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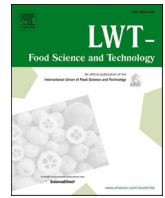
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Relative role of leaching and chemical degradation in the loss of water-soluble vitamins C and B9 from frozen vegetables cooked in water

Catherine M.G.C. Renard^{a,b}, Hanna Brick^a, Jean-Francois Maingonnat^a, Claus Kadelka^c, Nicolas Delchier^{d,e,*}

^a UMR SQPOV, INRAE, Avignon University, F-84000, Avignon, France

^b TRANSFORM, INRAE, F-44300, Nantes, France

^c Department of Mathematics, Iowa State University, Ames, IA, 50011, United States

^d Chair of Analytical Food Chemistry, Technische Universität München, Alte Akademie 10, D-85354, Freising, Germany

^e Department of Food Science and Human Nutrition, Iowa State University, Ames, IA, 50011, United States

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ABSTRACT

Losses of vitamins C and folates due to chemical degradation and leaching were modelled in green peas and Brussels sprouts. Vegetables were treated at four temperatures from 25 to 85 °C. They were directly placed in water (diffusion and thermal degradation) or hermetically sealed bags (only thermal degradation). Chemical degradation was modelled according to a pseudo-first-order and leaching according to Ficks' second law. While in contact with water, losses of vitamins appeared to be ten times higher for vitamin C and two times for folates. In both vegetables, vitamin C diffused faster than folates, and the chemical degradation was faster in peas than in Brussels sprouts. For both vitamins, increasing of the temperature augmented the degradation speed and, to a lesser extent, the leaching. Chemical degradation of folates plateaued at approximately 53% and 63% of initial contents for pea and Brussels sprouts, respectively. The relative importance of degradation and leaching changed with vegetable size and temperature. In peas, leaching was the faster mechanism and notably predominant at low temperatures, probably due to the smaller size of the vegetable. An accurate understanding of vitamin loss requires simultaneous consideration of the two mechanisms studied here, degradation and leaching, which have different parameters.

1. Introduction

Fruits and vegetables are known to provide health benefits, as they contribute to an essential source of fibres, minerals, and vitamins, including vitamin C (L-(+)-ascorbic acid and dehydroascorbic acid), folates (vitamin B9) and provitamin A (α - and β -carotene, β -cryptoxanthin) (Agte, Tarwadi, Mengale, Hinge, & Chiplonkar, 2002; Mahima et al., 2013). There is a consensus that micronutrients and other bioactive compounds from fruits and vegetables can prevent the development of chronic diseases such as cancer and heart disease (Plumb et al., 1996).

Fruits and vegetables are commonly consumed after industrial or domestic processing (Agte et al., 2002). As cooking causes considerable vitamins loss, information on the vitamin content of processed foods is essential for assessing the adequacy of vitamin intakes. While vitamin C and folates exhibit higher stabilities due to increased antioxidant

content, are susceptible to heat, pH, pressure, temperature, and oxygen (Barrett & Lloyd, 2012; Chen & Cooper, 1979; Delchier, Reich, & Renard, 2012; Delchier, Herbig, Rychlik, & Renard, 2016; Demiray, Tulek, & Yilmaz, 2013; Deutsch, 1997; IndrawatiArroqui, Messagie, Nguyen, Van Loey, & Hendrickx, 2004a; IndrawatiVerlinde et al., 2004; Laing, Schlueter, & Labuza, 1978; Li, Alli, & Kermasha, 1989; Liu et al., 2012; Mieszczakowska-Fraç, Celejewska, & Plochanski, 2021; Oey, Verlinde, Hendrickx, & Van Loey, 2006; Ruddick, Vanderstoep, & Richards, 1980; Viberg, Jägerstad, Öste, & Sjöholm, 1997). Thus, it is crucial to understand how processing affects fruits, vegetables, and their vitamin contents.

Most studies on the degradation of vitamin C and folates in fruits and vegetables were conducted on single processing operations, demonstrating the high impact of heat treatment (Delchier et al., 2016; Gianakourou & Taoukis, 2021). Leaching has been recognised for years as a factor in increasing vitamin C losses (Lathrop & Leung, 1980). Delchier

* Corresponding author. Iowa State University, Department of Food Science and Human Nutrition, 536 Farm House lane, Ames, IA, 50011, United States.

E-mail address: delchier@iastate.edu (N. Delchier).

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et al. (2013) also observed a 20% folate loss due to leaching in green beans during the canning process. This result was confirmed by Bureau et al. (2015), who assessed the effect of domestic cooking of frozen vegetables on folate losses. These authors also found indications of losses caused by water-soluble vitamins diffusion in all procedures tested.

Interestingly, cooking in water consistently led to the most significant losses (compared to steaming, microwave, or pressure-cooker). These losses are accompanied by attenuated dry matter, which is more marked in vegetables with a high surface/volume ratio. Islam, Liu, Jiang, Zhang, and Liang (2021) obtained similar results when comparing losses of folates in fresh corn either after boiling (with an average retention of 45% for sweet corn cultivars and 65% for waxy cultivars) or steaming (77% and 98% average retention, respectively for the two cultivars) or microwaving (80% and 90% average retention, respectively for the two cultivars).

Surprisingly, studies that explicitly model the loss of vitamins in vegetables by both degradation and leaching are scarce in the scientific literature despite evidence that leaching may be responsible for more vitamin loss than degradation at the beginning of cooking (Delchier et al., 2016). The first models studying the diffusion of folates were developed by Delchier, Ringling, Maingonnat, Rychlik, and Renard (2014), where folate diffusivity constants were determined for green beans and spinach. They determined the impact of the food matrix (green beans vs. spinach) on folate diffusivity, with the highest one for green beans. The temperature did not greatly impact the diffusivity constant, but it seemed to affect the residual vitamin concentration. Thermal degradation, however, was not differentiated in this study. More recently, Coffigniez et al. (2019) used a multi-response modelling approach to assess the folate evolution in cowpea during soaking at different temperatures. They reported a predominance of diffusion at 60 °C and 95 °C, while at 30 °C, enzymatic interconversion was predominant. After 1 h at 60 °C or 30 min at 95 °C, about 55% of the initial content of 5-methyltetrahydrofolate (5-CH₃-H₄ folate) remained in the cowpea seeds. While thermal degradation during processing is well established (Giannakourou & Taoukis, 2021; Mieszczakowska-Frać et al., 2021), and it has been known for over 40 years that diffusion is responsible for rapid vitamin C loss (Lathrop & Leung, 1980), data and models related to its diffusion remain limited.

Hence, when cooking vegetables in water, two loss mechanisms co-occur for water-soluble vitamins: chemical degradation and leaching into the surrounding cooking water (Delchier et al., 2016). This work aims to assess and integrate the respective importance of these two physicochemical mechanisms, which has never been done as far as we could ascertain. For this, vitamin content during cooking will be assessed in two experiments, one that only allows degradation (heating in hermetically closed bags) and another that allows degradation and leaching (diffusion in a large excess of water). Both experiments were performed at the same temperatures to determine the net contribution of the diffusion in the overall vitamin loss (diffusion and degradation). Vitamin loss in peas (*Pisum sativum* L.) and Brussels sprouts (*Brassica oleracea* L. var. gemmifera Zenker) will be determined. These vegetables were chosen for their relatively high vitamin C and folate contents and spherical shapes, enabling relatively simple diffusion modelling using Fick's second law.

