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A B S T R A C T

Folate losses in thermally treated foods are mainly due to oxidation. Other mechanisms and folate vitamers behaviour are poorly described.

Our study evaluated oxygen impact on total folate degradation and derivatives' evolution during thermal treatments.

Spinach and green bean purees were heated, in an instrumented reactor, in anaerobic conditions, under an oxygen partial pressure of 40 kPa.

Folates were stable in the absence of oxygen, whilst they were degraded under 40 kPa of oxygen. Total folate showed a sharp decrease in the first hour driven by the degradation of 5-$CH_3$-$H_4$folate, followed by a plateau due to the formyl derivatives and minor compounds stability.

The different evolution of the main derivatives was confirmed by the degradation of 5-$CH_3$-$H_4$folate and folic acid in solution, under the same conditions of oxygen concentrations. The stability of folic acid and the high susceptibility of 5-$CH_3$-$H_4$folate to degradation in the presence of oxygen were confirmed.

*Keywords:*
Vitamin
Heating
Vegetable
Oxidation
Spinach
Green bean
Kinetics

## 1. Introduction

Green vegetables are a good source of micronutrients, particularly of vitamins, and contribute to 40% of folate intake in the French diet (Lafay, 2009). Folates (vitamin B9) are well known to be involved in reducing the risk of neural tube defects (Czeizel & Dudás, 1992). Green vegetables, such as spinach and green beans, are usually consumed cooked and frequently industrially processed. Industrial processing has a positive impact due to inactivation of microorganism and a negative impact linked to the reduction of micronutrients.

Heat treatments, such as blanching or boiling, leads to folate losses of 20–80% in spinach and from 0% to 20% in green beans (Delchier, Reich, & Renard, 2012; DeSouza & Eitenmiller, 1986; Klein, Lee, Reynolds, & Wangles, 1979; McKillop et al., 2002; Melse-Boonstra et al., 2002). However, steaming and microwave cooking preserves folate content (Delchier et al., 2012; Klein et al., 1979; McKillop et al., 2002). One of the main mechanisms of folate losses is leaching to surrounding liquids (Scott, Rébeillé, & Fletcher, 2000), as we confirmed from observations of industrial processing chains (Delchier et al., 2013), and from studying folate diffusion from whole spinach and green beans (Delchier, Ringling, Maingonnat, Rychlik, & Renard, 2014).

Oxidation is the other main mechanism described in literature for folate loss. However, studies were carried out on model solutions and do not take strictly into account oxygen measurement, thus giving contrasting results. From 49 to 100 °C, activation energy of 5-$CH_3$-$H_4$folate degradation was calculated as 39.74 kJ/mol by Chen and Cooper (1979). In the same range of temperature and in presence or absence of oxygen, Barrett and Lund (1989) calculated activation energy as 68 and 97 kJ/mol, respectively, which is in the same order of magnitude as calculated by Viberg, Jägerstad, Öste, and Sjöholm (1997) 62 kJ/mol in presence of oxygen and 106 kJ/mol in absence of oxygen. Other studies on folate stability focussed on stability in different pH conditions. Paine-Wilson and Chen (1979) showed that pH has a profound influence on the thermal stability of folates, with optimal stability in neutral conditions. For 5-$CH_3$-$H_4$folate at 100 °C, the time to decrease the initial content by half was 8.77 min at pH 7 but 3.35 and

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3.45 min at pH 4 and 10, respectively. Indrawati, Verlinde, Ottoy, Van Loey, and Hendrickx (2004) determined the degradation rate constant $k$ at 90 °C for 5-$CH_3$-$H_4$folate in citrate–phosphate buffer (pH 4) as $115.08 \times 10^{-3}$ $min^{-1}$ and in phosphate buffer (pH 7) as $68.31 \times 10^{-3}$ $min^{-1}$. Folates thus appear to be quite vulnerable to heat, especially in slightly acidic conditions.

Our previous studies on folate degradation and diffusion in vegetable matrices under atmospheric conditions showed a different evolution depending on the nature of the derivatives. Moreover, total folate evolution during thermal degradation showed a non monotonous degradation in the beginning of the reaction kinetics and a plateau. Evolution of the different folate derivatives during thermal degradation seems to be a key point in the evolution of total folate.

The present study was designed to investigate the evolution of total folate and folate derivatives during thermal degradation of spinach and green bean purees. Moreover, in order to determine the role of oxygen, reaction kinetics were performed on vegetable purees and model solutions under two oxygen conditions (absence and twice the concentration in ambient atmosphere). For that, we used an instrumented reactor where all physico-chemical parameters, and especially oxygen, were strictly controlled.

