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ORIGINAL ARTICLE

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Kinetic modeling of four folates in a model solution at different temperatures and pH to mimic their behavior in foods during processing

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Abstract

This study aims to generate new kinetic data on 4 folate forms, namely folic acid (FA), 5-methyl-tetrahydrofolate (5MTHF), 10-formylfolic acid (10FFA) and tetrahydrofolate (THF) in the same conditions: model media at three temperatures (60°C, 80°C, and 100°C) and three pH values (3, 5, and 7). For the four forms, degradation could be described using a first-order model. At 100°C and pH 3, rates were 2.8×10^{-2}, 1.6×10^{-2}, 1.8×10^{-3}, and 4×10^{-5} min^{-1} for 5MTHF, THF, 10FFA and FA respectively. Therefore, the reactivity could be modulated by a factor of 2, 10, and up to 200-fold for folic acid. pH increase had a positive effect on retention for THF and FA and has the lowest impact on 5MTHF degradation. Product formation from 5MTHF and 10 FFA were studied by multiresponse modeling. Both vitamers generated intermediary products that accumulated differently as a function of pH. These results give comprehension and kinetic insights into folate degradation during food processing from the formulation (pH lever) to the process (temperature lever).

Practical Applications

This article brings new insights into vitamins B_9 degradation during cooking. Folate is a group of vitamins, sharing a similar molecular structure while having different bioactivity. The lack of folate in diet can lead to severe health disorder. Their human metabolization and thermic degradation can be influenced by different factors, including their own structure. Therefore, it is of high importance to understand how each form is degraded and to determine the best formulation and process conditions to preserve them. Our work, in model mediums, allowed to identify four folate forms reactivity and degradation (including degradation pathway) in order to give more guidance, and offers an operational tool for manufacturer to determine the potential retention of folates as a function of pH, temperature and vitamer composition.

KEYWORDS

10-formylfolic acid, 5-methyl-, activation energy, degradation products, rate, tetrahydrofolate

1 | INTRODUCTION

Folates or B_9 vitamins are a group of vitamins, called vitamers, sharing the same molecular base structure, that of a pterin cycle bound to a *para*-aminobenzoic acid linked to a polyglutamate tail. B_9 vitamers play an important role in many reactions involved in major cellular processes including nucleic acid biosynthesis, amino acid metabolism, methyl group biogenesis, etc. Its deficiency is particularly dangerous before and during pregnancy as folates can help prevent certain major birth defects (Sobczyńska-Malefora, 2019). Humans are not able to synthesize folates, and food or supplements must supply the necessary amounts (Gmelch et al., 2020). It is recommended that healthy adults consume at least 330 μg/day of folate to prevent deficiency and 600 μg/day during pregnancy or lactation. To achieve these levels, American public policy promotes folic acid supplementation or food fortification in cereals for instance, while Europe suggests a regular consumption of folate-rich animal foods (offal) or folate-abundant plants (Araújo, Marchioni, Villavicencio, et al., 2012; Araújo, Marchioni, Zhao, et al., 2012; EFSA, 2014). Among the latter, the most important sources are leafy green vegetables and legumes that are gaining in interest because of their sustainability and affordability (Blancquaert et al., 2014; Coffigniez et al., 2019; Czarnowska-Kujawska et al., 2020; Hefni et al., 2010).

In plants, folates exist naturally in different polyglutamyl forms. The number of glutamates can vary from 2 to 8 residues (Delchier et al., 2016). In addition, they differ by their oxidation degree and substitution by a methyl or a formyl group. These different structures are associated with different biological properties which is important for nutritional considerations. As a consequence, individualization of their content in food is important (Vishnumohan et al., 2017). The most common, by order of occurrence in foods, are 5-methyl tetrahydrofolic acid (5MTHF) ($R_1 = CH_3$; $R_2 = H$; $R_3 = H$), 5-formyl tetrahydrofolic acid (5FTHF) ($R_1 = CHO$, $R_2 = H$, $R_3 = H$), tetrahydrofolic acid (THF) ($R_1 = H$; $R_2 = H$; $R_3 = H$), 10-formyl folic acid (10FFA) ($R_1 = N^5$; $R_2 = CHO$; $R_3 = N^8$), and 5-formyl tetrahydrofolic acid (5FTHF) ($R_1 = CHO$; $R_2 = H$; $R_3 = H$) (Figure 1), (Czarnowska-Kujawska et al., 2020; Vishnumohan et al., 2017). In the majority of green leafy vegetables and legumes, the predominant dietary folate form is 5MTHF (Coffigniez et al., 2019; Delchier et al., 2016; Hefni et al., 2010; Jha et al., 2015). As well as in many fruits, such as oranges, 40%–45% of folates are represented by 5MTHF pentaglutamate (Sobczyńska-Malefora, 2019). However, other raw vegetables considered as a source of folate present another composition. For example, 80% of total folate content in dried Jew's mellow is 10FFA (Hefni et al., 2010). Other formyl forms of folate can be abundant in some legumes, such as chickpea (Strandler et al., 2015).

During food processing, folate loss is significant and principally associated to thermal treatments (blanching, cooking, pasteurization, sterilization, etc.) when exposure to heat and oxygen is important. This is due to the chemical reactions which are activated by elevated temperature and the presence of oxygen (Delchier et al., 2014; Gazzali et al., 2016; Gmelch et al., 2020; Petersen, 1993; Stea et al., 2007). While there is no consensus on a single reaction mechanism, the degradation of folates leads to their cleavage into a pterin and a *p*-aminobenzoic acid linked to the polyglutamate tail with no residual biological activities (Araújo, Marchioni, Zhao, et al., 2012; Blair & Pearson, 1974; Delchier et al., 2016). This pathway was validated by multiresponse modeling on the most studied natural folate: 5-methyl tetrahydrofolic acid (Verlinde et al., 2009). To our knowledge, the degradation mechanisms of other natural forms were not elucidated.

Delchier et al. (2014) showed that the origin of folate loss was matrix-dependent: he proved that in spinach, leaching was the main mechanism causing folate loss while in green beans, the chemical degradation mainly led to folate reduction. Despite the necessity to understand and control B_9 vitamin loss during food processing, very little kinetic information is available. This lack of kinetic information comes from a combination of causes, the first one being analysis difficulties. Indeed, folates are found in several forms strongly linked to the food matrix and in particular to starches which makes them difficult to extract (Arcot & Shrestha, 2005). In addition, once they are successfully extracted from the raw material using complex trienzymatic actions, the number of polyglutamate forms is so high that it is complicated to identify and quantity them. Lastly, as some vitamins B_9 are very sensitive to oxidation, important loss or interconversions can occur during analysis leading to high uncertainty about the extent of their real loss during processing (Zhang et al., 2018).

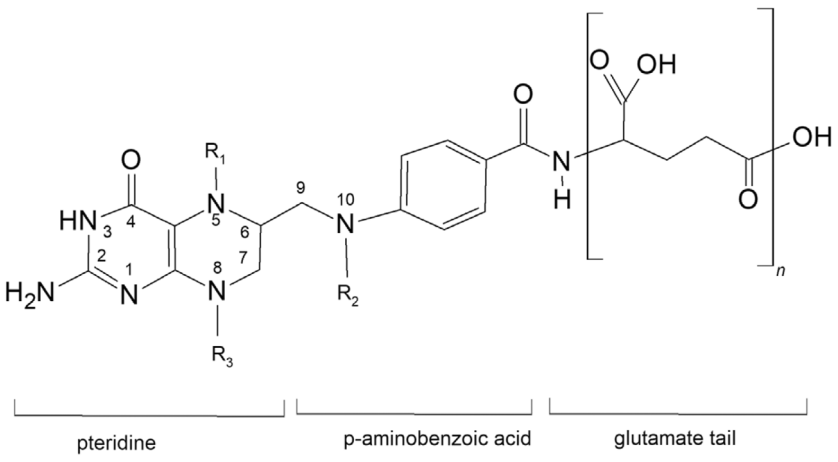

FIGURE 1 Chemical structure of the folate family. R_1, R_2, and R_3 correspond to substitution depending on the vitamer.