2. Materials and methods

2.1. Chemicals

L-(+)-ascorbic acid, L-dehydroascorbate, formic acid, trichloroacetic acid (TCA), and MES buffer were provided by Sigma-Aldrich (Steinheim, Germany). Acetonitrile HiPerSolv for HPLC from VWR (Leuven, Belgium). D,L-Dithiothreitol was from Fluka (St. Quentin Fallavier, France).

Folate conjugase from chicken pancreas was obtained from PatriCell (London, UK) or from Pel-Freez Biologicals (Rogers, USA), Rat serum

from Biozol (Eching, Germany), and folate binding protein (FBP) from Scripps Labs (San Diego, CA-USA).

Folic acid; (6*R,S*)-5-formyl-5,6,7,8-tetrahydrofolic acid, calcium salt (5-CHO-H₄ folate); (6*R,S*)-5-methyl-5,6,7,8-tetrahydrofolic acid calcium salt (5-CH₃-H₄ folate); (6*S*)-5,6,7,8-tetrahydrofolic acid (H₄ folate); pteroyldi-γ-L-glutamic acid were obtained from Schircks labs (Jona, Switzerland). [¹³C₅]-5-CH₃-H₄ folate, [¹³C₅]-5-HCO-H₄ folate, [¹³C₅]-PteGlu were from Merck (Eprova, Schaffhausen, Switzerland). The isotopically labelled folates [²H₄]-5-CH₃-H₄folate, [²H₄]-5-HCO-H₄folate, [²H₄]-H₄folate and [²H₄]-PteGlu were synthesised as reported by Freisleben, Schieberle, and Rychlik (2002).

2.2. Plant material and samples preparation

Frozen peas (*Pisum sativum* L.) and Brussels sprouts (*Brassica oleracea* L. var. Gemmifera Zenker) were bought at a local supermarket (Auchan, Avignon, France) in bags of 1 kg each, and stored at -20 °C until experiments. Vegetables were steamed with a SEB Vitacuisine Compact cooker (SEB, Selongey, France) before experimentation to ensure endogenous enzyme inactivation (potentially leading to vitamin degradation) and produce disrupted material amenable to leaching. Brussels sprouts were steamed for 25 min and peas for 18 min (typical times for domestic preparation). The vegetables were then directly cooled in an ice bath for 5 min and drained before experimentation.

In the present study, there were three reasons for using frozen material that was thawed by steaming:

- Focus of the work: we wanted to assess the physicochemical mechanisms (chemical degradation, diffusion) that may occur during cooking, hence eliminating phenomena that may occur at the start of processing [e.g., enzymatic activity (degradation or conversion) or membrane compartmentation], which would introduce bias
- Limit raw material variability (e.g., variety, pedoclimatic conditions and post-harvest)
- Convenience for executing the experiments.

Thermal degradation kinetics were performed for 300 min with few exceptions (Supplementary Table 1), by placing 50 g of steamed peas or 400 g of steamed Brussels sprouts into vacuum-sealed plastic bags (low-density polyethylene bags, 100 μm thickness, Toutemba, Octeville-sur-mer, France) to avoid the leaching of the vitamins. Diffusion kinetics were performed by placing 50 g of steamed peas or 400 g of steamed Brussels sprouts into a 1 mm mesh cotton bag. Bags (hermetic for the thermal degradation or mesh for the diffusion) were then immersed into a 15 L water bath set up at four different temperatures (25, 45, 65 and 85 °C) for the kinetic time course (Supplementary Table 1). All experiments were performed in aerobic conditions. Two full replicates (different bags on different days) were carried out for each vegetables, temperature, and condition. For each time point, samples were transferred into an air-blast freezer (4 m/s), ground in liquid nitrogen, and stored at -80 °C.

2.3. Analytical

2.3.1. Total folates

Total folate content was determined by HPLC coupled to fluorimetric detection using the conversion procedure of Ndaw, Bergaentzlé, Aoudé-Werner, Lahély, and Hasselmann (2001) and adapted by Delchier et al. (2012). In brief, folates were extracted from homogenised ground samples, with phosphate buffer 0.1 mol/L, pH 7, with 0.1 g/L of ascorbic acid, for 10 min at 100 °C. Glutamate chains were deconjugated using a chicken pancreas carboxypeptidase. Then, all derivatives were converted into 5-CH₃-H₄ folate utilising a combination of reduction and methylation described by Ndaw et al. (2001). Finally, folates were purified by affinity chromatography using an FBP. An HPLC coupled to a fluorimeter (λ_{EX} = 295 nm and λ_{EM} = 356 nm; RF-10AXL, Shimadzu Inc.,

Kyoto, Japan) was utilized for folate quantification. The injection system was set at 4 °C, and folates were separated on a Lichrospher 100RP-18 column (250 × 4.6 mm I.D.; 5 µm particle size; Alltech, Epemon, France) and guarded by a precolumn (Lichrospher 7.5 × 4.6 mm, Alltech, Epemon, France). Mobile phases consisted of ultrapure water with 1 mL/L formic acid (A) and acetonitrile (B). The gradient started with 5% B, linearly increased to 58% B within 14 min, increased to 100% B within 3 min and held at 100% B for 11 min. At the end of the run, the solution was decreased to 5% B within 2 min and kept for 5 min. The flow rate was 0.8 mL/min, the oven temperature was 30 °C, and the injection volume was 25 µL. Quantification was performed with external calibration against 5-CH₃-H₄ folate monoglutamate and 5-CH₃-H₄folate diglutamate obtained from the reduction of pteroyl-diglutamate, and total folates expressed as 5-CH₃-H₄folate calculated using the respective molecular weights.

2.3.2. Folate vitamers

Folate vitamers were quantified by the method adapted from Ringling and Rychlik (2013). Briefly, samples were extracted with MES buffer at 100 °C for 10 min and cooled in an ice bath for 15 min. Folate deconjugation was carried out, overnight, using a chicken pancreas solution and rat serum, at 37 °C. Purification was performed on SPE SAX cartridges (500 mg, 3 mL, Phenomenex, Torrance, USA). Vitamers were HPLC separated (Shimadzu Inc., Kyoto, Japan) using a YMC Pack Pro C18 (150 × 3 mm; 3 µm, YMC America Inc.; Allenton, USA) and detected with a triple quadrupole mass spectrometer (API 4000 Q-Trap, AB-Sciex, Foster City, CA, USA). The mobile phase consisted of variable mixtures of 10 mL/L aqueous formic acid (A) and acetonitrile HPLC grade (B) acidified with formic acid (10 mL/L), with a flow rate of 0.2 mL/min. The elution gradient was started at 5% B and increased to 10% B in 5 min. The gradient was held at 10% B for 5 min, increased linearly to 15% B in 10 min and then to 50% B in 2 min. The composition remained constant for 2 min before being reduced to 5% B in 2 min. Finally, the gradient was held at 5% B for 9 min. The injection volume was 10 µL and the column temperature was 30 °C. During the first 5 min of the gradient programme, the column effluent was diverted to the waste reservoir. The spectrometer was operated using Multiple Reaction Monitoring (MRM) in the positive electrospray ionization mode (Ringling & Rychlik, 2013). The ionization voltage was set to 5500 V, the nebulizer gas pressure was 75 psi, auxiliary gas pressure was 75 psi, and the curtain gas pressure was 20 psi. The temperature of the heater was set to 550 °C.