## 2. Materials and methods

### 2.1. Plant material

Purees were prepared from canned spinach (net weight 265 g; capacity 425 ml) and green beans (net weight 220 g; capacity 425 ml), bought at a local supermarket. Cans were opened and vegetables were drained by sieving. 100 ml of deionised water was added to 50 g of vegetables and ground with an ultraturax at 12,000 rpm for 1 min (S25 18G, IKA, Staufen, Germany). Spinach purees were further diluted by half with deionised water.

### 2.2. Stock solutions

Folic acid and 5-$CH_3$-$H_4$folate were obtained from Schirks labs. (Jona, Switzerland). Stock solutions were prepared in phosphate citrate buffer (pH 5; 0.1 mol/L), divided in 1 ml aliquot and stored at −20 °C until experiments. Concentrations of stock solutions were $1 \times 10^{-4}$ mol/L, both for folic acid and 5-$CH_3$-$H_4$folate.

### 2.3. Experimental device

All reaction kinetics were performed in an instrumented reactor, composed of a thermostated chamber wherein the glass reactor was put (EasyMax, Mettler Toledo, Viroflay, France), a gas diluter (Gasmix, Alytech, Juvisy sur Orge, France) and an oxygen measurement apparatus (Fibox 3 LCD Trace, Presens, Regensburg, Germany).

Stirring was carried out using a cross-shaped magnetic bar with a length of 4 cm for each arm. The reactor had a glass plug with different apertures in order to introduce the temperature probe, condenser and sampling system, composed of a capillary hermetically bonded to a 10 ml removable syringe.

Studies on folate degradation in model solutions or in purees of spinach or green beans were performed either in the presence of a gas composed of 40% of oxygen and 60% of nitrogen, giving a partial pressure of 40 kPa of oxygen, or in anaerobic conditions (under a flow of 100% of nitrogen).

For experiments in the presence of 40 kPa of oxygen, the gas mix was obtained using the gas diluter connected to nitrogen and oxygen cylinders. The associated software was used to prepare the gas mix, with an outlet flow rate of 322 ml/min. For anaerobic experiments, nitrogen flow was directly introduced from cylinder into the reactor with a flow rate of 5.0 ml/min.

In both cases, gas flow was applied into the reactor through a capillary and throughout the experiment. Oxygen concentration at the beginning, during and at the end of each reaction kinetic was determined using the oxygen sensor optical Fibox 3 LCD trace (Presens, Regensburg, Germany). This device allows a non-invasive measurement as the oxygen sensitive dye is immobilized in a sensor spot glued inside the glass reactor, and the measurement is done through the transparent wall of the glass reactor by using an optical fibre.

### 2.4. Physico-chemical parameters

Reaction kinetics were studied using the purees of spinach and green beans or solutions of 5-$CH_3$-$H_4$folate and folic acid in the 0.1 mol/L phosphate buffer pH 7 or 0.1 mol/L phosphate citrate buffer pH 5.

Two oxygen conditions were used,

(i) Under anaerobic conditions (flow of 100% of nitrogen).
(ii) In the presence of 40 kPa of oxygen.

Reaction kinetics were performed at different temperatures:

(i) 45 and 65 °C for purees in the presence of 40 kPa of oxygen.
(ii) 45, 65 and 85 °C for purees under anaerobic conditions.
(iii) 25, 45, 65 and 85 °C for folic acid and 5-$CH_3$-$H_4$folate solutions in the presence of 40 kPa of oxygen and under anaerobic conditions.

### 2.5. Reaction kinetics

Buffers or purees were firstly bubbled at room temperature until the desired oxygen content was reached. Oxygen content was followed in buffers, purees and reactor's headspace using the Fibox 3LCD trace. Then, buffers or purees were heated until the temperature setpoint was reached.

At this moment, for spinach and green bean purees, reaction kinetics were started by sampling 2 ml of purees which were directly put at −20 °C and stored until analysis. For each condition (temperature and oxygen), 2 batches were independently followed during 4 h. For folic acid and 5-$CH_3$-$H_4$folate solutions, reaction kinetics were started by adding 100 µl of stock solutions to the buffer in the reactor for a final concentration of $1 \times 10^{-7}$ mol/L, an aliquot was immediately collected and stored at −20 °C. Reaction kinetics were monitored during 3 h.

Kinetic data was adjusted using a first order kinetics, directly fitted to the raw data. First order kinetics is described below (Eq. 1):

$$C = C_0 \times e^{(-kt)} \quad (1)$$

where $C$ is the folate concentration, $C_0$ is the initial folate concentration, $k$ is the degradation rate constant and $t$ the time.

