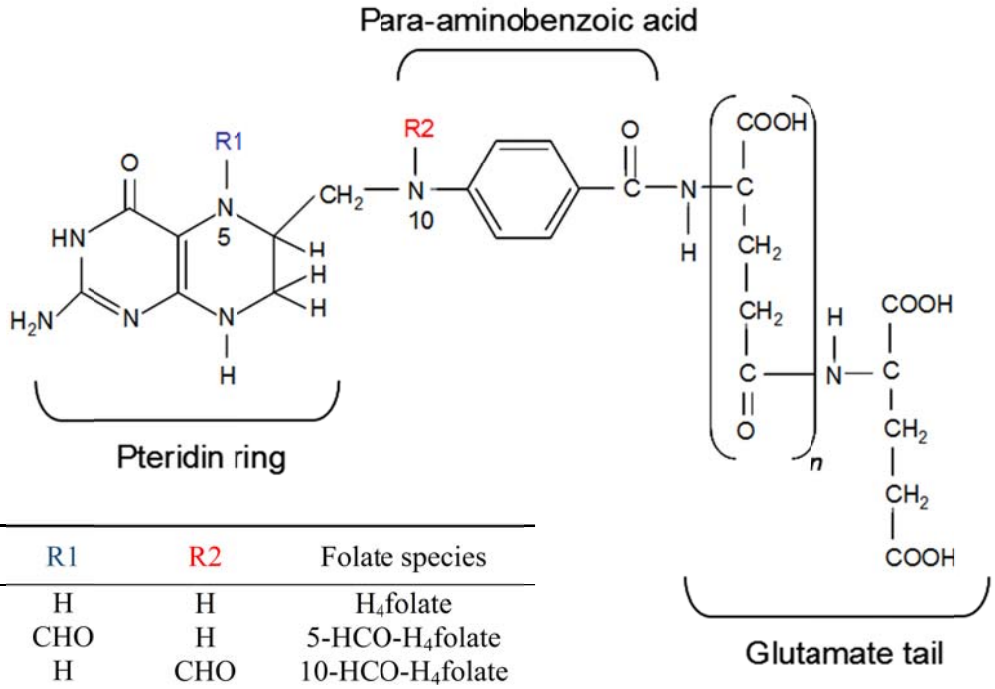

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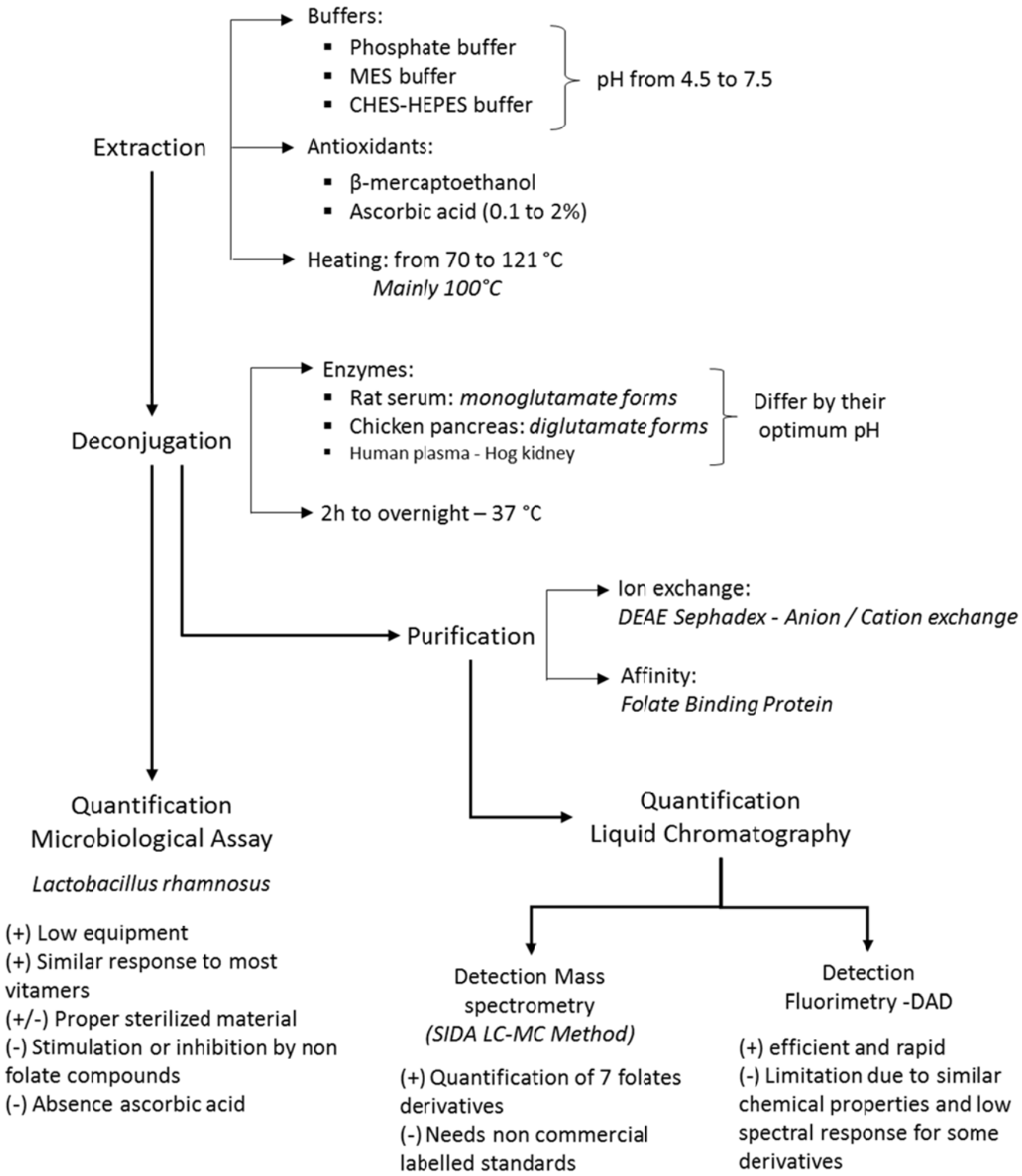
R1	R2	Folate species
H	H	H_4folate
CHO	H	5-HCO-H_4folate
H	CHO	10-HCO-H_4folate
CH_3	H	5-CH_3-H_4folate
-CH_2-		5,10-CH_2-H_4folate
$^{+}$=CH-		5,10-CH^{+}-H_4folate

Figure 2.


Extraction
Buffers:
Phosphate buffer
MES buffer
CHES-HEPES buffer
pH from 4.5 to 7.5
Antioxidants:
β-mercaptoethanol
Ascorbic acid (0.1 to 2%)
Heating: from 70 to 121 °C
Mainly 100°C
Deconjugation
Enzymes:
Rat serum: monoglutamate forms
Chicken pancreas: diglutamate forms
Human plasma - Hog kidney
Differ by their optimum pH
2h to overnight – 37 °C
Purification
Ion exchange:
DEAE Sephadex - Anion / Cation exchange
Affinity:
Folate Binding Protein
Quantification
Microbiological Assay
Lactobacillus rhamnosus
(+) Low equipment
(+) Similar response to most vitamers
(+/-) Proper sterilized material
(-) Stimulation or inhibition by non folate compounds
(-) Absence ascorbic acid
Quantification
Liquid Chromatography
Detection Mass spectrometry
(SIDA LC-MC Method)
(+) Quantification of 7 folates derivatives
(-) Needs non commercial labelled standards
Detection
Fluorimetry -DAD
(+) efficient and rapid
(-) Limitation due to similar chemical properties and low spectral response for some derivatives