2.3.3. Vitamin C

The method developed by Stevens, Buret, Garchery, Carretero, and Causse (2006) was used to measure vitamin C concentrations. Briefly, vitamin C was extracted with 0.6 mL of TCA (60 g/L) from 250 mg of Brussels sprouts or 500 mg of peas and centrifuged at 9000×g (1K15; Bioblock Scientific, Illkirch, France) and 4 °C for 15 min. The extract was transferred to 1.5 mL tubes and maintained on ice. Total vitamin C was determined by transferring an aliquot (20 µL) to a 96-well plate. The sample was reduced, for 20 min at 37 °C in the dark, by 20 µL of D, L-dithiothreitol (5 mmol/L in phosphate buffer; pH 7.4). The excess of D,L-dithiothreitol was eliminated by adding 10 µL of a 5 g/L N-ethyl maleimide solution. Ascorbic acid was quantified using a second aliquot, diluted in 30 µL of phosphate buffer. All aliquots were further derivatised for 40 min at 37 °C by 80 µL of an extemporaneously 2,2'-bipyridine solution. The 2,2'-bipyridine solution was prepared by mixing 6.6 mL of solution A (263 g/L orthophosphoric acid, 46 g/L TCA, 6 g/L FeCl₃) and 2.4 mL of solution B (40 g/L 2,2'-bipyridine in 700 mL/L ethanol). Adducts were detected at 525 nm using a microplate reader (Safas Xenius, Monaco). For each sample, two analytical replicates were carried out, and quantification was performed by external calibration against a standard curve of L-ascorbic acid (from 0 to 2.5 g/L). Dehydroascorbic acid content was determined by calculating the difference between the total vitamin C and the ascorbic acid content.

2.4. Theoretical considerations

2.4.1. Reaction kinetics

Since Al Fata, Geogé, André, and Renard (2017) showed that a temperature over 100 °C is needed for vitamin C dehydration to become significant, the oxidation-reductive pathway was only considered. Moreover, the oxygen concentration was assumed constant at each temperature and therefore, not explicitly integrated into the model. Thus, a pseudo-first-order kinetic was assumed for vitamin C degradation. Folate degradation was modelled using the same first-order kinetics equation (Eq. 1), as already Delchier et al. (2016) described.

$$\text{First-order kinetics: } C_t = C_0 \cdot \exp(-k(T) \cdot t) \quad (\text{Eq. 1})$$

where C_t is the concentration at time t in seconds, $C_0=1$ is the initial (normalised) concentration, and $k=k(T)$ is the rate constant, which depends on the temperature T .

Activation energy was determined according to the Arrhenius law:

$$k = e^{Ea/RT} \quad (\text{Eq. 2})$$

where k is the rate constant, Ea is the activation energy, R is the ideal gas constant, and T is the temperature.

2.4.2. Diffusion

Brussels sprouts and peas were chosen due to their spherical shape, simplifying the integration of Fick's second law. The general equation of Fick's second law is the following:

$$\frac{\partial C}{\partial t} = -D(T) \frac{\partial^2 C}{\partial r^2} \quad (\text{Eq. 3})$$

where $C=C(r,t)$ is the concentration of the molecule under consideration at time t (in seconds) and at distance r (in meters) from the centre of the sphere, and $D = D(T)$ is the diffusion constant, which depends on the temperature T .

The solution of Equation (3) for spheres is given by (Crank, 1975), assuming an initially uniform distribution of the molecule in the plant material:

$$C_t = C_{eq} + (C_0 - C_{eq}) \frac{6}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{n^2} \exp\left(-\frac{D(T)\pi^2 n^2 t}{a^2}\right) \quad (\text{Eq. 4})$$

Here, C_{eq} is the equilibrium concentration, which would be reached if the molecules were present at the same concentration in all compartments, and a is the radius of the sphere in metres. This radius was determined as the average of 20 measurements performed with a calliper for both vegetables. For Brussels sprouts, $\alpha = 12.5$ mm, while for peas, $\alpha = 2.5$ mm.

As degradation and diffusion happen at the same time, we solved a diffusion equation with first-order degradation:

$$\frac{\partial C}{\partial t} = -D(T) \frac{\partial^2 C}{\partial r^2} - k(T)C \quad (\text{Eq. 5})$$

Similar to Crank (1975), the solution is given by the following equation:

$$C_t = \exp(-k(T)t) * \left[C_{eq} + (C_0 - C_{eq}) \frac{6}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{n^2} \exp\left(-\frac{D(T)\pi^2 n^2 t}{a^2}\right) \right] \quad (\text{Eq.6})$$

The sum in Equation (6) was developed up to $n = 100$, and the equation contains both the rate constant $k(T)$ and the diffusion constant $D(T)$. Fitting both constants simultaneously is not feasible due to their high correlation (linked to the acceleration of degradation and diffusion with increased temperature) and the limited number of data points. Fig. 1 illustrates the collinearity issue by evaluating Equation 6 for different $k(T)$ and $D(T)$ values at a randomly chosen fixed time point of t

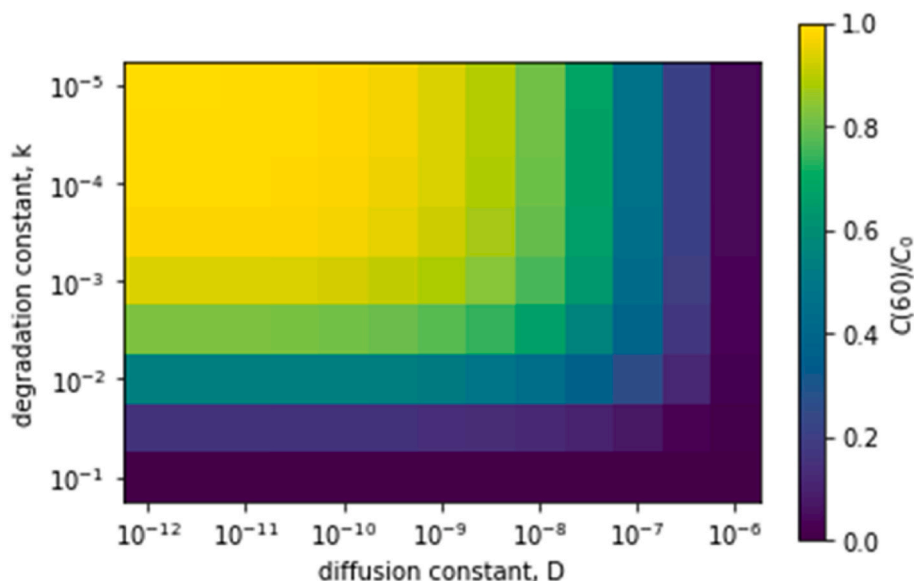


Fig. 1. Model-predicted concentration (Equation 6), relative to the initial concentration C_0 , for different values of the two collinear parameters, the rate constant k and the diffusion constant D .

= 60. The solution (Equation 6) decreases as $k(T)$ increases and as $D(T)$ increases. For this reason, one can find many good fits to a time series with low sample size where the specific choices for $k(T)$ and $D(T)$ vary widely between the fits. In this study, we are particularly interested in the specific $k(T)$ and $D(T)$ values and not best fit for predictive purposes. Therefore, it is not prudent to fit two collinear parameters simultaneously. While the fit would be good (i.e., marginally better than just fitting one at a time), the results would be uninterpretable. Therefore, we opted to run two separate experiments, one with thermal degradation only and one that allows additional diffusion. From the first experiment, we obtained best fit values for the rate constants $k(T)$ following the modelling described in 2.4.1 (using the geometric mean across duplicates). These estimated rate constants are then assumed as the ground truth when deriving the best fit value for $D(T)$ from the second experiment.