### 2.6. Analytical procedures

#### 2.6.1. Folic acid and 5-$CH_3$-$H_4$folate measurement

A solution of ascorbic acid (100 µl; 250 g/L) was added to 900 µl of samples. Solutions of 5-$CH_3$-$H_4$folate were diluted 10 times in phosphate buffer (pH 7; 0.1 mol/L) for HPLC analysis.

Folic acid and 5-$CH_3$-$H_4$folate quantification was carried out independently on a HPLC equipped with a Diode Array Detector (SPD-M-20A, Shimadzu, Kyoto, Japan) at 280 nm for folic acid and equipped with a fluorimetric detector (RF-10AXL, Shimadzu Inc.,

Kyoto, Japan) operated at an excitation wavelength of 295 nm and an emission wavelength of 356 nm for 5-$CH_3$-$H_4$folate. A LiChrospher 100RP-18 (250 × 4.5 mm; 5 μm, Altech, France) equipped with a guard column LiChrospher RP-18 All Guard (7.5 × 4.6 mm; 5 μm, Altech, France) was used for the two compounds. The mobile phase was a gradient of water with formic acid (10 ml/L) and acetonitrile HPLC grade at a flow rate of 0.8 ml/min. The injection volume was 100 μl for folic acid and 25 μl for 5-$CH_3$-$H_4$folate.

For folic acid the gradient used started at 5% of acetonitrile, increased linearly to 35% in 25 min, then to 100% in 5 min and held for 10 min, followed by a linear decrease to 5% in 5 min. Column was equilibrated during 15 min at 5% of acetonitrile. Quantification was based on an external calibration against folic acid from $1 \times 10^{-9}$ to $1 \times 10^{-6}$ mol/L ($r^2 = 0.999$).

For 5-$CH_3$-$H_4$folate the elution gradient started at 5% of acetonitrile, increased linearly to 100% in 25 min, held for 10 min, followed by linear decrease to 5% in 10 min and equilibrated for 10 min. Quantification was based on an external calibration against 5-$CH_3$-$H_4$folate monoglutamate from $1 \times 10^{-9}$ to $6 \times 10^{-8}$ mol/L ($r^2 = 0.996$).

#### 2.6.2. Measurement of folate vitamers

Individual folate vitamers were quantified by stable isotope dilution assay according to Ringling and Rychlik (2013). Briefly, before extraction, labelled standards of folate vitamers were added and all folates were deconjugated into their monoglutamate forms. Derivatives were purified on SPE SAX cartridges after adding acetonitrile (10 ml) and centrifugation. Folate analysis was carried out on an HPLC (Shimadzu Inc., Kyoto, Japan) coupled with a triple quadrupole mass spectrometer (API 4000 Q-Trap, AB-Sciex, Foster City, CA, USA). A Pro-C18 HPLC-column (150 × 3, 3 μm, 130 Å, YMC, Japan) with water plus 0.1% (v/v) formic acid (A) and acetonitrile plus 0.1% (v/v) formic acid (B) as mobile phases was used. The gradient started at 5% B. After a linear increase to 10% B and holding this condition for 5 min, another linear increase to 15% B during 10 min and to 50% B in 2 min, which was hold for 2 min followed. Within 2 min B was decreased linearly to 5% and the column was equilibrated for 9 min.

Concentration of each single vitamer in the food samples was calculated using the response factors reported recently (Ringling & Rychlik, 2013). For experimental details see Ringling and Rychlik (2013).

### 2.7. Results expression

Total folate amounts were determined by calculating the sum, in equivalent of folic acid, of folate derivatives (5-$CH_3$-$H_4$folate, 5-CHO-$H_4$folate, 10-CHO-PteGlu, folic acid, $H_4$folate, 5,10-$CH^+$-$H_4$ folate and 10-CHO-$H_2$folate).

Results are expressed as $C/C_0$ calculated as the ratio between the concentration at the end of the reaction kinetics and the initial concentration.

## 3. Results

### 3.1. Folate evolution in purees during thermal treatments under anaerobic conditions

$C/C_0$ varied from 1.12 at 45 °C to 1.09 at 85 °C for spinach and was 1.02 both at 45 and 85 °C for green beans. So, in anaerobic conditions there was no quantifiable folate degradation over 4 h neither in spinach nor in green beans, at 45, 65 and 85 °C (data not shown).

### 3.2. Folate evolution in purees during thermal treatment in the presence of 40 kPa of oxygen

#### 3.2.1. Total folate

For spinach, initial folate concentrations were relatively similar in all batches. However, for green beans, initial folate concentrations showed some variability between batches hence the expression in $C/C_0$.