The little literature about folate degradation kinetics during thermal treatments reports a first order degradation trend (Chen & Cooper, 1979; Mnkeni & Beveridge, 1983; Paine-Wilson & Chen, 1979). Some authors studied the effect of dissolved oxygen. Chen and Cooper (1979) showed that oxygen accelerated the degradation of THF by nearly 10-fold and 5MTHF to a lesser extent. The same trend was noticed in real food. Delchier et al. (2014) found that folate degradation was not significant in green beans and spinach in an anaerobic environment, while marked degradation of 5MTHF was observed in the presence of dissolved oxygen (Delchier et al., 2014). Chen and Cooper (1979) also analyzed the effect of an antioxidant (0.1% ascorbate) on the degradation rate of 5MTHF at 100°C in aqueous solution and proved that this reducing agent led to a significant diminution of loss. In all cases, dissolved oxygen content during food cooking may generate marked folate oxidation. Kinetic data can also be found on the effect of temperature. A lot of publications about this topic are available on FA mainly at storage temperature, the most stable form used for supplementation purposes (Araújo, Marchioni, Villavicencio, et al., 2012; Cooper et al., 1978; Gazzali et al., 2016; Vora et al., 2002). Concerning folates, and even more in food matrixes, degradation kinetic data is much scarcer. Indeed, measuring the effect of temperature on chemical reactions in a food matrix is complex because temperature influences not only oxidation and cleavage degradation rates but also diffusion coefficients. Coffigniez et al. (2019) noticed marked differences in reactivity of certain folates. The authors showed that in cowpea, the formyl folate structures (10FFA and 5FTHF) were less reactive than 5MTHF. However, because enzymatic interconversion was concomitant, the specific chemical degradation rates were not given. For their parts, Nguyen et al. (2001) showed that in model media at neutral pH, 5MTHF degradation occurred within of a few minutes at or above 60°C compared to within 1 h for folic acid (Nguyen et al., 2001).

Paine-Wilson and Chen (1979) followed FA, 5FTHF, 5MTHF, and THF degradation at pH between 1 and 12 at a temperature of 100°C. These authors reported that the two first vitamins were the most stable for 10 h heating at 100°C while the others were degraded in a few minutes. For all folates, the degradation rates increased at acidic pH, lower than 4. This behavior was also confirmed by Mnkeni and Beveridge (1983). However, Delchier et al. (2014) stated that 5MTHF and FA were more stable at pH 5 than at pH 7. Therefore, while the effect of temperature is coherent on folate degradation kinetics in literature, the pH impact is more controversial, probably because of the different buffer formulations and the synergies or opposition of the chemical environment and pH conditions (Paine-Wilson & Chen, 1979).

The objective of this study is to gain insight into the reactivity of four different folate forms. Therefore, two commons bioactive reduced forms, involved in one-carbon transfer reactions, (5-methyl tetrahydrofolic acid and tetrahydrofolic acid) and two usual oxydised forms which have to be metabolized and converted by human body (folic acid and 10-formyl-folic acid) were chosen. Their reactivities were studied as a function of temperature and pH in model media in order to exclude other factors of loss such as leaching, enzymatic interconversion or matrix effect. The principal objective is to follow and understand the degradation rate of folate in different pH-temperature conditions but also to gain insight into their degradation product dynamic. This practical information is essential to better process rich-folate food from the formulation (pH lever) to the process (mainly temperature lever) and therefore optimize their nutritional quality.

2 | MATERIALS AND METHODS

2.1 | Chemicals

All reagents and solvents used in the experiments were purchased from Sigma-Aldrich (St. Louis, USA) and were of HPLC grades. 5,6,7,8-tetrahydrofolic acid (THF), 5-methyl tetrahydrofolic acid (5MTHF), 10 formyl folic acid (10FFA), and folic acid (FA) standards were purchased from Schircks Laboratories (Bauma, Switzerland).

Stock solutions of FA and 5MTHF were prepared at, respectively, 10 and 330 $\mu g \cdot mL^{-1}$ in distilled water. THF and 10FFA were prepared at, respectively, 60 and 100 $\mu g \cdot mL^{-1}$ in sodium hydroxide solution 50 mM to facilitate solubilization. Citrate buffer, used to standardize the pH of solutions, was prepared at 0.1 M for every pH selected.

2.2 | pH and temperature treatments

Working solutions were made with different dilutions of stocks solutions with citrate buffer at pH 3, 5, and 7 to reach a desired final concentration in folate with a minimal impact on buffer molarity. Afterward, samples were placed in sealed tubes of 15 mL in the heated device in order to reach the defined temperature within 2 min.

According to the preliminary experiments and the scientific information available, THF and 5MTHF were heated for 10, 20, 40, 60, and 140 min at 3 temperatures, 60°C, 80°C, and 100°C and 3 pH (pH 3, 5, and 7). FA and 10FFA were only heated at 80°C and 100°C since no degradation was observed at 60°C within the experiment time of 200 h. Finally, 10FFA was heated for 24, 47, 71, 95, and 119 h while FA was heated 27, 48, 120, 168, and 287 h.

For extended operation times, the heating device used was an oven model 500 from Memmert (Schwabach, Germany) in order to ensure a constant temperature without deviation over time. For short-time kinetics, the heating device was a Polystat 24 water bath (Fisher scientific, Waltham, USA). After treatment, 2 mL of the sample was filtered through a syringe-filter of 0.45 µm in 2 mL vials prior to HPLC analysis. An average of 63 measures were done for THF and 5MTHF and 36 for 10FFA and FA. Dissolved oxygen was measured at 8 ppm for all samples.

2.3 | HPLC quantification and identification

Folate separation and quantification were achieved using UPLC Agilent infinity II (Agilent, Santa Clara, USA) coupled with a diode array detector (DAD) and a fluorescence detector (FLD). Injection volume

was 20 µL. The column used was a Kinetex C18 2.6 µm × 150 mm × 4.6 mm (Phenomenex, Torrance, USA) set at a temperature of 30°C. Mobile phase consisted of phosphate buffer 33 mM at pH 2.3 (solvent A) and acetonitrile 100% (solvent B). Gradient was set as follow: 6–2 min: solvent A 100%; 2–16 min: solvent A from 100% to 85% and solvent B from 0% to 15%; 16–18 min solvent A 100% in order to come back to initial conditions. Flow rate was set at 1 mL·min^{-1}. Quantifications of undegraded vitamins were carried out thanks to the individual calibration curves, which were set for each form considering their maximum absorption. DAD was set at 210 nm for 5MTHF and 10FFA, and 280 nm for THF and FA. Degradation products were followed at the same wavelengths. FLD was set at $\lambda_{excitation}$ of 295 nm and a $\lambda_{emission}$ of 356 nm.