2.5. Statistics

All degradation and diffusion experiments were carried out in duplicates. Since models are the topic of this article, no inference testing was used in time curves, and we chose to present all individual points instead. Model fitting was performed using Python 3.8.8 and minimising the sum of squares of the differences between predicted and observed concentrations C_t . Given the nonlinear nature of the models and the slightly varying number of observations per time series, the goodness of fit was evaluated using mean squared errors (MSE in Table 2). We averaged the rate constants, $k(T)$ and $D(T)$, using the geometric mean across duplicates.

Our data were analysed using the good practices for data

Table 1

Individual folate vitamers contents (mg/kg FW) in four batches of the initial pea and Brussels sprouts (mg/kg FW) used for diffusion experiments.

Samples	5-CH ₃ -H ₄ folate	5-CHO-H ₄ folate	Folic Acid	H ₄ folate	Sum
Peas	557 ± 185	68 ± 6	32 ± 9	31 ± 5	689 ± 187
Brussels sprouts	499 ± 242	262 ± 118	37 ± 32	47 ± 20	842 ± 379
P-value	0.04	0.01	0.65	0.13	0.17

P-value for difference between the two vegetables.

presentation outlined by Renard (2021). Dispersion of vitamin content in the raw material is described using boxplots (16 replicates), providing richer and more accurate information than reporting the mean and standard deviation. Individual folate vitamers were analysed in two to four replicates in selected points of the time curves and are presented as mean and standard deviation. Using Excel, two-way ANOVA was carried out to identify relative impact of treatment (thermal or thermal plus diffusion) and temperature on the evolution of ascorbic acid and total folates using Excel.

3. Results and discussion

3.1. Variability in initial concentrations of vitamin C and folates

Initial ($t = 0$) vitamin C (ascorbic acid and dehydroascorbic acid) and total folate concentrations were measured in the peas and Brussels sprouts for each kinetic experiment (16 samples per vegetable) at four temperatures, and are presented in Fig. 2. Average vitamin C concentrations were 165 mg/kg in peas and 683 mg/kg in Brussels sprouts. In peas, a relatively high proportion of dehydroascorbic acid could be observed (about one-third of all vitamin C). Total folate concentrations were on average 687 and 495 $\mu\text{g}/\text{kg}$ 5-CH₃-H₄ folate equivalents in peas and Brussels sprouts, respectively.

Four biological replicates were analysed for each vegetables. High variabilities were observed for the 5-CH₃-H₄ folate content in peas, and all vitamers in Brussels sprouts, in line with the total folate determination. The main folate vitamer, in peas and Brussels sprouts was 5-CH₃-H₄ folate, representing respectively about 80% and 58% of all detected folate vitamers (Table 1). The second most abundant vitamer was 5-CHO-H₄ folate, which was more abundant in Brussels sprouts representing almost one-third of folate vitamers. H₄ folate accounted for about 5% of total folates in both vegetables, while folic acid had similar average concentrations (no significant difference between species for folic acid and H₄ folate). The distribution of the different folate vitamers agrees with previous studies reviewed by Delchier et al. (2016). The sum of individual folates, determined by LC-MS, was slightly higher than the total folate content measured after conversion into 5-CH₃-H₄ folate, as expected due to a nonstoichiometric conversion of some vitamers (Ndaw et al., 2001).

Concentrations for both vitamin C and folates varied by a factor of two, although all samples presumably came from a single industrial

Table 2

Total folate and vitamin C residual content (percentage of the relative to initial content) in peas and Brussels sprouts of thermal degradation or diffusion kinetics; data are after 180 min for peas (or last point where the concentration was not below detection level) and 240 min for Brussel sprouts.

Samples	Temperature (°C)	Vitamin C		Folates	
		Thermal	Diffusion	Thermal	Diffusion
Peas	25	83 ± 7	2 ± 2	92 ± 20	61 ± 2 ^a
	45	66 ± 4	1.0 ± 0.2°	77 ± 14	29 ± 4
	65	49 ± 7	4 ± 2 ^b	61 ± 1	9 ± 2
	85	19 ± 3	3 ± 2 ^b	62 ± 6	3.0 ± 0.5
Brussels sprouts	25	93 ± 6	75 ± 7	92 ± 35	65 ± 9
	45	93 ± 4	69 ± 1	92 ± 3	56 ± 3
	65	75 ± 1	43.0 ± 0.5	102 ± 28	43 ± 5
	85	72 ± 2	40 ± 2	73 ± 11	43 ± 3
		Vitamin C		Folates	
		Pea	Brussel sprouts	Pea	Brussel sprouts
F-value thermal versus diffusion		295	103	55	13
P-value		<0.0001	<0.0001	<0.0001	0.007
F-value temperature		20	32	10	0.7
P-value		0.0004	<0.0001	0.004	NS
F-value interaction		21	1.7	0.86	0.6
P-value		0.0004	NS	NS	NS

^a residual content determined after 180 min; [°] residual content determined after 120 min and.

^b Residual content determined after 60 min; data are average of two experimental replicates and analytical duplicates.

process of vegetables (same brand, same batch number), and relatively large batches were used in our study (400 g of Brussels sprouts, 50 g of peas, i.e., about 100 peas). The relative abundance of dehydroascorbic acid in peas was surprising and likely indicates that measuring only ascorbic acid may significantly underestimate of the vitamin C content. Folate content showed higher variability in Brussels sprouts than in peas, highlighting the influence of the matrix on folate content. This variation in the vitamin content of foods has also been reported by Loznjak, Striegel, Díaz De la Garza, Rychlik, and Jakobsen (2020), though employing a limited number of samples (1–4) for each food, and Upadhyaya et al. (2017) for ripe tomatoes (from 14 to 46 µg/100 g fresh weight in 100 accessions).

Finally, diffusion of the different water-soluble nutrients is not possible from raw matrices due to their intact structures and all their controlled transport across cell membranes. Freezing partially disrupts the organisation of the plant tissue enabling the diffusion of the different water-soluble compounds. However, no diffusion can occur in the frozen state. Thermal treatment is required for the reliable elimination of endogenous enzymes (in a preliminary experiment some browning was observed in non-steamed material at 25 °C) and of cell membranes so that the vitamins could diffuse freely. Steaming appeared to be the most efficient process to defrost the plant material for our study due to its low impact on the degradation of the vitamins, about 10% for vitamin C and no significant loss for folate (Bureau et al., 2015).

We do not think over-processing occurred, as related to modelling, and the products were not meant for human consumption. The vegetables stayed coherent throughout the time series; the range of temperatures (up to 85 °C) was chosen to avoid the loss of individual leaves in Brussels sprouts. Indeed, despite the different treatments already endured by the vegetables before the kinetics, concentrations (C_0) determined were in good agreement with previous studies. Reported vitamin C contents were, on average, 16 mg/100 g in boiled peas, 12 mg/100 g in canned peas, and 18.5 mg/100 g and 60 mg/100 g after blanching or boiling Brussels sprouts (Lathrop & Leung, 1980; Selman, 1994; Souci, Fachmann, & Kraut, 2000). Similarly, total folate content in frozen green peas varied between 680 and 970 µg/kg, depending on the sample and quantification method, 270 µg/kg in boiled peas, and between 0.950 and 1.36 mg/kg in boiled Brussels sprouts (Leichter, Landymore, & Krundieck, 1979; Selman, 1994; Westenbrink, Jansen-van der Vliet, & van Rossum, 2012). Industrial blanching is performed to eliminate of the interference of enzyme activity (Coffigniez et al., 2019; Leichter et al., 1979).

