Food Chemistry 139 (2013) 815–824

Contents lists available at SciVerse ScienceDirect

Food Chemistry

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

Effects of industrial processing on folate content in green vegetables

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ARTICLE INFO

Article history:
Received 6 August 2012
Received in revised form 11 December 2012
Accepted 24 January 2013
Available online 8 February 2013

Keywords:
Vitamin
Blanching
Heating
Freezing
Leaching
Sterilisation
Green beans
Spinach

ABSTRACT

Folates are described to be sensitive to different physical parameters such as heat, light, pH and leaching. Most studies on folates degradation during processing or cooking treatments were carried out on model solutions or vegetables only with thermal treatments.

Our aim was to identify which steps were involved in folates loss in industrial processing chains, and which mechanisms were underlying these losses. For this, the folates contents were monitored along an industrial canning chain of green beans and along an industrial freezing chain of spinach.

Folates contents decreased significantly by 25% during the washing step for spinach in the freezing process, and by 30% in the green beans canning process after sterilisation, with 20% of the initial amount being transferred into the covering liquid. The main mechanism involved in folate loss during both canning green beans and freezing spinach was leaching.

Limiting the contact between vegetables and water or using steaming seems to be an adequate measure to limit folates losses during processing.

1. Introduction

Folate is the generic term for different derivatives of the pterin molecule such as folic acid (PteGlu), 5-methyltetrahydrofolic acid (5-CH_3-H_4folate), 5-formyltetrahydrofolic acid (5-HCO-H_4folate), 10-formylfolic acid (10-HCO-PteGlu), tetrahydrofolate (H_4folate), 5,10-methenyltetrahydrofolic acid (5,10-CH^+-H_4folate) and 10-formyldihydrofolic acid (10-HCO-H_2folate). They are important vitamins, necessary for preventing of neural tube defects with a risk reduction from 58% (Laurence, James, Miller, Tennant, & Campbell, 1981) to 100% (Czeizel & Dudás, 1992). They can also be involved in the protection against the development of cardiovascular (Robinson, 2000) and neurodegenerative diseases (Snowdon, Tully, Smith, Riley, & Markesbery, 2000).

Recommended daily intakes in the USA are 400 µg per day for both men and women and 600 µg per day for pregnant women (USDA, 2011). In France, authorities recommend a folate intake of 330 µg per day for men and 300 µg per day for women with an increase to 1 mg per day for pregnant women (ANSES, 2012).

INCA 1 and 2 studies report that in France there is a gap between the real and the recommended intake of around 20% for women and 15% for men and that vegetables contribute significantly to this folate intake (40%) (Lafay, 2009). The evolution of life styles means that most fruits and vegetables, particularly green vegetables, are consumed as processed products such as cans or frozen products. Therefore, there is a need to better understand folates losses during industrial processing so as to be able to alleviate this loss in process optimisation. This motivated our study in folates losses along two representative industrial chains, i.e. canning for green beans and freezing for spinach.

Green beans and spinach were chosen for their high folate content and high share of processed products in their consumption. Moreover, industrial canning and freezing chains were studied because they combine processing steps frequently found for fruits and vegetables such as washing and blanching, but with different intensities of heat treatments, such as blanching and sterilisation.

Relatively little is known about the fate of folate during fruits and vegetables processing.

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http://dx.doi.org/10.1016/j.foodchem.2013.01.067

On the one hand, folate degradation during heat treatments was studied on model solutions. Chen in 1979 showed that heating 5-CH_3-H_4folate in aqueous solution at 100 °C during 60 min involve a loss of 90%, while heating at 78 °C involve a loss of 50% under the same conditions (Chen & Cooper, 1979). Chen determined that the rate of 5-CH_3-H_4folate destruction is temperature dependant conforming to the Arrhenius equation (Chen & Cooper, 1979) with an activation energy of 9.5 kcal/mol. Paine-Wilson and Chen (1979) showed that pH has a profound influence on the thermal stability of folate, with optimal stability in neutral conditions. For 5-CH_3-H_4folate, $t_{1/2}$ is 8.77 min at pH 7 but $t_{1/2}$ is 3.35 and 3.45 min at pH 4 and 10, respectively (Paine-Wilson & Chen, 1979). Indrawati determined the degradation rate constant k for 5-CH_3-H_4folate in citrate–phosphate buffer (pH 4) at $115.08 \times 10^{-3}\ min^{-1}$ and in phosphate buffer (pH 7) at $68.31 \times 10^{-3}\ min^{-1}$ (Indrawati et al., 2004). Folates appear to be quite vulnerable to heat, especially in slightly acidic conditions.

On the other hand, there are some studies on folates losses in vegetables during cooking, blanching or freezing. McKillop in 2002 determined that spinach blanching for 3.5 min involves a folate loss of 51% (McKillop et al., 2002). Holasova et al. obtained similar results with a percentage of retention of around 40% after 12 min boiling (Holasova, Vlasta, & Slavomira, 2008). De Souza in 1986, studying the impact of different treatments such as blanching and freezing on folate loss in spinach, showed 17% of retention after blanching at 100 °C for 4 min. De Souza also reported that canning involved a folate decrease of 50% in the vegetable and determined the folate amount in canning medium as 0.34 mg/kg of fresh weight. Folate content in frozen spinach decreased after 3 months by 72% (DeSouza & Eitenmiller, 1986).