LC–MS analysis was performed on an Acquity H-Class UPLC system (Waters Corp., Milford, MA), equipped with the same Kinetex C18 2.6 µm × 100 mm × 2.1 mm column. The set-up was the same as described. The mass spectrometer was a Synapt G2-S HDMS system (Waters Corp., Milford, MA) with electrospray ionization source operating in high-resolution mode. Samples were analyzed in the positive ionization mode with a capillary voltage of 1 kV. The TOF mass analyzer was calibrated using phosphoric acid in 1:1 (v:v) acetonitrile: H_2O from 50 to 1200 m/z to obtain mass accuracy within 3 ppm. The Synapt parameters were optimized as follows: the sample cone was set at 20 V, the source and desolvation temperature were set at 140°C and 450°C, respectively. Each sample was processed with MassLynx (V4.1) software.

2.4 | Kinetic modeling of folate degradation

Individual folate change versus time can be described by Equation (1).

$$\frac{d[X]}{dt} = -k[X] \quad (1)$$

where [X] represents concentration (mol·L^{-1}), t the time (min), and k the reaction rate constant (min^{-1}).

The uncertainty of the rate constants obtained with the Levenberg–Marquardt algorithm was calculated by nonlinear error propagation using Matlab software (The Mathworks Inc., USA).

The rate constants k, were assumed to vary with the temperature according to the Arrhenius law (Equation 2).

$$k = k_{ref} \exp\left(\frac{-E_a}{R}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right) \quad (2)$$

where k_{ref} is the rate constant at the reference temperature chosen in the middle of the studied temperature range (80°C), E_a, T, and R, respectively, the activation energy (J·mol^{-1}), the medium temperature (K), and the gas constant (8.314 J·mol^{-1}·K^{-1}).

Kinetic constants, and activation energies were identified by nonlinear regression thanks to the Levenberg–Marquardt minimization procedure using the Matlab software (The Mathworks Inc., Natick, MA, USA).

Confidence intervals of Arrhenius parameters (Equation 2) were obtained via the bootstrap simulation method. The principle is the generation of a high number (500) of resamples of the kinetic dataset added with a Gaussian perturbation within the experimental standard deviations. These virtual data were generated via Matlab software (The Mathworks, Inc., USA).

2.5 | Multiresponse modeling of the thermal degradation of folates

Degradation products, both oxidation and cleavage molecules, were monitored. From this set of data, a proposition of degradation scheme was proposed and translated into a system of differential equations. Optimization of the kinetic parameters was achieved with the Levenberg–Marquardt algorithm using the Matlab software (The Mathworks Inc., Natick, MA, USA).

2.6 | Statistical analysis

ANOVA analysis was done using Statistica 7.1 software (StatSoft inc, Maisons-Alfort, France).

3 | RESULTS AND DISCUSSION

3.1 | Part 1: pH and temperature effect on folate vitamins degradation kinetics

The kinetics of folate degradation are presented in Figure 2 for 5MTHF and THF and Figure 3 for FA and 10FFA. Firstly, the first-order kinetic model fitted better the experimental data ($0.91 < R^2 < 0.98$) than a second-order ($0.72 < R^2 < 0.87$) except for THF where both orders were equivalent ($R^2 \sim 0.95$). This observation is in accordance with Mnkeni and Beveridge (1983); Paine-Wilson and Chen (1979); and Viberg et al. (1997). The thermal degradation of 5MTHF and THF at 60°C, 80°C, and 100°C from 0 to 150 min showed that tetrahydrofolate forms were the most reactive molecules with a total degradation of both forms in less than 1 h at pH 3. A slight discrepancy with the first-order kinetic model was observable for THF especially at pH 5. The rate constant was high for the first few minutes of thermal treatment and decreased as a function of time. In their study of 5MTHF oxidation, Viberg et al. (1997) observed that its degradation rate was oxygen-dependent and that a possible limitation of dissolved oxygen during the experiment could change the reaction order. Indrawati et al. (2004) also observed a rupture of the first-order kinetics during degradation of 5MTHF when β-mercaptoethanol, acting like an antioxidant, was added. Therefore, the rapid initial rate of THF degradation and the discrepancy from the first-order trend could be explained by the oxygen consumption in the media for this molecule that is very prone to oxidation (Blair & Pearson, 1974). In acidic conditions of pH 3, rate constants of 5MTHF and THF were similar with values between 3×10^{-4} to

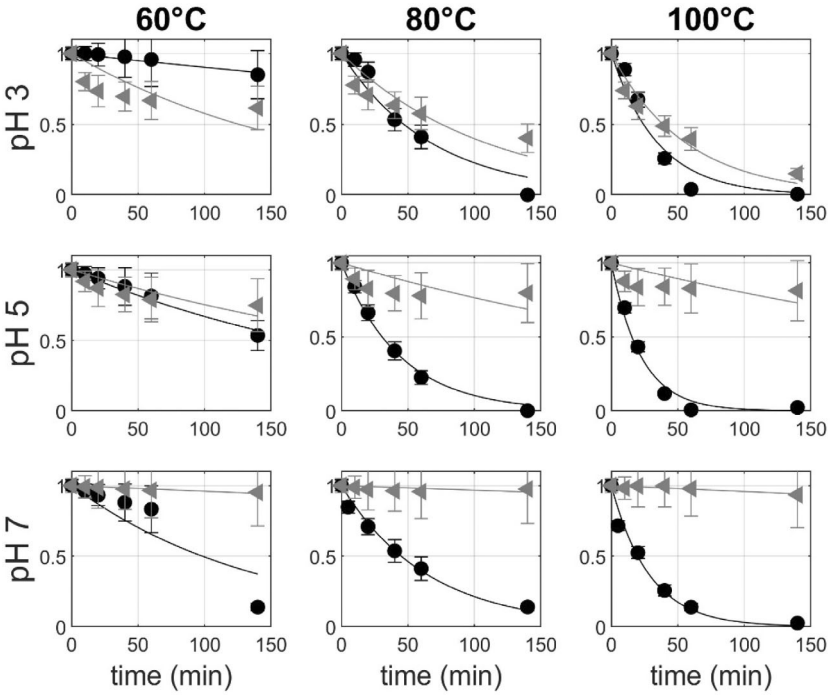

FIGURE 2 Degradation kinetics of 5MTHF (●) and THF (◄), at 60°C, 80°C, and 100°C and pH 3, 5, and 7. Dots represents the experimental mean ($n = 3$) and lines the first-order kinetic model.

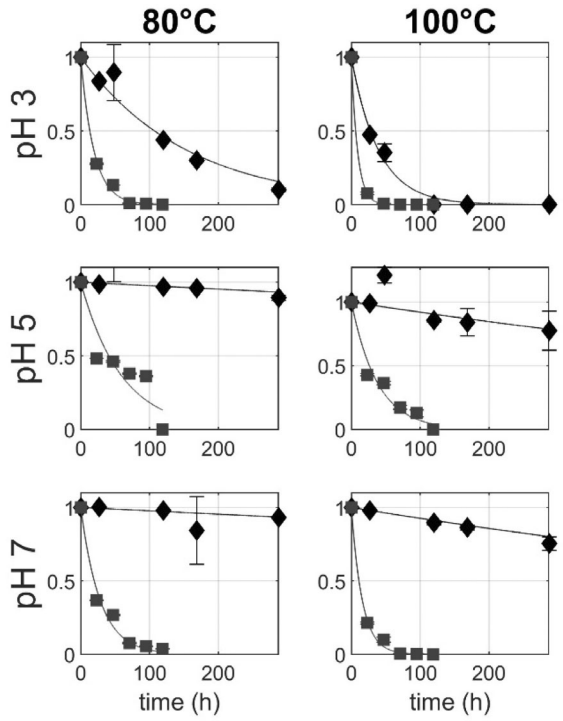

FIGURE 3 Degradation kinetics of FA (◆) and 10FFA (■), at 80°C and 100°C and pH 3, 5, and 7. Dots represents the experimental mean ($n = 3$) and lines the first-order kinetic model.