However, to confirm that the treatments (industrial and steaming)

carried out prior to the different kinetics experiments have no impact on the diffusion and the thermal degradation constants of the vitamins, similar kinetics should be performed from steamed raw material.

3.2. Thermal degradation kinetics

Thermal degradation was carried out on samples wrapped in a plastic bag (not allowing any diffusion) at four temperatures. As shown in Fig. 3, vitamin C and folate concentrations were measured at several time points (specific times for each kinetics are presented in Supplementary Table 1). The residual concentrations at the end of the kinetics or when reaching the plateau are presented in Table 2. Although the exact mechanisms of vitamin C and folate degradation are complex, the interconversion between the different vitamers, the role of oxygen, nor antioxidant-mediated folate protection were not evaluated here.

Higher temperatures led to faster vitamin C degradation in peas (Fig. 3). Residual content after 240 min varied from 83% at 25 °C to 19% of the initial content at 85 °C (Table 2). Folate degradation in peas was slower (Fig. 3), with about 40% lost after 240 min at 65 °C and 85 °C (Table 2). In Brussels sprouts, the degradation of both vitamins was reduced (Fig. 3). Substantial degradation was only observed at 85 °C for folates (27% loss after 240 min), and at both 65 °C (25% loss) and 85 °C (28% loss) for vitamin C. Except for vitamin C in peas (45, 65 and 85 °C), the vitamins were surprisingly not highly degraded under the conditions tested. The impact of the plant matrix might be involved in vitamin protection during the heat treatment, as proposed by Capuano, Oliviero, and van Boekel (2018).

As Van Boekel (2008) described, reliable degradation modelling using a first-order reaction law requires at least 70% loss. Kinetic data do not favour using a first-order reaction law for the thermal degradation of the two vitamins, which exhibit a limited degradation level (Table 2). The second data modelling approach would have been to use a pseudo-first-order with a plateau, as done by Delchier et al. (2014). However, the data did not suggest the appearance of a plateau during the thermal degradation kinetics. Therefore, we utilised a first-order modelling approach for the thermal degradation of the vitamins, as reported previously (Delchier et al., 2014; Lima, Elizondo, & Bohuon, 2010; Oey et al., 2006; Ruddick et al., 1980; Van den Broeck, Ludi-khuyze, Weemaes, Van Loey, & Hendrickx, 1998; Verbeyst, Bogaerts, Van der Plancken, Hendrickx, & Van Loey, 2013; Verliende, Oey, Deborggraeve, Hendrickx, & Van Loey, 2009), even if this has some limitations regarding our data. Results for both vitamin C and folate in peas and Brussels sprouts are shown in Fig. 3 and the associated kinetic

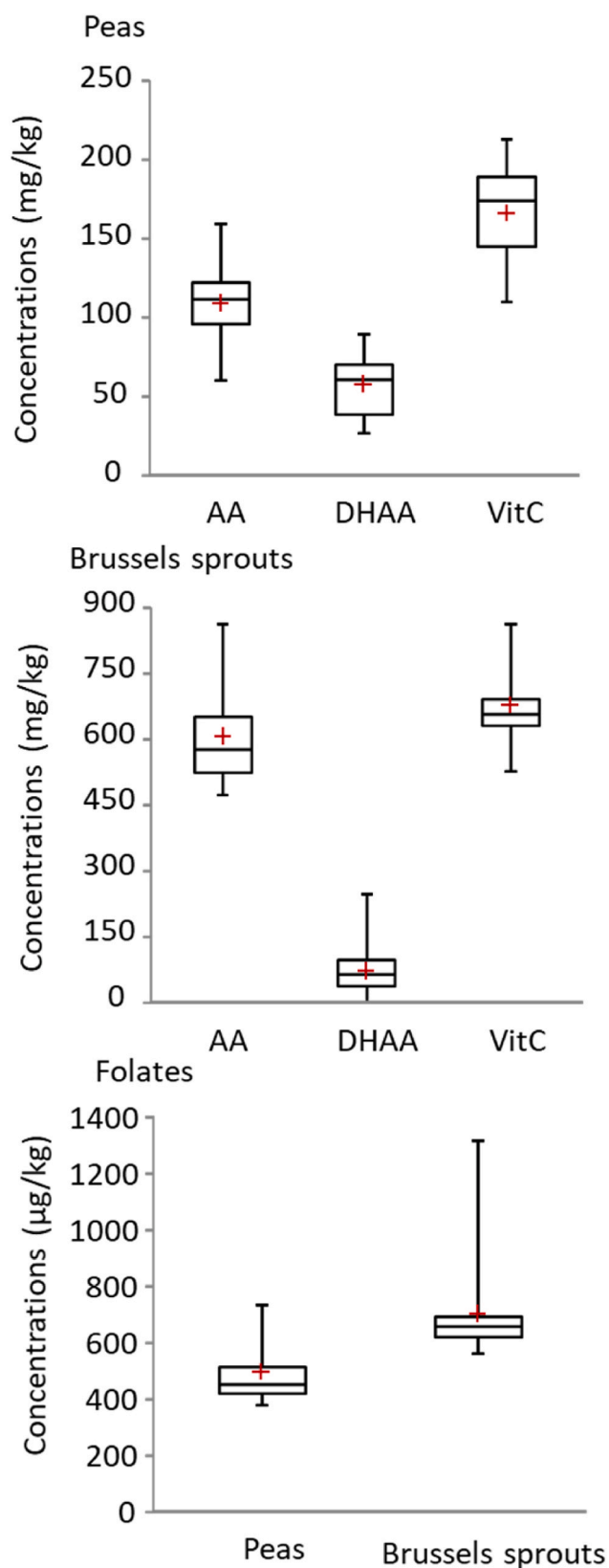


Fig. 2. Vitamin content in the peas and Brussels sprouts at the beginning of experimentation ($t = 0$). The boxplots represent the variability in the concentrations in all initial raw materials (C_0). AA: ascorbic acid; DHAA: dehydroascorbic acid; folates in 5-CH₃-H₄ folate equivalents.

constants k are presented in Table 3.

For the vitamin C in peas, the reaction rate was ten times higher at 85 °C than at 25 °C, and it was about seven times higher in peas than in Brussels sprouts at 85 °C (Table 3). The temperature was significant factor affecting the speed of the vitamin C degradation for both vegetables and folate degradation in peas. Activation energies were calculated as 34.2×10^3 J/mol ($R^2 = 0.97$) and 141.0×10^3 J/mol ($R^2 = 0.7$) for vitamin C and folate in peas; and as 23.1×10^3 J/mol ($R^2 = 0.85$) for vitamin C in Brussels sprouts. This result corresponds to the range of commonly reported activations energies for ascorbic acid in fruits and vegetables. Garrote, Silva, and Roa (2009) reported a $k_{100^\circ\text{C}} = 8.98 \times 10^{-4}$ s⁻¹ and $E_a = 60.8$ kJ/mol in green peas, Van den Broeck et al. (1998) reported a $k_{120^\circ\text{C}} = 0.049 \times 10^{-4}$ min⁻¹ and $E_a = 25$ kcal/mol in squeezed tomatoes, Verbeyst et al. (2013) reported a $k_{100^\circ\text{C}} = 11 \times 10^{-2}$ min⁻¹ and 22×10^{-2} min⁻¹, for $E_a = 29$ kJ/mol and 67 kJ/mol in strawberry and raspberry, respectively.