These losses actually appear even higher than what can be explained by thermal degradation alone. Therefore, leaching as additional mechanism for folate loss in vegetable was mentioned (Scott, Rébeillé, & Fletcher, 2000). Unfortunately, most of studies were carried out at laboratory scale, taking into account only single processing steps.

Our aim was to measure folate loss after each step of green beans canning and spinach freezing, under industrial conditions, and to identify which are the underlying mechanisms.

To that end, green beans and spinach were sampled along the corresponding industrial chain at different steps of the process, and analysed for their total folate content and folate distribution.

2. Materials and methods

2.1. Chemicals

The following chemicals were obtained commercially from suppliers given in parentheses: Ascorbic acid, sodium borohydride, Tris buffer, formaldehyde 37%, DL-dithiothreitol, acetic acid, formic acid (Sigma–Aldrich, France); Sodium hydroxide, trifluoroacetic acid (VWR, France); 2-octanol, hydrochloric acid 37% (Merck, France); Acetonitrile (Fischer Scientific, France); Chicken pancreas (Patricell Ltd., London, UK); Folate Binding Protein (Scripps labs., San Diego, CA, USA).

MES Hydrate (Sigma–Aldrich, Germany); Ascorbic acid (VWR, Germany); β-mercaptoethanol, sodium acetate, hexane, methanol, acetonitrile (Merck, Germany); Chicken pancreas (Difco labs., Detroit, MI, USA), rat serum (Biozol, Germany).

5-CH_3-H_4folate, 5-HCO-H_4folate, 10-HCO-PteGlu, H_4folate, PteGlu (Schircks laboratories, Jona, Switzerland).

5-CH_3-H_4folate diglutamate was obtained from reduction of Pteroyl-γ-diglutamate (Schircks laboratories, Jona, Switzerland) according to Ndaw, Bergaentzlé, Aoudé-Werner, Lahély, and Hasselmann (2001).

Labelled standards ([$^2H_4$]-5-CH_3-H_4folate, [$^2H_4$]-5-HCO-H_4folate, [$^2H_4$]-10-HCO-PteGlu, [$^2H_4$]-H_4folate, [$^2H_4$]-PteGlu), were synthetised as reported by Freisleben, Schieberle, and Rychlik (2003a).

2.2. Plant material

2.2.1. Spinach sampling

Spinach sampling along an industrial freezing chain was carried out in October 2010 in Bonduelle's factory in Péronne (North of France). Spinach was processed as follows and as described in Fig. 1.

Upon arrival at the factory, the spinach leaves were first washed during 1–3 min at 15–25 °C by immersion into water. The spinach leaves were then blanched by aspersion of water during 70–120 s at 90–95 °C, and directly cooled by aspersion of water during 2–5 min. Cooling water temperature was between 15 and 25 °C. The spinach leaves were then carried out to the forming step to be transformed into frozen quoits during 30–60 min at a temperature between −30 and −18 °C.

Spinach sampling was performed:

- Upon arrival at the factory the raw spinach leaves were collected and called "Raw material";
- Before and after the washing step, the samples were called "Before washing" and "After washing" respectively;
- After the coupled blanching and cooling step, the samples were called "After blanching";
- At the end of the processing chain, after the freezing, the samples were called "Final product";

Three batches of spinach leaves were followed along the freezing chain (each batch being one truckload of about 25 tons). At each sampling point, three samples were collected for each batch and directly stabilised in liquid nitrogen, except for "Final product".

The samples were ground in liquid nitrogen and stored at −80 °C until analysis.

All three batches of spinach were from the same cultivar: *Spinacea oleracea* cv. Dolphin, but from different producers.

2.2.2. Green beans sampling

Sampling of green beans was separated into two parts: the first one was dedicated to the evaluation of the impact of each step of the process on folates contents. With this first sampling (2 cultivars, 5 lots), the green beans were followed along the industrial chain (Fig. 2). The second sampling was totally independent from the first one. It aimed at evaluating the dispersion of folates contents in different raw materials (5 cultivars, 11 batches) and final products (4 cultivars, 9 batches) during the harvest and production period for the canned green beans, independently from the impact of individual process steps.

2.2.2.1. Sampling on canning chain to evaluate the impact of each step of the process. Green beans sampling along the canning chain was performed at Bonduelle's factory in Renescure (North of France), on two consecutive days, in August 2010. The process is described in Fig. 2.

Upon arrival at the factory, the green beans were washed in water during 30 s–1 min and trimmed for 20 min–1 h at room temperature. Then, green beans were blanched either by aspersion of water or by immersion into a water bath during 4–8 min. The temperature of the blanching water used was from 85 to 95 °C. Green beans were then quickly cooled by aspersion of water for both blanching modes, during a maximal time of 1 min from 85