1398 **Figure 3.**

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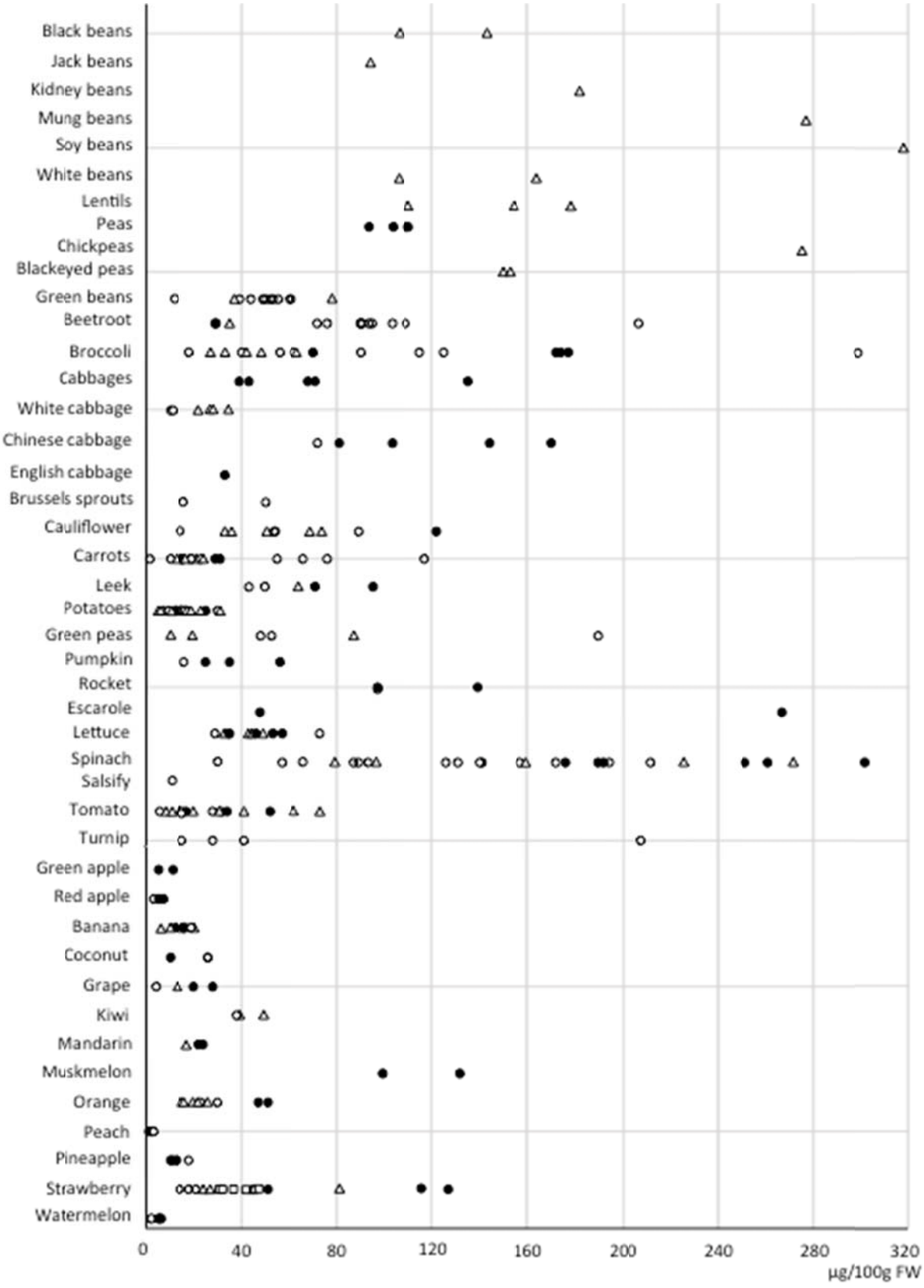
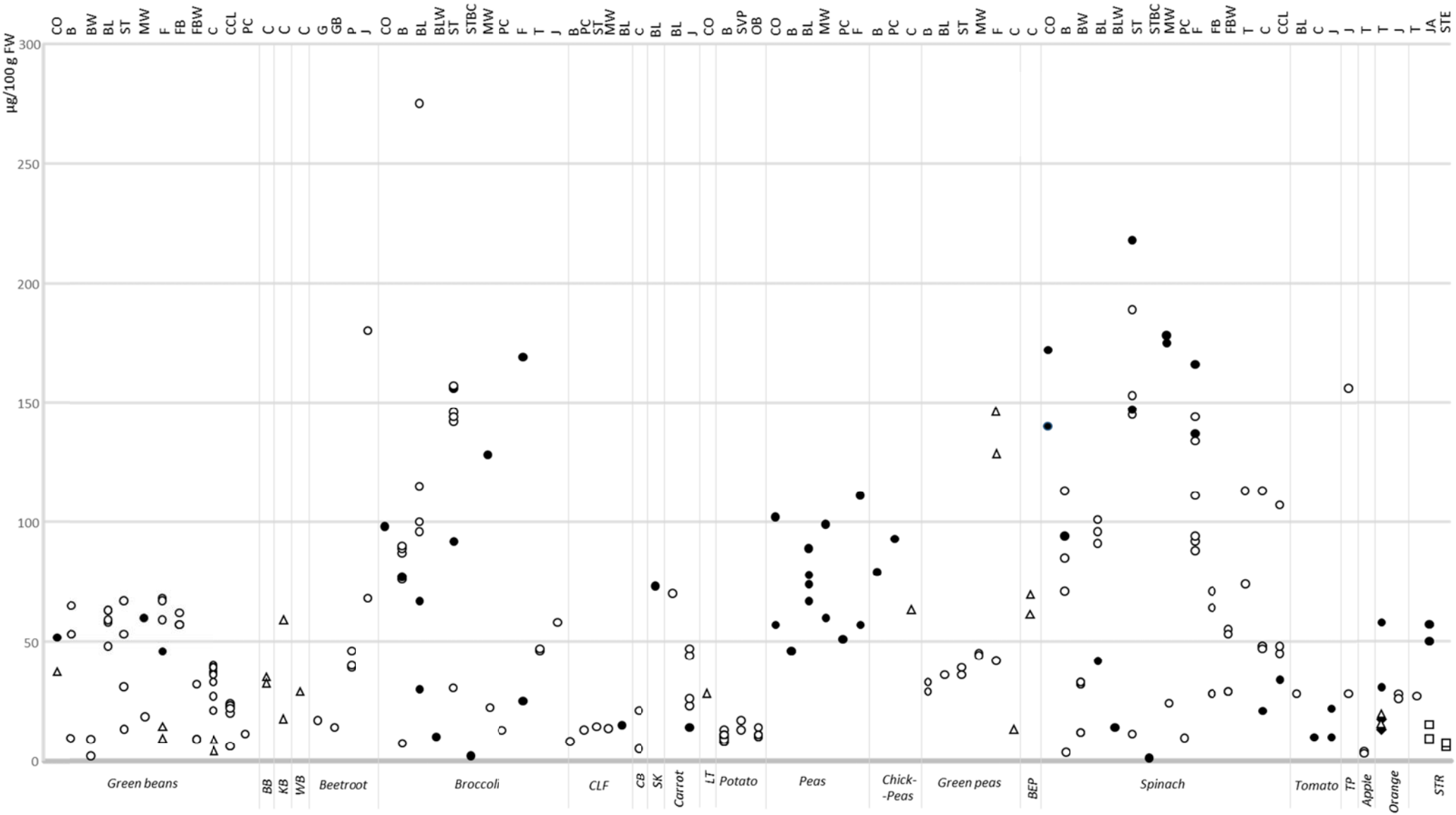