For 5-CH₃-H₄ folate in buffer, Ruddick et al. (1980) reported a $k_{75^\circ\text{C}} = 0.053$ min⁻¹ and $E_a = 7.10$ kcal/mol (pH 7.3), and Oey et al. (2006) reported a $k_{60^\circ\text{C}} = 47$ min⁻¹ and $E_a = 90$ kJ/mol (pH 7). In vegetables, Coffigniez et al. (2019) reported for cowpea a $k_{90^\circ\text{C}} = 7.7 \times 10^{-5}$ s⁻¹, very close to the values obtained in our study for peas, and Delchier et al. (2014) reported 20×10^{-3} min⁻¹ and 25×10^{-3} min⁻¹ at 45 °C in spinach and green beans, respectively. No reliable estimations could be obtained for folates in Brussels sprouts with an $R^2 = 0.07$, which could related to the observation of Herbig, Delchier, Striegel, Rychlik, and Renard (2019), who found no degradation of this vitamer in apple puree in the presence of ascorbic acid. Half-lives were determined for vitamin C at 85 °C and were 79 min in peas and 516 min in Brussels sprouts. Bureau et al. (2015) found that 66%, 71% and 9% of vitamin C was retained in water-cooked peas, Brussels sprouts, or spinach, respectively, while 100%, 93% and 74% were retained in steamed peas, Brussels sprouts, or spinach after 10 min of cooking. For comparison, Al Fata (2017) reported a half-life of ascorbic acid of 80 min in air and >320 min under strict anaerobic conditions in model solutions at 95 °C and pH 3.5. A possible explanation might be the difference in the initial vitamer compositions, with Brussels sprouts containing less dehydroascorbic acid than peas. For folates, Delchier et al. (2014) observed that folate degradation in spinach and green beans was limited by a plateau (at about $C/C_0 = 0.35$) when submitted to a high oxygen concentration ($pO_2 = 0.04$ MPa), i.e., at a lower C/C_0 than final levels found in the present study.

3.3. Loss by diffusion

Vitamin C and folate diffusion were studied for peas and Brussels sprouts at 25, 45, 65 and 85 °C in a net to allow the diffusion, into excess water. Time curves are presented in Fig. 3 (empty symbols). At the end of the kinetic runs, are presented in Table 2, and the evolution of folates proportions at 120 and 240 min is presented in Fig. 4.

Vitamin C residual contents were approximately 0% in peas, irrespective of the temperatures, while they varied from 40% (at 85 °C) to 75% (at 25 °C) in Brussels sprouts (Table 2). Compared to vitamin C, residual folate content was higher in peas, and influenced by the temperature, ranging from 3% (at 85 °C) to 35% (at 25 °C). In Brussels sprouts, residual folate content was similar to vitamin C, varying from 43% (at 65 and 85 °C) to 65% (at 25 °C). All residual contents were lower when allowing diffusion, rather than when only allowing thermal degradation.

The difference between purely thermal degradation and degradation plus diffusion was the main factor for the levels of residual vitamins (with F-values higher than those observed for temperatures and with highly significant differences, Table 2), confirming the prominent role of diffusion and leaching. The temperature impact was significant in peas for both vitamins, but only vitamin C was influenced in Brussels sprouts due to a high content and limited degradation.

Diffusion kinetics were modelled according to an adapted Fick's

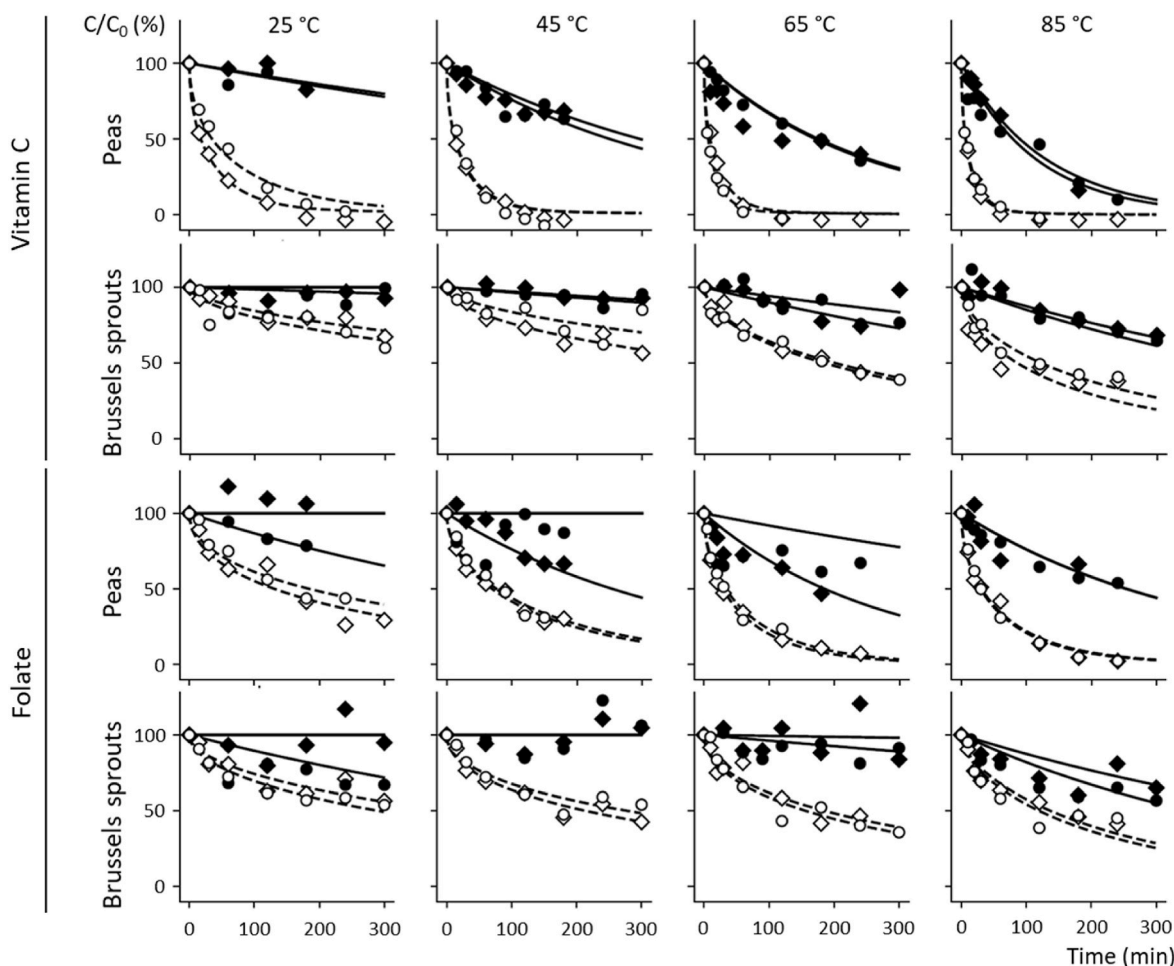


Fig. 3. Thermal degradation and thermal degradation plus diffusion of vitamin C and folates in peas and Brussels sprouts at 25, 45, 65 and 85 °C. Solid lines represent the model-predicted thermal degradation (samples sealed in the plastic bags), full circles and diamonds represent the observed data from the two replicates. Dashed lines represent the model-predicted thermal degradation when allowing for diffusion, empty circles and diamonds represent the observed data from the two replicates. Best-fit model parameters are presented in Table 3.