2.8×10^{-2} min^{-1} and from 4.6×10^{-3} to 1.7×10^{-2} min^{-1}, respectively. Paine (1978) found the same range of rate constant for 5MTHF (pH 3) of 8.3×10^{-2} min^{-1} at 100°C. However, the author found that THF was more reactive with rates of 0.1 min^{-1} at pH 2 and 100°C. These variations may be due to the difference of oxygenation that is not always specified and buffer types that may affects (up to 5-fold) the reactivity of the reduced forms of folates (Paine, 1978; Viberg et al., 1997). At pH 3, the sensitivity of temperature was slightly higher for 5MTHF than THF with activation energies of 51.0 ± 1.2 and 35.6 ± 2.8 $kJ{\cdot}mol^{-1}$ respectively. No comparison in literature can be found at such a pH, which is however a classic food product pH for food pasteurization.

The degradation behavior of these two folates and their sensitivity were different as a function of pH. The THF degradation pattern was very different at a pH greater than 5, which was consistent with literature. Paine-Wilson and Chen (1979) found that rates dropped from 0.56, 0.21, and 0.15 min^{-1} at pH 4, 6, and 8, respectively, for THF at 100°C that is to say a reduction of 4-fold between pH 4 and 8. In our case, THF degradation rate dropped from 1.6×10^{-2} to 5.0×10^{-5} min^{-1} between pH 3 and 7, that is, a decrease by 300-fold. However, Chen and Cooper (1979) found a rate superior to 1 min^{-1} for THF with 90% degradation in less than 2 min at pH 7. Paine-Wilson and Chen (1979) noticed a great discrepancy in the literature about THF reactivity because the degradation of this compound may be very dependent on the media and its reducing capacity. Therefore, the degradation rates of THF need further research on side composition effect and, until this is done, should not be used for prediction purposes. However, despite the value range, the THF degradation rate trend according to pH was consistent with literature describing a stability which increases with pH. Besides, Paine (1978) found a linear relation between –log(k) and pH.

Regarding 5MTHF behavior toward pH, at 100°C, the rate values varied from 2.8 to 4.4 to 3.4×10^{-2} min^{-1} from pH 3, 5, and 7, respectively. Therefore, 5MTHF was less sensitive to an increase of pH. Paine (1978) also found a different pH effect on 5MTHF that was much less marked than on THF. Plotting –log(k) versus pH, the same

author found a bell-shaped curve for 5MTHF with minimal rates obtained at pH 6 and 8. In our case, it is difficult to conclude since at 60°C, the highest rate was at pH 7, but at 100°C, the highest was at pH 5. Many degradation rates can be found in literature for 5MTHF especially at pH 7. Therefore, we can say that our value of 3.4×10^{-2} min^{-1} obtained at 100°C is very comparable to that of 3.2×10^{-2} min^{-1} found by Paine-Wilson and Chen (1979) in the same conditions or of 2.8×10^{-2} min^{-1} by Nguyen et al. (2001) at 80°C. However, some published rates were as high as 4.6×10^{-2} min^{-1} at 60°C or 0.3 min^{-1} at 110°C in the works of Indrawati et al. (2004) and Viberg et al. (1997). This discrepancy, which is usual for kinetic rates, is much less significant than that of THF rates.

Finally, kinetic rates followed the Arhenius model well (Figure 4), and activation energies (E_a) for the 3 pH are presented in Table 1. 5MTHF E_a were of 58, 41, and 45 $kJ{\cdot}mol^{-1}$ for pH 3, 5, and 7, respectively. This trend is consistent with Mnkeni and Beveridge (1983) who found an E_a of 19.0–19.8 $kcal{\cdot}mol^{-1}$ at pH 3 and 6 that is to say a mean value of 80 $kJ{\cdot}mol^{-1}$ whatever the pH. Viberg et al. (1997) modified oxygenation conditions and found Ea varying from 62 to 106 $kJ{\cdot}mol^{-1}$. Finally, Paine-Wilson and Chen (1979) found an activation energy of 9.5 $kcal{\cdot}mol^{-1}$ that is to say about 40 $kJ{\cdot}mol^{-1}$, which is close to our result.

Therefore, reduced folates are the most sensitive forms with an important degradation that can be achieved within 10–60 min during thermal treatment depending on the pH and temperature. These compounds are also the most bioactive forms. The pH, and other solute effects such as dissolved oxygen, have a marked impact on THF degradation rates. However, the effect of pH was less important on 5MTHF that has a degradation rate close to 0.03 min^{-1} at 100°C, that is to say a decimal reduction time (D) of about 80 min at this temperature. As a comparison, vitamin C average rate value at 100°C is 1–3×10^{-3} min^{-1} (Dhuique-Mayer et al., 2007; Kadakal et al., 2018), meaning that 5MTHF folate is about 10-fold more thermal sensitive.

Degradation of folic acid forms, FA and 10FFA are presented in Figure 3 for 80°C and 100°C, at pH 3, 5, and 7. The rates identified by the first-order kinetic model are reported in Table 1. The folic acid structure exhibited degradation rates that were 10- to 100-fold lower than that of the tetrahydrofolate forms. As a comparison, rates at 100°C and pH 7, were of 1×10^{-3} min^{-1} and 1×10^{-5} min^{-1} for 10FFA and FA, that is, 30- to 3000-fold inferior to that of 5MTHF. Paine-Wilson and Chen (1979) found rates of 0.07 h^{-1} for FA at 100°C versus 4×10^{-4} min^{-1} (0.02 h^{-1}) in our study. The authors also found that at pH 3, the 5-formyl tetrahydrofolate was degraded by 10% after 3 h of thermal treatment at 100°C, leading to a degradation rate of 3×10^{-2} h^{-1} versus 8×10^{-2} min^{-1} for 5MTHF in the same conditions. Therefore, the formyl group may contribute to a higher stability. In our case, at 100°C, 10FFA exhibited rates of 1.8×10^{-3} min^{-1} (0.1 h^{-1}) at pH 3 confirming that the formyl group substitution, in the structure that was reduced or not, make it more stable than the methyl group substitution.

Folic acid was the most stable form whatever the pH and temperature which is consistent with literature (Araújo, Marchioni, Zhao, et al., 2012; Gazzali et al., 2016). The lowest stability was obtained at the most acidic pH. Indeed, at 100°C and pH 3, the degradation rate was equal to 1×10^{-4} min^{-1} that is 10-fold higher than the rate at pH 7. As for THF, the rate was a nonlinear decreasing function of pH. The effect of pH was different for 10FFA: its stability was the highest at the intermediate pH of 5 with rates of 4×10^{-4} min^{-1} at 100°C that is to say 2–5 times lower than pH 7 and 3, respectively.

