Food Chemistry 157 (2014) 439–447

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Mechanisms of folate losses during processing: Diffusion *vs.* heat degradation

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ARTICLE INFO

Article history:
Received 9 November 2013
Received in revised form 23 January 2014
Accepted 12 February 2014
Available online 22 February 2014

Keywords:
Vitamins
Folate
Vegetables
Process
Leaching
Heat degradation
Kinetics
Fick's second law

ABSTRACT

Though folates are sensitive to heat treatments, leaching appears to be a major mechanism involved in folate losses in vegetables during processing. The aim of our study was to study folate diffusivity and degradation from spinach and green beans, in order to determine the proportion of each mechanism involved in folate losses.

Folate diffusivity constant, calculated according to Fick's second law (Crank, 1975), was 7.4×10^{-12} m^2/s for spinach and 5.8×10^{-10} m^2/s for green beans, which is the same order of magnitude as for sugars and acids for each vegetable considered. Folate thermal degradation kinetics was not monotonous in spinach and green beans especially at 45 °C and did not follow a first order reaction. The proportion of vitamers changed markedly after thermal treatment, with a better retention of formyl derivatives. For spinach, folate losses were mainly due to diffusion while for green beans thermal degradation seemed to be preponderant.

1. Introduction

Folate is the generic term used for different water-soluble vitamers which differ by the nature of carbon groups linked to nitrogen 5 or 10, the oxidative state and the length of the glutamate tail. They are involved in the "one carbon metabolism" especially being donor of methyl group during DNA synthesis. It is well established that folates can protect against neural tube defects (Czeizel & Dudás, 1992) and neurodegenerative diseases (Snowdon, Tully, Smith, Riley, & Markesbery, 2000). Folates are also involved in the methylation of homocysteine, which is one risk factor for heart diseases (Robinson, 2000).

One of the main contributors for folate intake are vegetables, and particularly green vegetables, which represent circa 40% of the folate intake in the French diet (Lafay, 2009). In France, authorities recommend an intake of folate from 300 μg per day for women to 330 μg per day for men, with an increase to 1 mg per day during pregnancy (ANSES, 2012). However, there is a gap between the real and the recommended intake from around 20% for women to 15% for men (Lafay, 2009).

Evolution of lifestyles means that most fruits and vegetables are consumed after processing, whether domestic processing (cooking, heating, microwaves) or industrial processing, such as canning or freezing, hence there is a need to better understand the impact of processing on folate content.

Folate losses from spinach during boiling or blanching represent 20–80% of initial folates 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 did not cause folate losses (Delchier et al., 2012; Klein et al., 1979; McKillop et al., 2002). Few studies measured folates in cooking liquids but Delchier et al. (2012) showed that leached folates represent half of folate losses from fresh spinach and the whole of folates losses from frozen spinach and green beans, after boiling in water. Data concerning the impact of industrial processing on folate losses is really scarce. Our previous study showed that blanching had no effect on folate loss both during the spinach freezing process and the green beans canning process. Losses occur

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http://dx.doi.org/10.1016/j.foodchem.2014.02.054
0308-8146/

during the washing step for spinach and after sterilization for green beans, with folate found in the covering liquid (Delchier et al., 2013).

This study on folate loss during industrial processing led us to suspect that diffusion may play a major role in folate loss, especially when heating steps are limited.

Therefore, this study aims to determine the relative importance of diffusion and thermal degradation of spinach and green beans during heat treatments. For this, two parallel experiments were set up: one in which vegetables were only subjected to heat, and one in which they were subjected to heat and diffusion.

2. Materials and methods

2.1. Plant material

2.1.1. Diffusion

Fresh spinach and green beans were bought at a local supermarket on the day of the experiments, or stored at 4 °C for maximum of 48 h after purchase. Spinach and green beans were first blanched in phosphate buffer pH 7 (0.01 mol/l) or in citrate phosphate buffer pH 5 (0.01 mol/l) for 10 min at 100 °C with solid–liquid ratio of 50 and 100 g/l, respectively, in order to inactivate enzymes and destroy cell compartmentalization. After blanching, spinach and green beans were drained, weighted and immediately put into a large receptacle (with the same solid–liquid ratio of 50 g/l), to start the diffusion.