1400

Figure 4.


µg/100 g FW
300
250
200
150
100
50
0
CO B BW BL ST MW F FB FBW C CCL PC
C C C
G GB
P J
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B PC ST MW BL
C
BL
BL J
CO
B SVP OB
CO B BL MW PC F
B PC
C
B BL ST MW F
C C
CO B BW BL BLW ST STBC MW PC F FB FBW T C CCL
BL C J
J T
T J T JA STE
Green beans
BB
KB
WB
Beetroot
Broccoli
CLF
CB
SK
Carrot
LT
Potato
Peas
Chick--Peas
Green peas
BEP
Spinach
Tomato
TP
Apple
Orange
STR

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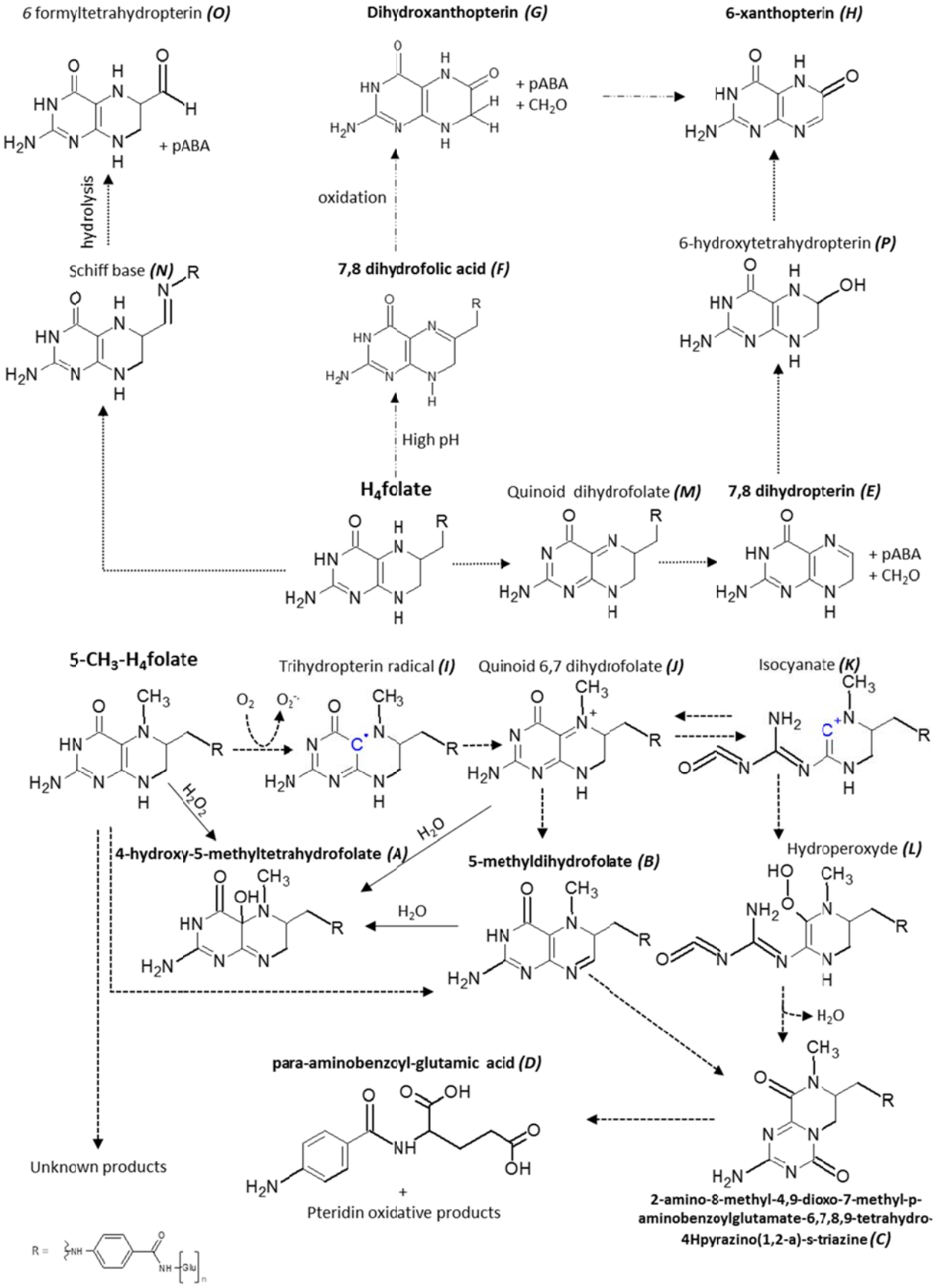
1403