Finally, THF, FA, and 10FFA degradations were markedly dependent on pH while this factor was significantly less sensitive for 5MTHF. THF and FA were more stable at a higher pH of 7 while 10FFA was more stable at pH 5. Among the folate studied, the

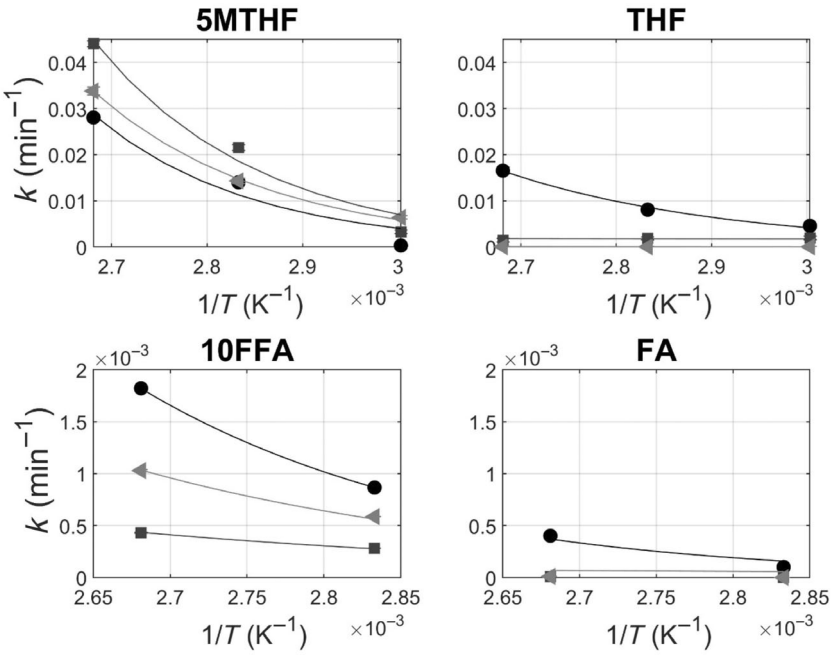

FIGURE 4 Arrhenius representation for 5MTHF, THF, FA, and 10FFA degradation for pH 3 (●), 5 (◄), and 7 (■). Dots are rate constants identified from the kinetics of Figures 1 and 2. Lines represent the Arrhenius model associated to parameters presented in Table 1.

TABLE 1 Rate constants k (min^{-1}) and Arrhenius parameters for 5 MTHF, THF, FA, and 10FFA with k_{ref} the reference constant at 80°C (min^{-1}), and E_a the activation energy ($kJ \cdot min^{-1}$).

		5-Methyl tetrahydrofolic acid				Tetrahydrofolic acid			
		k (min^{-1})		k_{ref} (80°C)	E_a	k (min^{-1})		k_{ref} (80°C)	E_a
		Average	Std Dev	(min^{-1})	($kJ \cdot mol^{-1}$)	Average	Std Dev	(min^{-1})	($kJ \cdot mol^{-1}$)
pH 3	60°C	3.0×10^{-4}	(2.0×10^{-4})	1.1×10^{-2}	51	4.6×10^{-3}	(4.0×10^{-4})	8.6×10^{-3}	35.6
	80°C	1.4×10^{-2}	(4.0×10^{-4})	(2.8×10^{-4})	(1.2)	8.1×10^{-3}	(6.0×10^{-4})	(3.6×10^{-4})	(2.8)
	100°C	2.8×10^{-2}	(5.0×10^{-4})			1.7×10^{-2}	(7.0×10^{-4})		
pH 5	60°C	3.3×10^{-3}	(4.0×10^{-4})	1.7×10^{-2}	48.1	2.0×10^{-3}	(4.0×10^{-4})	1.8×10^{-3}	1.1
	80°C	2.2×10^{-2}	(6.0×10^{-4})	(3.4×10^{-4})	(1.1)	1.9×10^{-3}	(3.0×10^{-4})	(2.4×10^{-4})	(0.6)
	100°C	4.4×10^{-2}	(6.0×10^{-4})			1.4×10^{-3}	(4.0×10^{-4})		
pH 7	60°C	6.4×10^{-3}	(3.0×10^{-4})	1.5×10^{-2}	45.4	3.0×10^{-5}	(1.5×10^{-5})	7.6×10^{-5}	14.6
	80°C	1.4×10^{-2}	(6.0×10^{-4})	(3.8×10^{-4})	(1.9)	4.0×10^{-5}	(2.0×10^{-5})	(4.0×10^{-5})	(7.8)
	100°C	3.4×10^{-2}	(9.0×10^{-4})			5.0×10^{-5}	(2.0×10^{-5})		
		Folic acid				10 Formyl folic acid			
		k (min^{-1})		k_{ref} (80°C)	E_a	k (min^{-1})		k_{ref} (80°C)	E_a
		Average	Std Dev	(min^{-1})	($kJ \cdot mol^{-1}$)	Average	Std Dev	(min^{-1})	($kJ \cdot mol^{-1}$)
pH 3	80°C	1.0×10^{-4}	(3.0×10^{-6})	1.6×10^{-4}	46.2	9.0×10^{-4}	(2.0×10^{-6})	8.7×10^{-4}	40.5
	100°C	4.0×10^{-4}	(9.0×10^{-6})	(1.6×10^{-6})	(0.7)	1.8×10^{-3}	(2.0×10^{-5})	(2.2×10^{-6})	(0.8)
pH 5	80°C	3.0×10^{-6}	(4.0×10^{-7})	5.9×10^{-5}	8.4	3.0×10^{-4}	(4.0×10^{-7})	2.8×10^{-4}	24.7
	100°C	9.0×10^{-6}	(2.0×10^{-6})	(4.3×10^{-7})	(0.1)	4.0×10^{-4}	(3.0×10^{-6})	(4.5×10^{-7})	(0.3)
pH 7	80°C	1.0×10^{-6}	(5.0×10^{-7})	5.9×10^{-5}	8.6	6.0×10^{-4}	(1.0×10^{-6})	5.6×10^{-4}	33.3
	100°C	1.0×10^{-5}	(5.0×10^{-7})	(2.5×10^{-7})	(0.1)	1.0×10^{-3}	(9.0×10^{-6})	(1.2×10^{-6})	(0.4)

Note: Standard deviation is between brackets.

presence of a formyl group seems to lead to more stable vitamins. This information is interesting to select formyl-rich legumes or leafy green vegetables to maximize diet deficiencies and may explain why this form is prevalent or residual in processed vegetables like dried Jew mallow (Hefni et al., 2010).

3.2 | Part 2: pH and temperature effect on folate degradation products

3.2.1 | Case of 5MTHF

The 5MTHF degradation scheme has been studied the most. Blair and Pearson (1974) and Verlinde et al. (2009) explained that 5MTHF was subjected to complex oxidative degradation during thermal treatment. This complex reaction pathway involving different steps and chemical species may explain why kinetic reaction model, that is, order, differ from one to another study. Different oxidized forms were identified as intermediary oxidized compounds. Verlinde et al. (2009) studied its degradation under pressure-thermal treatments in a model medium and found s-triazine derivative, 5-methyldihydrofolic acid and *p*-aminobenzoyl-L-glutamate (PABA-GLU) as predominant degradation products. The major oxidized compound found is called xanthopterin which is yellow. The oxidized forms can be cleaved at the C(9)-N (10) covalent bond leading to the pterin moiety on one side and the PABA-GLU fragment on the other, resulting in an irreversible loss of vitamin activity (Delchier et al., 2016; Gazzali et al., 2016). In our case, some experimental HPLC chromatograms at pH 3, 5, and 7 are presented in Figure 5. By order of elution, we found peak **3** being the cleavage compound ($m/z = 363$), 5MTHF as peak **1** ($m/z = 460$) and the oxidized product 2 as peak **2** ($m/z = 474$). Other degradation compounds are present as minor peaks at all pH values. From this experimental observation and the previous literature on degradation pathways, we built the reaction scheme in Figure 6 and converted it into a set of ordinary differential equations. This scheme assumes a direct cleavage of 5MTFH (k_1), oxidation (k_2), and degradation into an unknown compound (k_3). This multiresponse kinetic model was confronted to the kinetic evolution of peak **1** (5MTHF), peak **2** (oxidized product), and peak **3** (cleavage product) obtained by HPLC-MS.