For both vegetables studied, temperature had an effect on folate degradation, with a faster decrease between 0 and 60 min at 65 °C than at 45 °C (Fig. 1).

For spinach, the initial decrease with time was not monotonous. At 45 °C, folate concentrations seemed to increase during the first 5 min whereas at 65 °C, after an initial sharp decrease, an increase was observed between 10 and 30 min (Fig. 1A and B). As for spinach, folate decrease in green beans was not monotonous, at 45 °C total folate concentrations increased in the first minutes whereas at 65 °C folate concentrations increased between 10 and 30 min after a sharp decrease especially for one of the two batches (Fig. 1C and D).

For spinach, folates decreased until reaching a plateau at 90 min both at 45 and 65 °C. Irrespective of the temperature, folate concentrations in green beans decreased during the thermal treatment in the presence of 40 kPa of oxygen. A plateau was reached at 120 and 60 min at 45 and 65 °C, respectively.

Folate residual concentrations were 30% and 28% at 45 °C and 45% and 25% at 65 °C for spinach and green beans, respectively.

Folates were strongly degraded during thermal treatments in the presence of 40 kPa of oxygen, thus confirming that oxygen was involved in folate degradation.

Because of this behaviour, linearisation of total folate decrease in spinach and green beans for all temperatures by first order with partial conversion or second order law was not satisfying. To understand the causes of this unexpected behaviour, individual vitamers were analysed.

#### 3.2.2. Evolution of the different folate vitamers

*3.2.2.1. 5-$CH_3$-$H_4$folate.* 5-$CH_3$-$H_4$folate represented 70% and 75% of total folate in spinach and green beans respectively, and was the predominantly degraded vitamer both in spinach and in green beans during kinetics (Supplementary data). Degradation of 5-$CH_3$-$H_4$folate was almost complete over the 4 h of thermal treatment for both vegetables and followed a first order law with a correlation coefficient of 0.96 and 0.99 at 45 °C and 0.86 and 0.91 at 65 °C for spinach and green beans, respectively (Fig. 2). The rate constants $k$ obtained were $25 \times 10^{-3}$ and $20 \times 10^{-3}$ $min^{-1}$ at 45 °C and $70 \times 10^{-3}$ and $80 \times 10^{-3}$ $min^{-1}$ at 65 °C for spinach and green beans, respectively.

After 4 h of incubation, 4.5% of the initial 5-$CH_3$-$H_4$folate was still present in spinach, both at 45 and 65 °C, and 5.5% and 2% at 45 °C and 65 °C for green beans, respectively.

*3.2.2.2. Formyl derivatives.* Among formyl derivatives, 5-CHO-$H_4$ folate represented about 15% of total folate and 10-CHO-PteGlu about 10% both in spinach and green beans. In spinach, 5-HCO-$H_4$ folate concentrations increased in the first 30 min at 45 °C and in the first 60 min at 65 °C and then decreased to reach a plateau at 90 min for both temperatures. 88% of initial 5-CHO-$H_4$folate was still present in ground spinach after 4 h at 45 °C and 82% at 65 °C. In green beans, 5-HCO-$H_4$folate was stable during the thermal treatment, with a slight decrease (up to 30 min). Final concentrations of 5-HCO-$H_4$folate represented 96% of initial concentrations at 45 °C and 93% at 65 °C (Fig. 3).

10-HCO-PteGlu was not degraded over 4 h thermal treatments in spinach. Actually, a slight increase in concentrations over time was observed. Concentrations at the end of the thermal treatment were 111% of the initial concentration both at 45 and 65 °C. For