Figure 5.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Dihydroxanthopterin (G)
6-xanthopterin (H)
+ pABA
+ pABA
+ CH2O
hydrolysis
oxidation
Schiff base (N)
7,8 dihydrofolic acid (F)
6-hydroxytetrahydropterin (P)
High pH
H4folate
Quinoid dihydrofolate (M)
7,8 dihydropterin (E)
+ pABA
+ CH2O
5-CH3-H4folate
Trihydropterin radical (I)
Quinoid 6,7 dihydrofolate (J)
Isocyanate (K)
H2O2
H2O
4-hydroxy-5-methyltetrahydrofolate (A)
5-methyldihydrofolate (B)
Hydroperoxyde (L)
H2O
para-aminobenzoyl-glutamic acid (D)
Unknown products
+
Pteridin oxidative products
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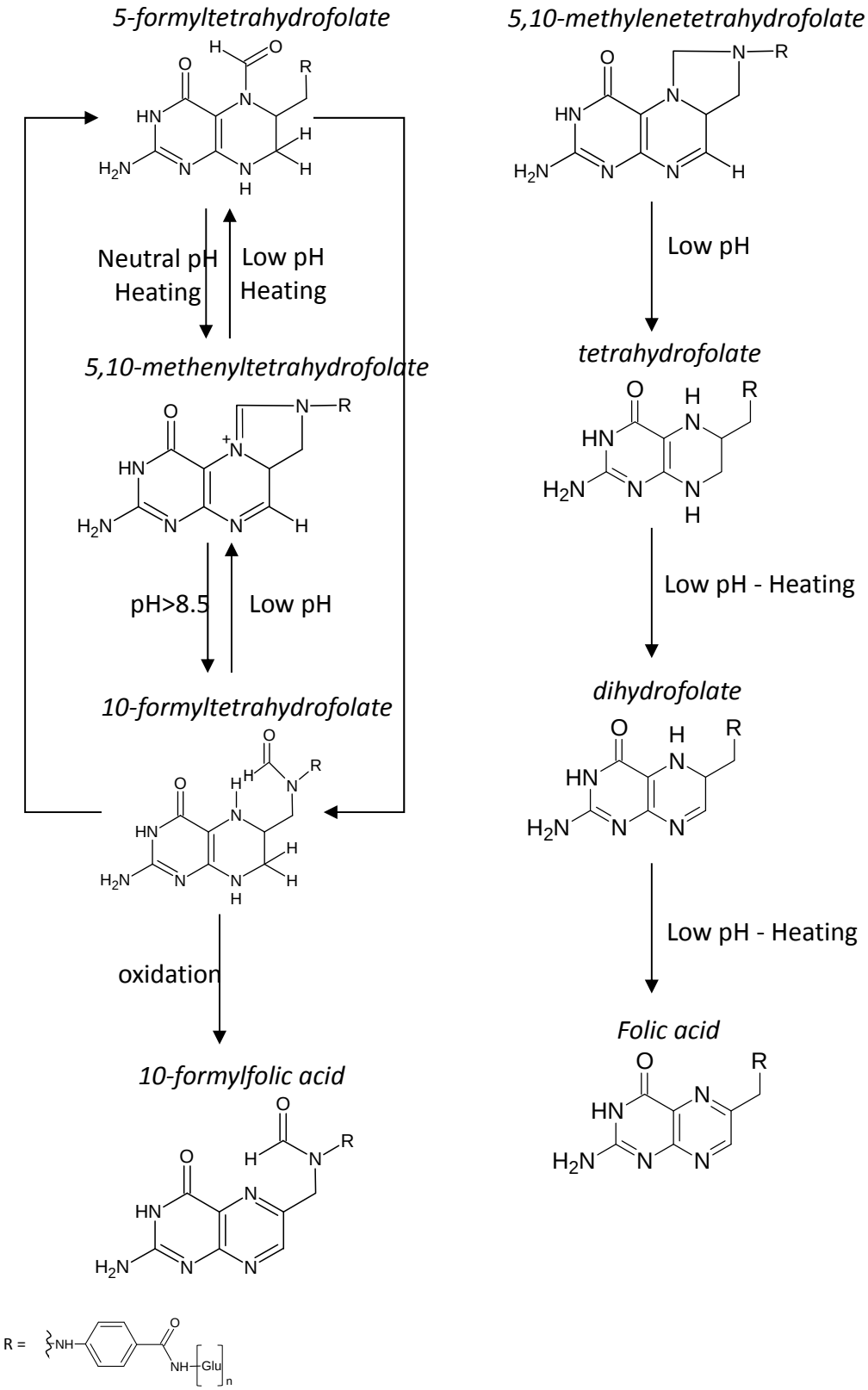
R =