Figure 7 shows that modeled data fitted the experimental data well of 5MTHF degradation, (●) as well as production of intermediate product 2 (■) and cleavage product 3 (◆). We can observe that there were different amounts of product 2 and 3 as a function of pH. Indeed, cleavage compounds were the main degradation products at pH 3, while there were more oxidized compounds at a pH superior to 5. The temperature had no significant influence on this trend, but on the rate of production of compound 2 or 3. Therefore, even if pH has a minor impact on the global degradation rate of 5MTHF, this

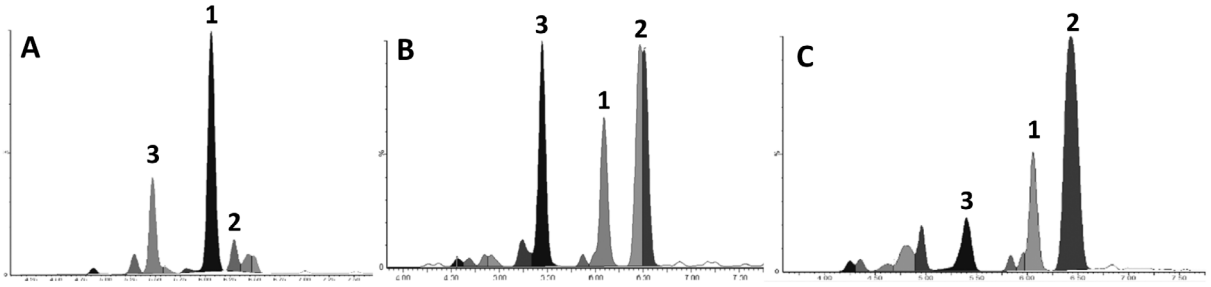

FIGURE 5 Chromatograms of one of the kinetic points (30 min) obtained after the degradation at pH 3 (a), 5 (b), and 7 (c) and at 100°C for 5MTHF. 1, 2, and 3 refers to peaks of 5MTHF, an oxidized product and a cleavage compound respectively.

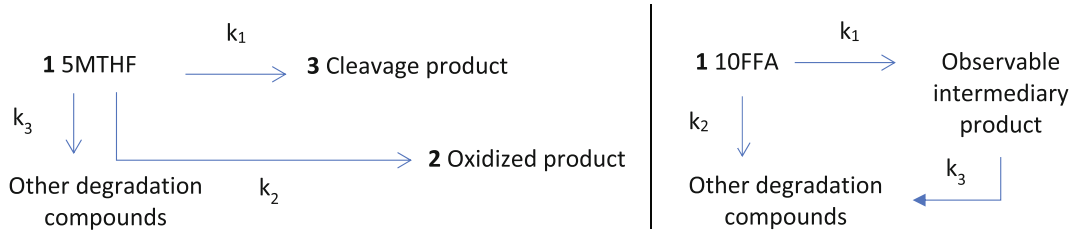

FIGURE 6 5MTHF and 10FFA degradation schemes according to experimental data and literature.

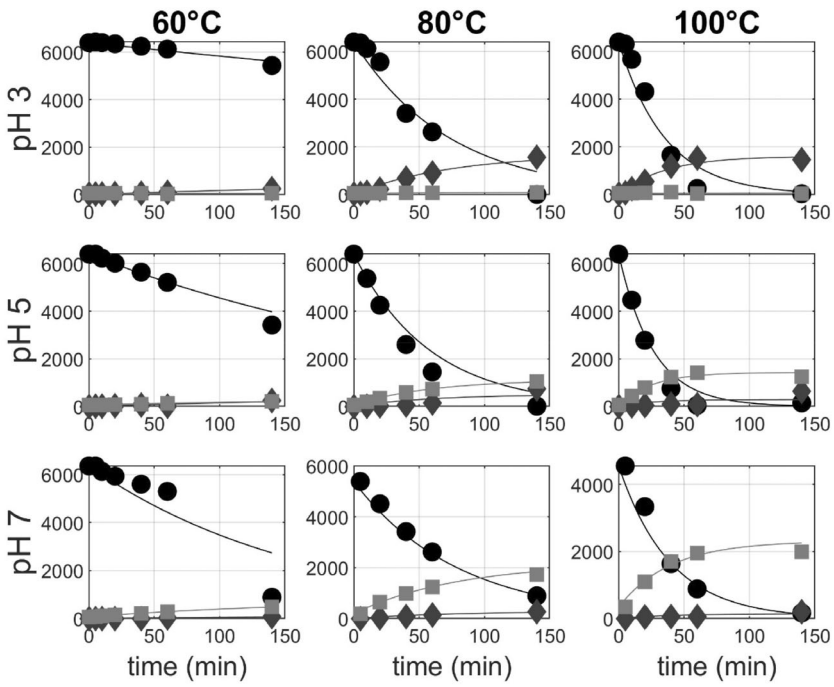

FIGURE 7 Multiresponse modeling of 5MTHF degradation (●) and supposed intermediate oxidized products (■) and final cleavage product (◆) generation during thermal treatments at 60°C, 80°C, and 100°C and at pH 3, 5, and 7.

parameter significantly influences the degradation pathway and the final composition of the media in terms of degradation compounds of 5MTHF whose biological activity is unknown.

In all conditions, the highest rate was $k_{3,5MTHF \rightarrow degradation}$, that is, the transformation of 5MTHF in other degradation products. This rate was about 3-fold the value of $k_{1,5MTHF \rightarrow cleavage}$ at pH 3 and up to 5-fold $k_{2,5MTHF \rightarrow oxidation}$ at pH 5. It is noteworthy that k_3 was close to k_2 at pH 7. Therefore, at pH 7, 5MTHF was degraded but the degradation product was not a cleaved, but an oxidized product. At pH 3, k_1 was higher by 50- to 100-fold to that of k_2. This was the reverse trend at pH 7 where k_2 was higher than k_1 by 6- to 14-fold.

3.2.2 | Case of 10FFA

During 10FFA degradation, only one compound was identified by HPLC as an intermediary product because its concentration increased and decreased at pH 3, which was probably consumed to form

another end-product which was not detected but we can assume it is an intermediary oxidation product. In this case, the model proposed is a degradation of 10FFA to form this observable compound (k_1) and degradation to form undetectable compounds directly from 10FFA (k_2) or from the observable compound (k_3). The model fitted the experimental data well as observed in Figure 8.

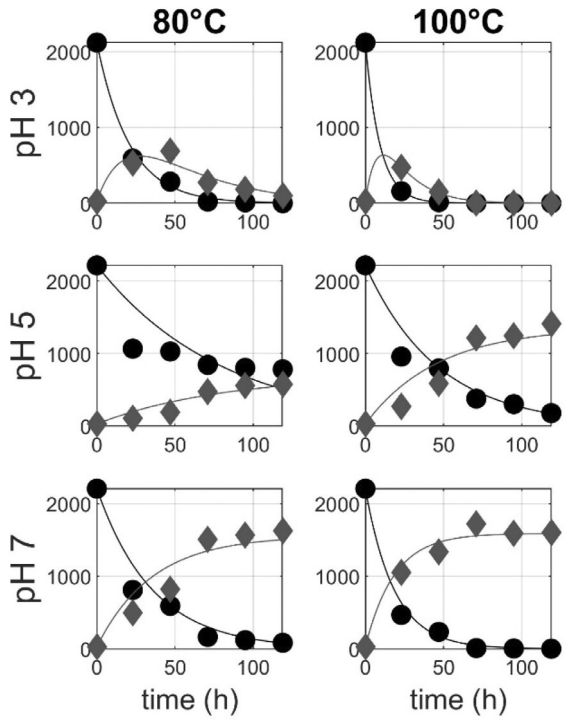

FIGURE 8 Multiresponse modeling of 10FFA degradation (●) and observable product (◆) generation during thermal treatments at 80°C and 100°C and at pH 3, 5, and 7.