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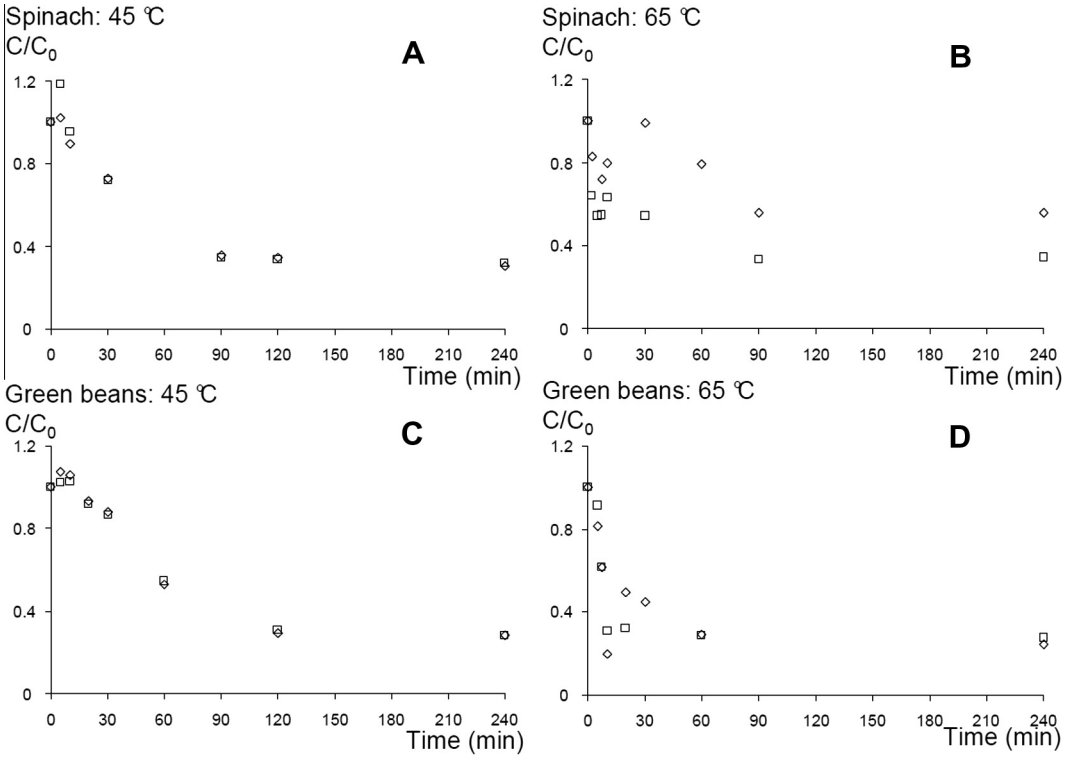


**Fig. 1.** Thermal degradation of total folate in spinach and green beans in presence of 40 kPa of oxygen at 45 and 65 °C. Thermal degradation assays of total folate represented by the ratio $C/C_0$ at 45 and 65 °C in presence of 40 kPa of oxygen are presented on the top for spinach (A and B) and on the bottom for green beans (C and D). For each temperature, $C/C_0$ of the two batches followed are presented, empty lozenge corresponding to the batch 1 and empty square to the batch 2.

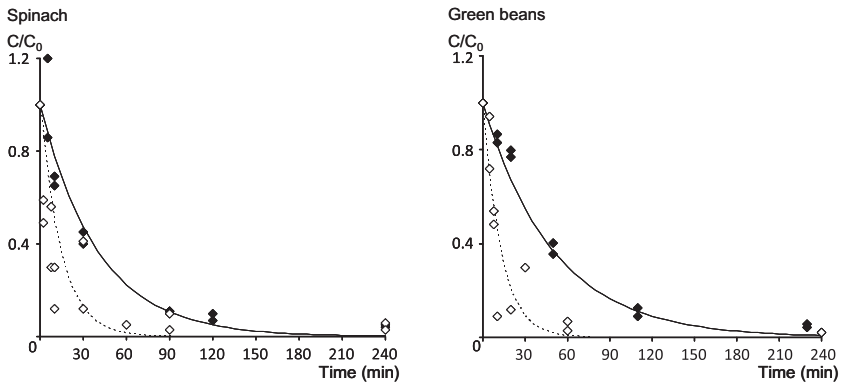


**Fig. 2.** Thermal degradation of 5-$CH_3$-$H_4$folate in spinach and green beans under 40 kPa oxygen at 45 and 65 °C. Evolution of $C/C_0$ of 5-$CH_3$-$H_4$folate under 40 kPa of oxygen and first order kinetic fitting is presented on the left for spinach and on the right for green beans, at 45 °C (full lozenges) and at 65 °C (empty lozenges). Full line represents the first order kinetic model of thermal degradation at 45 °C, dot line at 65 °C.

green beans, concentrations of 10-HCO-PteGlu increased slightly over incubation. Final concentrations were 117% of the initial concentration at 45 °C, and 108% at 65 °C (Fig. 3). Relative standard deviation for experiments at 45 and 65 °C varied from 0.01 to 0.12 for spinach and from 0.01 to 0.26 for green beans.

*3.2.2.3. Minor derivatives.* Minor derivatives in spinach and green beans (Supplementary data) were $H_4$folate; folic acid (PteGlu); 5,10-$CH^+$-$H_4$folate and 10-HCO-$H_2$folate, representing all together 5% of total folates. For these compounds, the very low and variable initial concentrations made interpretation difficult.

