Food Chemistry 159 (2014) 122–127

Contents lists available at ScienceDirect

Food Chemistry

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

Degradation of folic acid in fortified vitamin juices during long term storage

Lara Frommherz [a,*], Yvonne Martiniak [b], Thorsten Heuer [b], Alexander Roth [c], Sabine E. Kulling [a], Ingrid Hoffmann [b]

[a] Max Rubner-Institut, Federal Research Institute of Nutrition and Food, Department of Safety and Quality of Fruit and Vegetables, Haid-und-Neu-Straße 9, 76131 Karlsruhe, Germany
[b] Max Rubner-Institut, Federal Research Institute of Nutrition and Food, Department of Nutritional Behaviour, Haid-und-Neu-Straße 9, 76131 Karlsruhe, Germany
[c] Max Rubner-Institut, Federal Research Institute of Nutrition and Food, Department of Physiology and Biochemistry of Nutrition, Haid-und-Neu-Straße 9, 76131 Karlsruhe, Germany

ARTICLE INFO

Article history:
Received 11 June 2013
Received in revised form 22 January 2014
Accepted 27 February 2014
Available online 12 March 2014

Keywords:
Folic acid
Fortified vitamin juices
HPLC-MS/MS

ABSTRACT

Folic acid (FA) concentrations of nine fortified vitamin juices were determined with the aim to study the FA degradation and to investigate the deviation from the declared label value. The juices were received shortly after bottling and were analyzed monthly during controlled storage conditions (light and dark) over one year. The analyses were performed by HPLC–MS/MS, which included a fast "dilute and shoot" sample preparation. Average decreases in FA concentration of 46% were observed after one year. Fresh juices (shortly after bottling) showed the highest deviations from the declared label value (up to + 89%). Label values did not reflect the actual concentration of FA in these products, making it difficult to determine the intake of this vitamin.

1. Introduction

The generic term "folates" refers to a group of naturally occurring B-vitamins. They share a similar chemical structure and as being essential vitamins, folates must be obtained from dietary sources. Previous studies have shown a low intake of folates in Germany and the most European countries (Elmadfa, 2009; Krems, Walter, Heuer, & Hoffmann, 2012). Pteroyl-L-monoglutamic acid (FA) a synthetic form of folate, is approved by the EC regulation No. 1925/2006 (European Parliament, 2006) for the fortification of food to enable an adequate vitamin intake. This regulation does not specify a maximum fortification amount of FA in food products.

Folates are known to be sensitive to heat, oxygen, light and low pH (Akhtar, Khan, & Ahmad, 1999; McKillop et al., 2002). Degradation occurs during storage. This is especially true for vitamin juices, mixtures of different fruit juices fortified with several vitamins, where a low pH (3.5) prevails. Producers of vitamin juices declare the concentration of fortified FA on the label as the sum of natural and added amount and guarantee for that amount until the expiration date. In order to do so they need to add a higher amount when producing the fresh juices to compensate for the loss that occurs during storage.

At this point there is no European mandatory regulation as to how far the added amount of vitamins may exceed the declared concentration on the label. There are recommended practices on national bases like a position paper worked out by the German Food Chemical Society (LChG) regarding recommendations concerning the tolerance of nutrient declarations (LChG, 2009). For vitamins a tolerance of ±30% for FA compared to the label declaration is generally accepted. Due to instability of some vitamins an additional dosage of up to 50% is considered necessary and may even be exceeded in some cases, like the fortification of fruit juices.

Most recently the EU commission has provided a draft on guiding principles with reference to the setting of tolerances for nutrient values (European Commission, 2012). According to this draft the acceptable deviation of the declared label value regarding vitamins is +50%. So far this draft has no legal status but acknowledges the need for a regulation in this matter.

Due to the mentioned instability and degradation of vitamins in fruit juices, we expected producers to add significantly higher concentrations of vitamins to ensure the labeled value until the expiration date. We were particularly interested in the concentration of added FA as most recent studies have pointed out some possible ambivalent health effects. While an adequate folate intake is beneficial during pregnancy to prevent congenital malformations such as neural tube defects (Scholl & Johnson, 2000), adverse

* Corresponding author. Tel.: +49 (0)721 6625 545; fax: +49 (0)721 6625 453.
E-mail addresses: lara.frommherz@mri.bund.de (L. Frommherz), yvonne.martiniak@mri.bund.de (Y. Martiniak), thorsten.heuer@mri.bund.de (T. Heuer), alexander.roth@mri.bund.de (A. Roth), sabine.kulling@mri.bund.de (S.E. Kulling), ingrid.hoffmann@mri.bund.de (I. Hoffmann).

http://dx.doi.org/10.1016/j.foodchem.2014.02.156
0308-8146/

effects of a high FA intake are in discussion. An intake above the tolerable upper intake level of 1 mg/day FA (EFSA, 2006) can mask a vitamin B12 deficiency, which untreated will lead to neurological damages (Clarke et al., 2003). New studies also suggest that a high FA intake accelerates the malignant transformation of existing neoplasms (Kim, 2007a,b; Stolzenberg-Solomon et al., 2006) and reduces the efficacy of antifolate drugs used for instance for the treatment of rheumatoid arthritis or psoriasis (Khanna et al., 2005; Salim, Tan, Ilchyshyn, & Berth-Jones, 2006).

Our aim was to investigate the degradation of FA during the storage duration of twelve months and to compare the producers' declaration on the label with the concentrations found in the juices. We also studied the influence of light on the degradation process. We opted for a simple and quick yet accurate and precise method to analyze the juices.