Figure 8 shows that after 20 h at pH 3, the degradation of both FFA and observable intermediary compound is important. At pH 5 and 7, the observable intermediary compound was more stable, and its production rate was higher than its degradation rate which explains its accumulation. Therefore, the intermediary compound that was absent at pH 3, may contribute to the highest stability of 10FFA at the intermediate pH of 5 and 7. At pH 7, the rate of intermediary product generation and its degradation was slightly higher than at pH 5, explaining a higher degradation rate of 10FFA, and the optimal stability at pH 5. This result confirms the dependence of 10FFA on pH and the pH-dependent formation of an intermediary compound that is preferentially degraded but at a lower rate than the direct degradation of 10FFA. Blackey (1996) highlighted that pH effect was significant for derivatives substituted with the formyl group (Blakley, 1969).

4 | CONCLUSION

These results improved knowledge on the retention of folate, which differs greatly as a function of the pH–temperature couple. Therefore, individualization of forms by identification of the main structures present in the food and the consideration of the specific reactivity constant is of significant importance. Indeed, at 100°C and pH 7, which are common conditions for thermal treatments of legumes, the rate constant of 10FFA was 30-fold lower than that of 5MTHF. Therefore, degradation of tetrahydrofolic acid forms, such as THF and 5MTHF, can occur during thermal treatment while FFA loss will be under 20%. This conclusion is illustrated by Figure 9 where FA and FFA forms that here account for 20% of total initial folates (which is the case in some legumes) can reach 50% of total folates after 60 min cooking at 100°C. This prediction tool must be improved in order to be use for food.

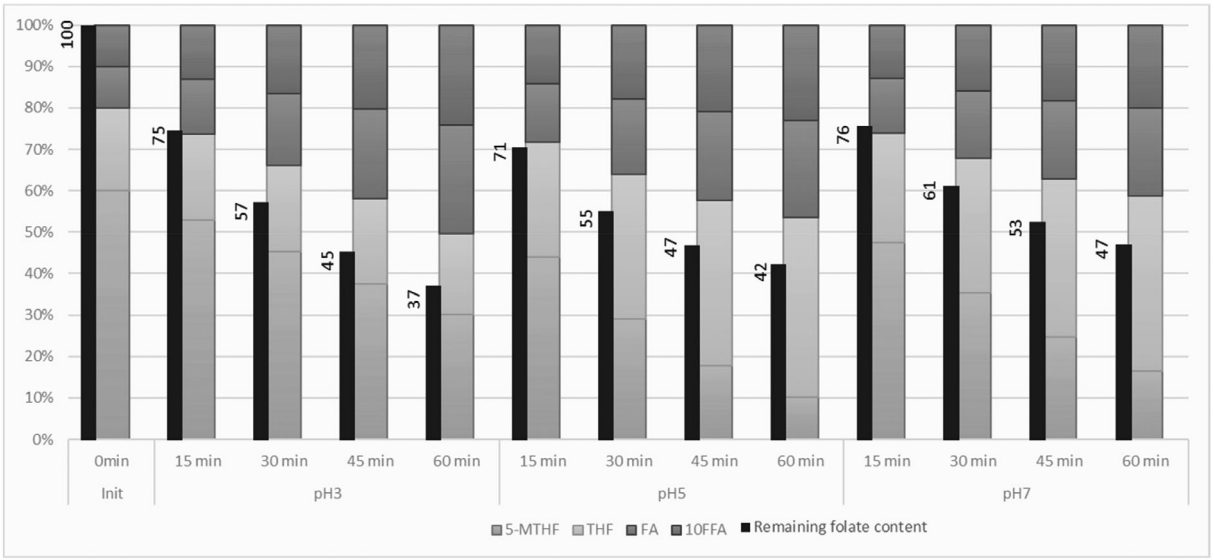

FIGURE 9 Simulation of folate degradation and final composition for a fixed fictive initial repartition of folates (80% reduced forms 5MTHF and THF and 20% of 10FFA and FA) at pH 3, 5, and 7 at 100°C during 15, 30, 45, and 60 min.

Our study also highlights the importance of the substitution, formyl or methyl, in the reactivity of folates. Each kind of ramification has its own reactivity and its own degradation kinetic pathway, and therefore the cooking process could be adapted accordingly. The study of the degradation mechanism by multi-response modeling confirmed that the degradation was pH-dependent because of the involvement of different intermediary or end-products of degradation. Furthers studies must be conducted on these molecules and their role.

To conclude, research is still needed to better understand the retention of bioactive folate in foods because its multifactorial phenomenon is dependent on composition, structure and the cooking process. This paper provides an objective comparison based on rate constants and activation energies in aqueous media. It would be very interesting in the future to compare this reactivity in more complex or real matrices to observe if this trend can be validated.

AUTHOR CONTRIBUTIONS

Adrien Servent: writing—original draft and review and editing, investigation, formal analysis, methodology, supervision, validation. **Guillaume Cazals:** Formal analysis, methodology, writing - review and editing. **Carmen Perfetto:** formal analysis, methodology, writing—review and editing. **Nawel Achir:** data curation, writing—original draft and review and editing, investigation, formal analysis, methodology, supervision, validation.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

Araújo, M. M., Marchioni, E., Villavicencio, A. L. C. H., Zhao, M., Zimmermann, P., El-Khoury, E., & Bergaentzle, M. (2012). Pressurized liquid extraction and HPLC quantification of folic acid in fortified wheat flours. *Journal of Agricultural and Food Chemistry*, *60*(31), 7629–7633. https://doi.org/10.1021/jf3025503

Araújo, M. M., Marchioni, E., Zhao, M., Kuntz, F., Di Pascoli, T., Villavicencio, A. L. C. H., & Bergaentzle, M. (2012). LC/MS/MS identification of some folic acid degradation products after E-beam irradiation. *International Meeting on Radiation Processing*, *81*(8), 1166–1169. https://doi.org/10.1016/j.radphyschem.2011.11.029

Arcot, J., & Shrestha, A. (2005). Folate: Methods of analysis. *EuroFoodFolate 2004*, *16*(6), 253–266. https://doi.org/10.1016/j.tifs.2005.03.013

Blair, J. A., & Pearson, A. J. (1974). Kinetics and mechanism of the autoxidation of the 2-amino-4-hydroxy-5,6,7,8-tetrahydropteridines. *The Journal of the Chemical Society, Perkin Transactions*, *2*(1), 80–88. https://doi.org/10.1039/P29740000080

Blakley, R. L. (1969). *The biochemistry of folic acid and related pteridines*. North-Holland Publishing Co.