In spinach, behaviour of the four minor compounds was relatively different and in most cases their concentrations increased at the beginning of the thermal treatment and decreased at the end. Final concentrations of $H_4$folate were 60% of initial concentration for both 45 and 65 °C. Folic acid was relatively constant throughout the kinetics or increased slightly at 65 °C, with final concentrations of 96% at 45 °C and 111% at 65 °C. Degradation of 5,10-$CH^+$-$H_4$folate was the same for both temperatures, with residual concentrations of about 70% of initial concentration. Finally, the 10-HCO-$H_2$folate decreased during the thermal treatment, with final concentrations on average of 70% of initial concentrations at 45 °C and 45% at 65 °C.

For green beans, folic acid, 5,10-$CH^+$-$H_4$folate, $H_4$folate and 10-HCO-$H_2$folate were relatively variable throughout the kinetics but were globally quite stable during the reaction.

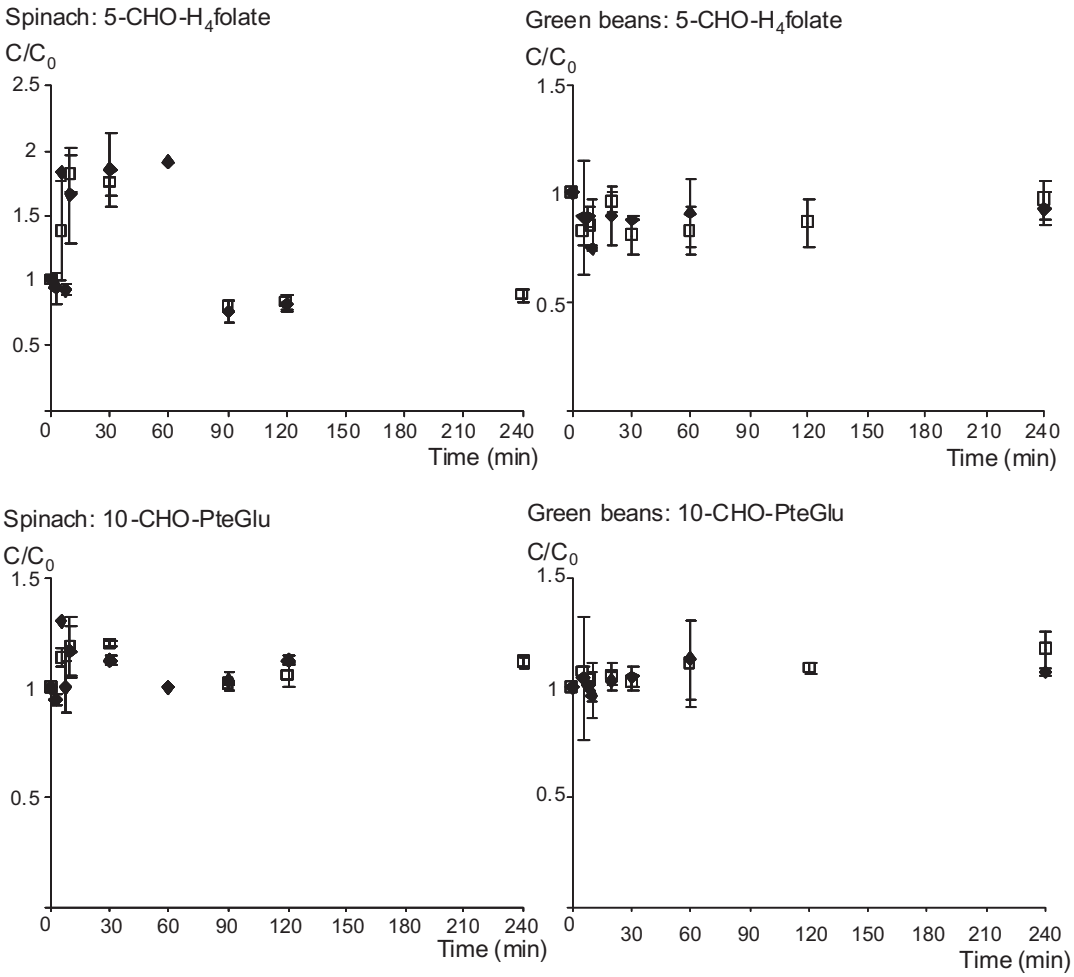


**Fig. 3.** Thermal degradation of formyl folate derivatives in spinach and green beans under 40 kPa of oxygen at 45 °C and 65 °C. Evolution of $C/C_0$ of 5-CHO-$H_4$folate is presented on the top and 10-CHO-PteGlu on the bottom of the figure for spinach (on the left) and for green beans (on the right) under 40 kPa of oxygen at 45 °C (empty squares) and at 65 °C (full lozenges).