2. Experimental/materials and methods

2.1. Reagents and materials

FA was purchased from Sigma Aldrich (Deisenhofen, Germany). Fivefold isotope-labeled FA (C13-FA) as internal reference substance (IS) was purchased from Merck&Cie (Schaffhausen, Switzerland). The purity of the substance was 99.7% with an isotope-labeling exclusively on the glutamate part of the molecule. Acetonitrile (HPLC grade), formic acid (99%) and sodium hydroxide were obtained from VWR (Darmstadt, Germany), 2-(cyclohexylamino) ethanesulfonic acid (CHES), 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES) and dithiothreitol (DTT) were from Sigma Aldrich (Deisenhofen, Germany). Analytical grade water was obtained from a LaboStar TM purification system, Siemens (Munich, Germany). Methanol was from Merck (Darmstadt, Germany). Phenex-PTFE syringe filters (15 mm, 0.2 μm) were obtained from Phenomenex (Aschaffenburg, Germany).

A HEPES/CHES buffer according to Wilson and Horne (Wilson & Horne, 1984) was used for diluting FA stock solutions and juice samples. Stock solutions of the FA standard and the IS (2 mg/ml) were prepared in an aqueous solution of NaOH (0.1 mol/l). These solutions were diluted with HEPES/CHES buffer and stored in small portions at −80 °C until use. The working solution of the IS was 1 μg/ml, various concentration of the FA standard were prepared by appropriate dilution with the HEPES/CHES buffer.

2.2. HPLC-MS conditions

HPLC analyses were carried out using an Agilent 1200 series HPLC system (Agilent, Waldbronn, Germany). The system comprised a vacuum degasser, binary pumps, a thermostated autosampler and column compartment. The column used for the chromatography was a Prontosil C-18 (Bischoff, 3 × 150 mm, 3 μm) at a flow rate of 0.5 ml/min and a column temperature of 35 °C. Injection volume was 10 μl. The HPLC mobile phases consisted of 0.1% aqueous formic acid (A) and acetonitrile (B). Following linear gradient was used (%B): 0–5 min (5%), 15 min (28%), and 16–20 min (100%). The column was then re-equilibrated for 10 min, making a total run time of 30 min. A switching valve was used in order to protect the instrument from unnecessary matrix pollution. The HPLC eluent flow between 12 and 18 min was directed into the mass spectrometer while the rest of the run went to waste. The FA signal was detected at 15 min.

The HPLC was coupled to a 3200 QTrap mass spectrometer (AB Sciex, Darmstadt, Germany). Ionization was achieved using positive electrospray ionization. Following conditions were found to be optimal: 21 V (DP), 20 psi (CUR), 450 °C (Source Temperature), 5000 V (Ion Spray Voltage), 55 psi/60 psi (Ion Gas 1 and 2, respectively). Data were recorded in the multiple reactions monitoring (MRM) mode using following transitions: FA 442.1 > 295.3 (CE 21 V), 442.1 > 176.2 (CE 53 V), C13-FA 447.1 > 295.3 (CE 23 V), 447.1 > 176.2 (CE 53 V). All aspects of system operation and data acquisition were controlled using Analyst 1.5.2 software (AB Sciex).

2.3. Sample preparation

Juice samples, matrix calibration samples and quality control (QC) samples were prepared equally: 1 ml juice was mixed with 1 ml 0.1% aqueous NaOH and 100 μl IS working solution. The samples were diluted with 2 ml HEPES/CHES buffer, homogenized and centrifuged. The supernatant was filtered through a syringe filter prior to analysis.

2.4. Quantitation and method validation

Quantitation of the FA concentration was done by using the peak area ratios of unlabeled to labeled compound using a matrix calibration curve. This curve was prepared with a non-fortified vitamin juice spiked in the following concentration levels: 50, 100, 200, and 400 μg/100 ml of FA.

Comparison of aqueous standard samples with matrix samples showed a process efficiency of 90% (±11%) (Matuszewski, Constanzer, & Chavez-Eng, 2003). The use of the IS compensated for the matrix effect.

A fortified vitamin juice was used as QC sample for each sample batch. One batch consisted of the packages of one juice throughout the entire year (13 sampling points with 3 individual packages). Our QC sample had been analyzed by three independent laboratories prior to our analyses. Measured mean concentrations of the QC samples (M = 146 μg/100 ml) were in good accordance to the results from the other laboratories (data of the inter-laboratory comparison can be found in the Supplemental). QC sample results of each batch were monitored via an individual moving range control chart (IMR-Chart). The precision of the QC samples over the range of the entire study (n = 16) was 6.5% (CV).

2.5. Juice samples

Samples (original packages) of nine commonly sold vitamin juices (juices from well-known brands as well as discounter products) were delivered from several producers in Germany right after the filling to the Max Rubner-Institut. Upon arrival the juices were stored in a dark room at 1 °C until the first sampling which marks the beginning of the study. We refer to the first sampling as the initial analyses. The packaging of the juices varied: glass bottles (brown and clear), PET (brown and clear) and cardboard boxes. The labeled FA concentration was 100 μg/100 ml, except for one juice with 60 μg/100 ml. A list of the vitamin juices with data of their packaging type, storage condition, label and storage time after filling can be found in Table 1.

2.6. Long term storage experiment

The study and therefore the initial analyses of the juices started as soon as all juices were available, which was between 11 and 32 days after filling by the producer.

The vitamin juices were stored for the duration of one year under controlled conditions. Samples from all juices (n = 9) were stored in the dark at 18 °C. Six of these came in light-transmissive packaging. Samples of these six juices were additionally stored under the influence of light (18 °C, 500 Lux for 10 h/day). These storage parameters were chosen to reflect common storage conditions, e.g., at a supermarket. Three independent juice packages