Blancquaert, D., De Steur, H., Gellynck, X., & Van Der Straeten, D. (2014). Present and future of folate biofortification of crop plants. *Journal of Experimental Botany*, *65*(4), 895–906. https://doi.org/10.1093/jxb/ert483

Chen, T.-S., & Cooper, R. G. (1979). Thermal destruction of folacin: Effect of ascorbic acid, oxygen and temperature. *Journal of Food Science*, *44*(3), 713–716. https://doi.org/10.1111/j.1365-2621.1979.tb08483.x

Coffigniez, F., Rychlik, M., Sanier, C., Mestres, C., Striegel, L., Bohuon, P., & Briffaz, A. (2019). Localization and modeling of reaction and diffusion to explain folate behavior during soaking of cowpea. *Journal of Food Engineering*, *253*, 49–58. https://doi.org/10.1016/j.jfoodeng.2019.02.012

Cooper, R., Chen, T., & King, M. (1978). Thermal destruction of folacin in microwave and conventional heating. *Journal of the American Dietetic Association*, *73*(4), 406–410.

Czarnowska-Kujawska, M., Draszanowska, A., & Gujska, E. (2020). Effect of different cooking Methods on folate content in chicken liver. *Food*, *9*(10), 1431. https://doi.org/10.3390/foods9101431

Delchier, N., Herbig, A.-L., Rychlik, M., & Renard, C. M. G. C. (2016). Folates in fruits and vegetables: Contents, processing, and stability. *Comprehensive Reviews in Food Science and Food Safety*, *15*(3), 506–528. https://doi.org/10.1111/1541-4337.12193

Delchier, N., Ringling, C., Cuvelier, M.-E., Courtois, F., Rychlik, M., & Renard, C. M. G. C. (2014). Thermal degradation of folates under varying oxygen conditions. *Food Chemistry*, *165*, 85–91. https://doi.org/10.1016/j.foodchem.2014.05.076

Dhuique-Mayer, C., Tbatou, M., Carail, M., Caris-Veyrat, C., Dornier, M., & Amiot, M. J. (2007). Thermal degradation of antioxidant micronutrients in citrus juice: Kinetics and newly formed compounds. *Journal of Agricultural and Food Chemistry*, *55*(10), 4209–4216.

EFSA. (2014). Scientific opinion on dietary reference values for folate. *EFSA Journal*, *12*(11), 3893.

Gazzali, A. M., Lobry, M., Colombeau, L., Acherar, S., Azaïs, H., Mordon, S., Arnoux, P., Baros, F., Vanderesse, R., & Frochot, C. (2016). Stability of folic acid under several parameters. *European Journal of Pharmaceutical Sciences*, *93*, 419–430. https://doi.org/10.1016/j.ejps.2016.08.045

Gmelch, L., Wirtz, D., Witting, M., Weber, N., Striegel, L., Schmitt-Kopplin, P., & Rychlik, M. (2020). Comprehensive vitamer profiling of folate mono- and polyglutamates in Baker's yeast (*Saccharomyces cerevisiae*) as a function of different sample preparation procedures. *Metabolites*, *10*(8), 301. https://doi.org/10.3390/metabo10080301

Hefni, M., Öhrvik, V., Tabekha, M., & Witthöft, C. (2010). Folate content in foods commonly consumed in Egypt. *Food Chemistry*, *121*(2), 540–545. https://doi.org/10.1016/j.foodchem.2009.12.044

Indrawati, I., Verlinde, P., Ottoy, F., Van Loey, A., & Hendrickx, M. (2004). Implications of β-mercaptoethanol in relation to folate stability and to determination of folate degradation kinetics during processing: A case study on [6 S]-5-methyltetrahydrofolic acid. *Journal of Agricultural and Food Chemistry*, *52*(26), 8247–8254. https://doi.org/10.1021/jf048801z

Jha, A. B., Ashokkumar, K., Diapari, M., Ambrose, S. J., Zhang, H., Tar'an, B., Bett, K. E., Vandenberg, A., Warkentin, T. D., & Purves, R. W. (2015). Genetic diversity of folate profiles in seeds of common bean, lentil, chickpea and pea. *Journal of Food Composition and Analysis*, *42*, 134–140. https://doi.org/10.1016/j.jfca.2015.03.006

Kadakal, C., Duman, T., & Ekinci, R. (2018). Thermal degradation kinetics of ascorbic acid, thiamine and riboflavin in rosehip (Rosa canina L) nectar. *Food Science and Technology*, *38*, 667–673.

Mnkeni, A. P., & Beveridge, T. (1983). Thermal destruction of 5-Methyltetrahydrofolic acid in buffer and model food systems. *Journal of Food Science*, *48*(2), 595–599. https://doi.org/10.1111/j.1365-2621.1983.tb10797.x

Nguyen, M., Francis, D., & Schwartz, S. (2001). Thermal isomerisation susceptibility of carotenoids in different tomato varieties. *Journal of the Science of Food and Agriculture*, *81*(9), 910–917.

Paine, B. L. (1978). *Thermal destruction of folacin: Effect of pH, buffer ions, and ionic strength*. Department of Home Economics, California State University.

Paine-Wilson, B., & Chen, T.-S. (1979). Thermal destruction of folacin: Effect of pH and buffer ions. *Journal of Food Science*, *44*(3), 717–722. https://doi.org/10.1111/j.1365-2621.1979.tb08484.x

Petersen, M. A. (1993). Influence of sous vide processing, steaming and boiling on vitamin retention and sensory quality in broccoli florets. *Zeitschrift für Lebensmittel-Untersuchung Und Forschung*, *197*(4), 375–380. https://doi.org/10.1007/BF01242064

Sobczyńska-Malefora, A. (2019). Chapter 11—Methods for assessment of folate (vitamin B9). In D. Harrington (Ed.), *Laboratory assessment of vitamin status* (pp. 219–264). Academic Press. https://doi.org/10.1016/B978-0-12-813050-6.00011-5

Stea, T. H., Johansson, M., Jägerstad, M., & Frølich, W. (2007). Retention of folates in cooked, stored and reheated peas, broccoli and potatoes for use in modern large-scale service systems. *Food Chemistry*, *101*(3), 1095–1107. https://doi.org/10.1016/j.foodchem.2006.03.009

Strandler, H., Patring, J., Jägerstad, M., & Jastrebova, J. (2015). Challenges in the determination of unsubstituted food folates: Impact of stabilities and conversions on analytical results. *Journal of Agricultural and Food Chemistry*, *63*(9), 2367–2377. https://doi.org/10.1021/jf504987n

Verlinde, P. H. C. J., Oey, I., Deborggraeve, W. M., Hendrickx, M. E., & Van Loey, A. M. (2009). Mechanism and related kinetics of 5-Methyltetrahydrofolic acid degradation during combined high hydrostatic pressure–thermal treatments. *Journal of Agricultural and Food Chemistry*, *57*(15), 6803–6814. https://doi.org/10.1021/jf900832g

Viberg, U., Jägerstad, M., Öste, R., & Sjöholm, I. (1997). Thermal processing of 5-methyltetrahydrofolic acid in the UHT region in the presence of oxygen. *Food Chemistry*, *59*(3), 381–386. https://doi.org/10.1016/S0308-8146(96)00251-8

Vishnumohan, S., Pickford, R., & Arcot, J. (2017). Naturally occurring folates in selected traditionally prepared foods in Southern India. *Journal of Food Science and Technology*, *54*(13), 4173–4180. https://doi.org/10.1007/s13197-017-2870-7

Vora, A., Riga, A., Dollimore, D., & Alexander, K. S. (2002). Thermal stability of folic acid. *Thermochimica Acta*, *392–393*, 209–220. https://doi.org/10.1016/S0040-6031(02)00103-X

Zhang, H., Jha, A. B., Warkentin, T. D., Vandenberg, A., & Purves, R. W. (2018). Folate stability and method optimization for folate extraction from seeds of pulse crops using LC-SRM MS. *Journal of Food Composition and Analysis*, *71*, 44–55. https://doi.org/10.1016/j.jfca.2018.04.008

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