### 3.3. Degradation in model solutions

#### 3.3.1. Folic acid

Folic acid was not degraded at 45, 65 and 85 °C under anaerobic conditions, neither at pH 5 or 7. In the presence of 40 kPa of oxygen, it was stable at pH 5 and only limited degradation (circa 10%) was observed at pH 7 over 3 h of incubation (data not shown).

#### 3.3.2. 5-$CH_3$-$H_4$folate

At pH 7 and under anaerobic conditions, no consistent behaviour was obtained for 5-$CH_3$-$H_4$folate neither at 25 °C or at 85 °C (Table 1). At pH 5, in some batches, its concentrations were stable (at 25 °C) while other showed a marked decrease, particularly rapid at 85 °C. Obviously some uncontrolled factors were still present in the experiments.

In the presence of 40 kPa of oxygen, 5-$CH_3$-$H_4$folate was degraded, both at pH 5 and 7 (Table 1). Residual concentrations of about 50% and 22% of initial concentrations were obtained after 3 h at pH 5, at 65 and 85 °C, respectively, while residual concentrations of about 5% of initial concentration were obtained at pH 7 and 85 °C after only 20 to 40 min.

Further, a neo-formed product appeared on the HPLC traces (Fig. 4), while the peak of 5-$CH_3$-$H_4$folate decreased markedly after only 2.5 min of incubation a slightly differently shaped peak reappears at the elution time after 10 min of incubation.

## 4. Discussion and conclusion

### 4.1. Impact of oxygen on folate degradation

Under anaerobic conditions in the media, folates were not degraded irrespective of the temperature applied (45, 65 or 85 °C) neither in spinach, green bean purees or in model solutions. The presence of 40 kPa of oxygen led to folate loss. Clearly oxygen was a factor for folate degradation during thermal treatment. However, the rate of folate loss was not doubled compared to our previous experiment under atmospheric (21 kPa oxygen) conditions (Delchier et al., 2014). Therefore, the exact role of oxygen (catalyst or reagent) and its stoichiometry still needs further investigation.

### 4.2. Evolution of folate derivatives during thermal treatments

Folate loss in spinach and green beans in the presence of 40 kPa of oxygen followed neither a first order nor a second order reaction as classically described in literature for folate losses during heat treatment (Barrett & Lund, 1989; Chen & Cooper, 1979; Mnkeni & Beveridge, 1983; Ruddick, Vanderstoep, & Richards, 1980; Viberg et al., 1997). The two points of this diversion were (i) the existence of a plateau at long treatment durations and (ii) an apparent and transitory increase in the early stages of thermal treatment. The same plateau was reached at the three

**Table 1**
Evolution of $C/C_0$ of 5-$CH_3$-$H_4$folate in solution, under anaerobic and 40 kPa oxygen, at pH 5 and 7, and at 25, 45, 65 or 85 °C.

| | pH | Temperature (°C) | Batch | Time (min) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 5 | 15 | 20 | 40 | 120 | 160 | 170 | 180 |
| Anaerobic | 5 | 25 | 1 | | | | | | 1.07 | | |
| | | 45 | 1 | | | | | | | | 1.06 |
| | | 65 | 1 | | | | | | | | 0.93 |
| | | 85 | 1 | | | | | | | | 1.02 |
| | 7 | 25 | 1 | | | | | 0.98 | | | |
| | | | 2 | | | | | | | | 1.21 |
| | | | 3 | | | | | | | | 0.32[a] |
| | | 85 | 1 | 0.11[a] | | | | | | | |
| | | | 2 | | 0.38[a] | | | | | | |
| | | | 3 | | | | | | | | 1.14 |
| 40 kPa oxygen | 5 | 25 | 1 | | | | | 0.90 | | | |
| | | | 2 | | | | | | | 0.85 | |
| | | 65 | 1 | | | | | | | | 0.52 |
| | | | 2 | | | | | | | | 0.51 |
| | | 85 | 1 | | | | | | | | 0.29 |
| | | | 2 | | | | | | | | 0.15 |
| | 7 | 25 | 1 | | | | | | | | 0.81 |
| | | 85 | 1 | | | | 0.05 | | | | |
| | | | 2 | | | 0.04 | | | | | |
| | | | 3 | 0.11 | | | | | | | |

[a] Unexplained result.