Table 3

Pseudo-first order reaction constants (s^{-1}) and effective diffusion constants ($m^2 s^{-1}$) for vitamin C and folates in peas and Brussel sprouts subjected to thermal treatment and thermal treatment with diffusion (calculated on duplicate kinetics for each condition).

	Peas				Brussels Sprouts				
		k (s^{-1})	MSE	D (m^2/s)	MSE	k (s^{-1})	MSE	D (m^2/s)	MSE
Vitamin C	25 °C	1.4×10^{-05}	4.1×10^{-05}	1.3×10^{-10}	2.4×10^{-05}	5.3×10^{-06}	8.2×10^{-05}	5.8×10^{-11}	4.4×10^{-05}
	45 °C	4.8×10^{-05}	4.8×10^{-05}	2.4×10^{-10}	2.4×10^{-05}	5.0×10^{-06}	1.3×10^{-05}	8.0×10^{-11}	5.7×10^{-05}
	65 °C	8.0×10^{-05}	9.8×10^{-05}	3.9×10^{-10}	8.3×10^{-06}	1.3×10^{-05}	8.0×10^{-05}	2.9×10^{-10}	2.3×10^{-05}
	85 °C	1.5×10^{-04}	6.6×10^{-05}	4.4×10^{-10}	4.4×10^{-06}	2.2×10^{-05}	3.8×10^{-05}	5.6×10^{-10}	7.56×10^{-05}
Folates	25 °C	2.4×10^{-09}	9.6×10^{-05}	2.2×10^{-11}	6.9×10^{-05}	6.0×10^{-06}	1.9×10^{-04}	1.8×10^{-10}	3.8×10^{-05}
	45 °C	2.6×10^{-05}	2.4×10^{-04}	3.4×10^{-11}	2.2×10^{-05}	4.1×10^{-15}	1.7×10^{-04}	3.5×10^{-10}	4.3×10^{-05}
	65 °C	5.9×10^{-05}	3.2×10^{-04}	8.1×10^{-11}	2.3×10^{-05}	4.8×10^{-06}	1.4×10^{-04}	4.4×10^{-10}	6.2×10^{-05}
	85 °C	5.0×10^{-05}	8.6×10^{-05}	8.8×10^{-11}	2.3×10^{-05}	3.4×10^{-05}	1.1×10^{-04}	2.9×10^{-10}	7.7×10^{-05}

MSE: Mean Square Error.

second law, as detailed in section 2.4.2. The modelling results are presented in Fig. 3, and the estimated diffusion constants and mean square errors are shown in Table 3. Leaching occurred with a higher rate in peas than in Brussels sprouts for both folates and vitamin C. Diffusivity constants for vitamin C increased linearly from $1.3 \times 10^{-10} m^2/s$ at 25 °C to $4.4 \times 10^{-10} m^2/s$ at 85 °C ($R^2 = 0.97$) in peas and from $5.81 \times 10^{-11} m^2/s$ to $5.62 \times 10^{-10} m^2/s$ ($R^2 = 0.89$) in Brussels sprouts. This result showed the impact of the temperature on vitamin C's diffusivity. Previously, Hough, Alzamora, Chirife, and Marini (1990) reported a diffusivity constant of $6.7 \times 10^{-10} m^2/s$ for vitamin C in peas at 100 °C.

Later, Delchier et al. (2014) reported diffusivity constant of $1.5\text{--}3 \times 10^{-10} m^2/s$ and 3×10^{-12} to $1 \times 10^{-11} m^2/s$ for glucose (structurally close to ascorbic acid) in green beans and in spinach, respectively. Interestingly, for monomeric sugars (e.g., fructose, glucose and galactose), diffusion constants of about $6 \times 10^{-10} m^2/s$ have been determined in agar gels (Miyamoto, Atsuyama, Ekino, & Shin, 2018) at 27.5 °C, hinting at a moderate effect of the plant's cellular structure on the diffusion of these small, hydrophilic molecules.

Similarly, for folates, the diffusivity constant increased linearly from $2.18 \times 10^{-11} m^2/s$ at 25 °C to $8.79 \times 10^{-11} m^2/s$ at 85 °C ($R^2 = 0.91$) in