2.1.2. Thermal degradation

Purees were prepared from spinach and green beans stored in cans bought at local supermarket in two batches for each temperature condition. Cans were opened and vegetables were drained. 200 g of vegetables were put into 400 ml of water and ground with an UltraTurax (S25 18G, IKA, Staufen, Germany) at 13,000 rpm for 1 min. Spinach purees were diluted, to facilitate stirring during the time course. For this purpose, 50 ml of water was added to 50 ml of spinach puree.

2.2. Time course experiments

2.2.1. Diffusion

Diffusions experiments were carried out in phosphate buffer pH 7 (0.01 mol/l) or citrate phosphate buffer pH 5 (0.01 mol/l), under stirring. Temperature and pH were monitored and controlled all along the time course, which were performed for three temperatures (25, 45 and 65 °C) and at pH 5 and pH 7 for 4 h. At pH 7, three batches of spinach and green beans were independently studied and two batches at pH 5.

For each kinetic point, an aliquot of 35 g of spinach or green beans was collected and directly stabilized by freezing in liquid nitrogen, and stored at −80 °C until analysis. The folate content was determined in the vegetables at each point along the time course.

2.2.2. Thermal degradation

Heat degradation was carried out in a beaker immersed in a water bath. Purees were stirred all along the experiments by a propeller stirrer of 55 mm diameter turning at 600 rpm (VOS 16, VWR, Fontenay sous bois, France). Time courses were performed in two independent batches for three temperatures: 45, 65 and 85 °C. Purees were heated and kinetics started when they were at the desired temperature. 10 ml of puree were sampled at different points for 4 h and directly put at −80 °C.

2.3. Modelling

2.3.1. Diffusion

Diffusivity constant (D) was calculated for folates, sugars and acids according to Fick's second law Eq. (1):

$$\frac{\partial C}{\partial t} = -D\frac{\partial^2 C}{\partial r^2} \tag{1}$$

where C represents the concentration, t the time and r a characteristic distance.

Spinach leaves were considered as a plane sheet where Fick's second law solution, given by Crank (1975), is Eq. (2):

$$\frac{C(t)-C_\infty}{C_0-C_\infty} = 1 - \sum_{n=0}^{\infty}\frac{8}{(2n+1)^2\pi^2}\exp\left(-\frac{D(2n+1)^2\pi^2 t}{4l^2}\right) \tag{2}$$

where $C(t)$ is the concentration at time t, C_0 is the initial concentration and C_∞ the concentration at infinite time, D the diffusivity constant and l the half thickness of the plane.

In the case of green beans, the diffusivity constant was determined according to the cylinder solution given by Crank (1975) Eq. (3):

$$\frac{C(t)-C_\infty}{C_0-C_\infty} = 1 - \sum_{n=1}^{\infty}\frac{4}{a^2\alpha_n^2}\exp(-D\alpha_n^2 t) \tag{3}$$

where $C(t)$ is the folates concentration at t time, C_0 is the initial concentration and C_∞ the concentration at infinite time, D the diffusivity constant and a the radius. In this equation α_n is the root of Bessel function of order 0 Eq. (4).

$$J_0(a\alpha_n) = 0 \tag{4}$$

The model was adjusted by maximizing the correlation coefficient, r^2, calculated as follow Eq. (5):

$$r^2 = 1 - \left(\frac{\sum(Exp-Th)^2}{\sum(Exp-m_{Th})^2}\right) \tag{5}$$

where Exp is the experimental concentration; Th is the theoretical data obtained by modelling, and m_{Th} is the mean of theoretical data obtained by modelling.

2.3.2. Thermal degradation

Linearization of folate thermal degradation was carried out according to the mean of the two batches for each temperature studied, using a first order with partial conversion model, as described below Eq. (6):

$$\ln\left(\frac{C}{C_0}\right) = -kt \tag{6}$$

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

2.4. Analytical procedures

2.4.1. Folate measurement

2.4.1.1. Total folates content. Total folate content was determined by HPLC with fluorimetric detection. After extraction all folate vitamers were deconjugated into mono and diglutamate, reduced and methylated into 5-CH_3-H_4folates. The latter were purified from the extract by affinity chromatography using Folate Binding Protein, and quantified by RP-HPLC with fluorimetric detection (RF-1AXL, Shimadzu Inc., Kyoto, Japan). For experimental details, see Delchier et al. (2013).

2.4.1.2. Stable isotope dilution assay. Before extraction, labelled standards of folate vitamers were added and all folates were