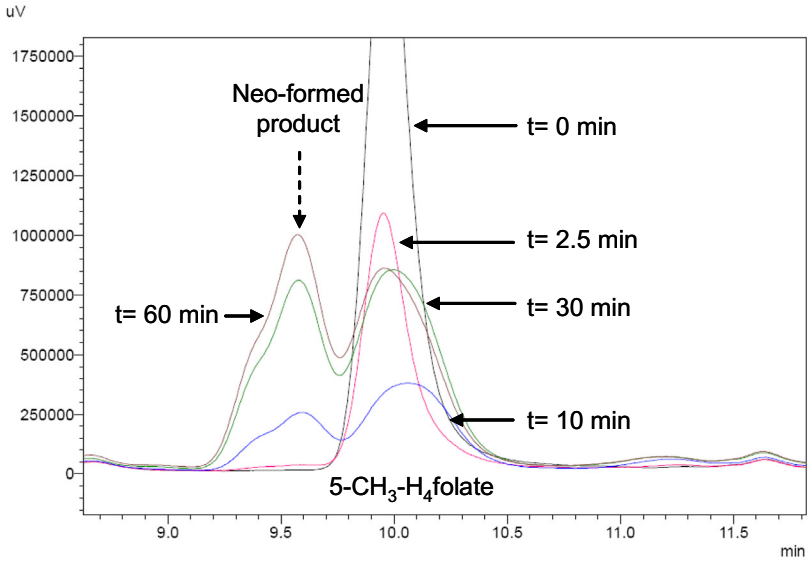


**Fig. 4.** Chromatograms of 5-$CH_3$-$H_4$folate solution over 60 min of thermal degradation under 40 kPa oxygen at 85 °C and pH 7.

temperatures used for spinach and green beans, though the initial rate of loss varied with temperature. This can be explained by the different evolution of folate derivatives, demonstrated by measuring the evolution of the different vitamers. In both vegetables, 5-$CH_3$-$H_4$folate was the main derivative, representing around 70% of total folate. Its evolution during incubation of the vegetables in the presence of 40 kPa of oxygen followed first order kinetics, in agreement with data obtained in literature for folates (Barrett & Lund, 1989; Chen & Cooper, 1979; Mnkeni & Beveridge, 1983; Oey, Verlinde, Hendrickx, & Van Loey, 2006; Ruddick et al., 1980). The rate constants were close for both vegetables, and comparable to those found in the literature: $110 \times 10^{-3}$ $min^{-1}$ at 100 °C (Paine-Wilson & Chen, 1979) $72 \times 10^{-3}$ $min^{-1}$ at 100 °C (Mnkeni & Beveridge, 1983), $47 \times 10^{-3}$ $min^{-1}$ at 60 °C for the (6*S*) enantiomer (Oey et al., 2006). However, the evolution of other derivatives, such as 5-HCO-$H_4$folate and 10-HCO-PteGlu, which represent 15 and 10% of total folate in spinach as well as 14 and 8% of total folate in green beans respectively, was different. Those two compounds were stable, with a slight decrease at the end of the incubation for 5-HCO-$H_4$folate and increase for 10-HCO-PteGlu. Moreover, minor compounds (folic acid, 5, 10-$CH^+$-$H_4$folate, 10-HCO-$H_2$folate and $H_4$folate) were globally stable during incubation over 3 h at 45–85 °C in the presence of 40 kPa of oxygen. Therefore, the stability of formyl and minor vitamers was responsible for the plateau observed during thermal treatments, whilst the decrease in total folate, in the beginning of the kinetics, was due to the degradation of 5-$CH_3$-$H_4$folate.

### *4.3. Folate degradation in model solutions*

To confirm this observation, two vitamers of contrasting behaviour, one highly degraded in vegetables purees (5-$CH_3$-$H_4$folate), and the other relatively stable (folic acid) were studied in solution at two different pH (5 and 7) and at temperatures from 25 to 85 °C. As the plateau of folate degradation in purees was reached in less

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than 3 h, the reaction kinetics of the 2 vitamers was followed only over 3 h.

Folic acid was stable in all conditions tested (temperature, pH and oxygen content), in agreement with O'Broin et al. (1975) and Paine-Wilson & Chen, 1979. 5-$CH_3$-$H_4$folate was stable at pH 5, when the medium is deprived of oxygen, and regardless of the temperature, again confirming the literature (Barrett & Lund, 1989; O'Broin et al., 1975; Paine-Wilson & Chen, 1979; Viberg et al., 1997). Marked degradation was observed for 5-$CH_3$-$H_4$folate at pH 5 and 7 and in the presence of 40 kPa of oxygen, particularly at high temperatures. Under these conditions, its degradation was more rapid than in the vegetables, which could be due to a protective effect of the plant matrices. One of the degradation products which we obtain was slightly more hydrophilic than 5-$CH_3$-$H_4$ folate (earlier elution on C18 column) but with similar fluorescence properties.

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