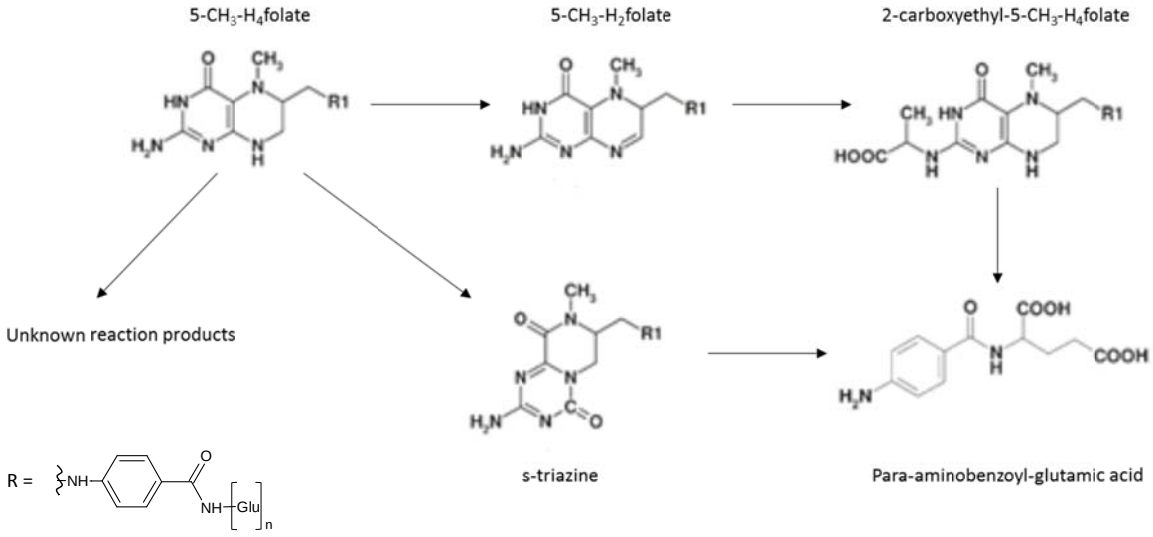
1405
1406

1407 **Figure 6.**



1408

Figure 7.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5-CH3-H2folate
2-carboxyethyl-5-CH3-H4folate
Unknown reaction products
s-triazine
Para-aminobenzoyl-glutamic acid
R =
NH
Glu
n