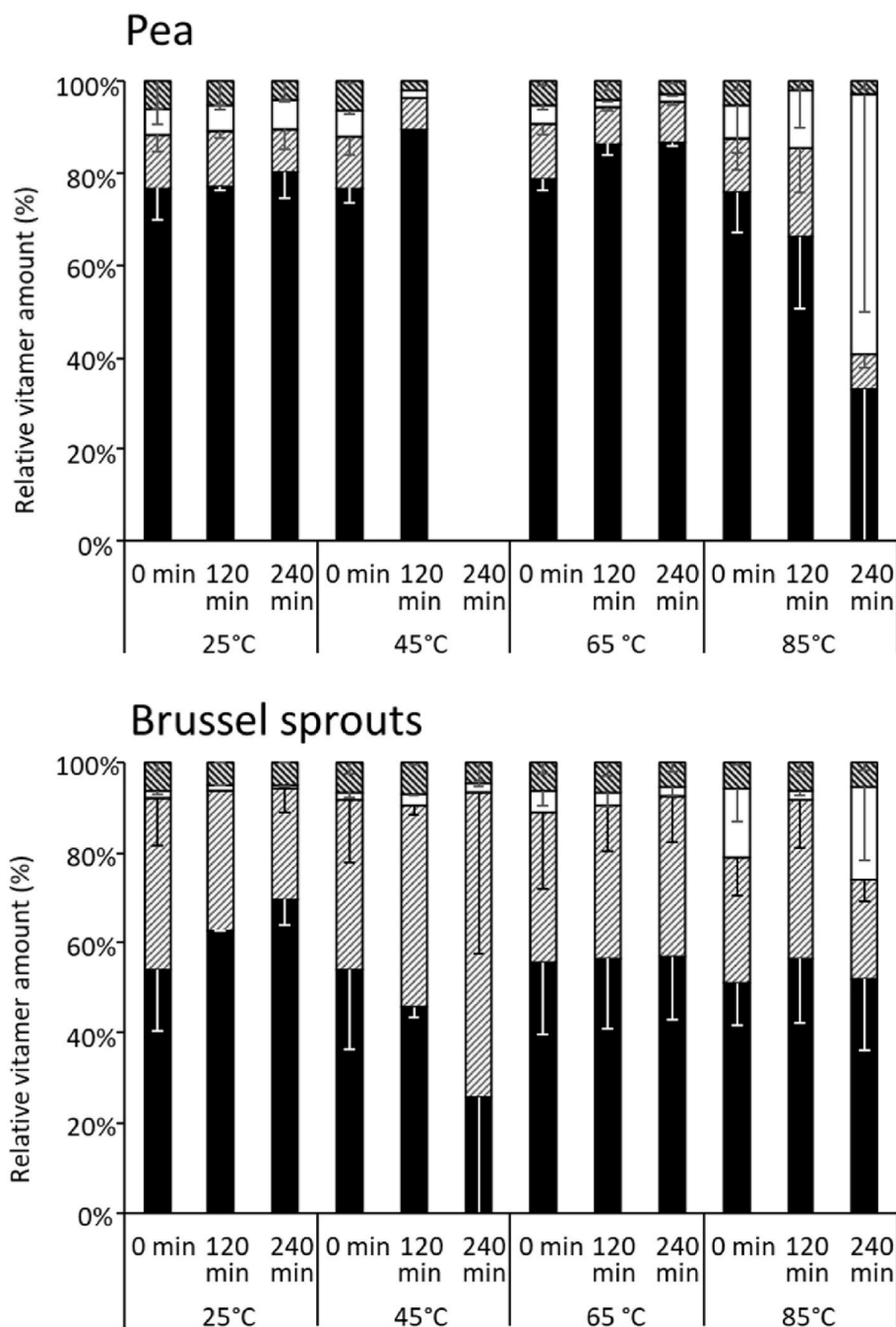


Fig. 4. Relative amounts of folate vitamers in the initial peas or Brussels sprouts (0 min) after thermal degradation plus leaching for the different temperatures (120 or 240 min). Data are averages and standard deviations from 2 to 4 samples.

peas. However, in Brussels sprouts the temperature did not affect the diffusivity constant of folates. In this case, the diffusivity constant varied between $1.79 \times 10^{-10} \text{ m}^2/\text{s}$ at 25 °C and $2.91 \times 10^{-10} \text{ m}^2/\text{s}$ at 85 °C.

This result might be due to the observed non-monotonic effect of the temperature on folate thermal degradation in Brussels sprouts. Experiments with more replicates could provide an answer in the future. Either way, folates appeared less affected by the diffusion than vitamin C, especially for peas and at a low temperature (25 °C and 45 °C). Moreover, the plant matrix had an impact on the diffusivity rate, with a faster diffusion from peas than Brussels sprouts. The complex multi-layered structure of Brussels sprouts could contribute to the difference in the diffusivity between peas and Brussels sprouts.

Folate diffusion constants determined for peas were in the same order of magnitude as those previously calculated by Coffigniez et al.

(2019) in cowpea seeds ($3\text{--}7 \times 10^{-11} \text{ m}^2/\text{s}$, between 30 and 90 °C), who adjusted the diffusion models with the enzymatic or thermal degradation constants. Our diffusivity constants were also in the same order of magnitude as those already reported by Delchier et al. (2014) for spinach and green beans at similar temperatures.

Finally, the diffusion model considers each vegetable's shape (spherical) and radius, which differs between Brussels sprouts and peas. This discrepancy could also explain why diffusion happens faster at the beginning of the kinetics in peas, evidenced by a higher slope between the two model curves. In contrast, the diffusion constants did not differ substantially between the two vegetables (Fig. 3 and Table 3).

Despite being variable, the proportions of the folate vitamers during and after leaching (Fig. 4) showed remarkably little evolution, except at the highest temperature and duration (enrichment in folic acid).

Therefore, the nature of the folate moiety was not a significant factor for diffusion, as might be expected given their close chemical structure; different results might have been found modelling diffusion as a function of the length of the polyglutamate tail. Delchier et al. (2013) registered marked evolution of the folate proportions in green beans and spinach after canning. This discrepancy might be linked to the much higher temperatures used for sterilisation than those used in the current experiment. Proportions of 5-CH₃-H₄ folate and 5-CHO-H₄ folate appeared to be relatively stable over the time of the diffusion kinetics. The one marked and similar variation was a relative increase in folic acid proportion at 85 °C, likely due to its stability (Delchier et al., 2016). Despite the correlation between the increase and the decrease of the vitamins distribution, interconversions between the different derivatives might occur, as previously described by Coffigniez et al. (2021), and Herbig et al. (2019).

3.4. Thermal degradation versus leaching

Models describing both the thermal degradation and the diffusion of vitamin C and folates during heat treatment involving water contact, in peas and Brussels sprouts seemed to favour leaching, all the more for short treatment times (Fig. 3). This observation is supported by others showing that blanching or cooking in water leads to a higher loss than steaming within culinary or industrial blanching durations, typically about 10 min or less for small vegetables (Bureau et al., 2015; Delchier et al., 2012; Islam et al., 2021). Moreover, the nature of the vegetable modulated the vitamin loss.

Capuano et al. (2018), discussed that modelling the chemical reactivity in foods has many challenges due to complex composition and compartmentation. Diffusion (or leaching) is rarely considered explicitly when modelling vitamin losses from fruits and vegetables. However, when comparing the results obtained in hermetically sealed bags versus open nets, two points seem generally relevant: i) much higher losses are observed with leaching, and ii) the evolution of the concentrations within the vegetables can be adequately modelled by using first-order kinetics. The two models give somewhat similar line shapes (Supplementary Fig. 1), the significant difference being that explicit modelling of leaching better explains the fast losses observed for short treatment durations (Fig. 3). Van Boekel (2008) demonstrated that assessing the order of a reaction in food processing is problematic for two reasons: i) reliably discriminating the models for different reaction orders demands reaching advanced reaction extents, more than 70%; ii) intrinsic variability in the food composition, as seen here, especially for Brussels sprouts. This result might account for why the first-order kinetic models are commonly used and how they explain the large disparities found for the degradation constants of vitamin C in real foods, depending on precise treatment conditions (i.e., plant material/water ratio). Compartmentation (a multi-layered structure of leaves, with relatively hydrophobic surfaces) could also explain some of the differences in the peas and Brussels sprout data.

Finally, the order of magnitude of k (ranging from 10^{-5} to 10^{-6} s⁻¹) and the order of magnitude of D (ranging from 10^{-10} to 10^{-11} m²/s) cannot be compared as such to explain the loss of the vitamins as they do not represent the same physicochemical mechanisms of loss and must be expressed in different units.

4. Conclusion

The current work brings quantitative models to a surprisingly neglected factor of vegetables' nutritional quality. In contrast to the abundance of papers on antioxidants, knowledge of vitamin losses is often ancient and very observatory, and current food policies are often based on mistaken preconceptions about vitamin fragility rather than data and models.

This experiment indicates two highly relevant points for further improvement in assessing the nutritional value of vegetables. First,

vitamin losses are about two-fold greater for folates (at 85 °C) and an order of magnitude faster for vitamin C (in peas at 25 °C) when these vegetables are processed in water. This result suggests that leaching may be more important than commonly believed. Second, vitamin C and folates are far more processing-resistant than previously thought. However, our initial study still has limitations. For example, there is a large variability in vitamin concentrations among different batches of the same vegetables, especially for Brussels sprouts. This intrinsic biological variability precludes fine adjustment for the models. Moreover, in the model, we assumed each vegetable to be a homogenous sphere, but in reality, peas are composed of two cotyledons included in a testa and Brussels sprouts are an agglomerate of ordered leaves. It is unclear how much the assumption of a simple sphere affects the outcomes.

CRedit authorship contribution statement

Catherine M.G.C. Renard: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Supervision. **Hanna Brick:** Investigation. **Jean-Francois Maingonnat:** Methodology, Investigation. **Claus Kadelka:** Methodology, Validation, Formal analysis. **Nicolas Delchier:** Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.lwt.2023.114694>.

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