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Thermal degradation of folates under varying oxygen conditions

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ABSTRACT

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_4folate, 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_4folate 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_4folate to degradation in the presence of oxygen were confirmed.

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_4folate 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_4folate 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_4folate in citrate–phosphate buffer (pH 4) as 115.08×10^{-3} min^{-1} and in phosphate buffer (pH 7) as 68.31×10^{-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_4folate 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 1×10^{-4} mol/L, both for folic acid and 5-CH_3-H_4folate.

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_4folate 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_4folate 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_4folate solutions, reaction kinetics were started by adding 100 µl of stock solutions to the buffer in the reactor for a final concentration of 1×10^{-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_4folate measurement

A solution of ascorbic acid (100 µl; 250 g/L) was added to 900 µl of samples. Solutions of 5-CH_3-H_4folate were diluted 10 times in phosphate buffer (pH 7; 0.1 mol/L) for HPLC analysis.

Folic acid and 5-CH_3-H_4folate 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.,