International Journal of Molecular Gastronomy
(Int J Mol Gast)

Time-Course Evolution of Two Water-soluble Vitamins (Ascorbic acid, Nicotinic acid) and Two Amino-Acids (L-Cysteine and L-Methionine) Following Thermal Processing in Water at 100 °C (part I)

Laetitia Le Falher,[a,b*] Vincent Faugeras,[a,b] Delphine Lioger,a Francisco X. Deolarte,[c] Hervé This[b, d]

[a] *Innit SAS, 76 rue de la pompe, F-75116 Paris, France.*
[b] *Groupe de Gastronomie Moléculaire, AgroParisTech-Inra, Laboratoire de Chimie Analytique, F-75005 Paris, France.*
[c] *Innit Inc, 600 Allerton – Suite 101 - Redwood city, California, United States.*
[d] *UMR Ingénierie Procédés Aliments, AgroParisTech, Inra, Université Paris-Saclay, 91300 Massy, France e*

Abstract:
Reactions of thermally processed vitamins and amino acids, in particular, ascorbic acid, nicotinic acid, L-cysteine and L-methionine were investigated in water at 100 °C. For such analysis, in situ quantitative nuclear magnetic resonance spectroscopy (*is* q ^{1}H NMR) was used which means that the samples were directly analyzed in water. This method has the advantage to be fast and non-invasive, without any extraction process. Under these experimental conditions, there were 18 % of ascorbic acid and 37 % of cysteine remaining after 96 h of reaction. Nicotinic acid and L-methionine were both found to be stable, even after 96 h of thermal processing.

Keywords:
Nutrients; time-course evolution; water; green analytical chemistry; *in situ* quantitative nuclear magnetic resonance.

Introduction

Macronutrients (proteins, amino-acids, saccharides and lipids) and micronutrients (vitamins, minerals and traces elements) are the key bioactive compounds in food#1#. During thermal treatments, their quantities in food are modified through chemical modifications and losses (compounds moving

outside of foods including the liquids from animal tissues during heat induced collagen contractions) [2,3]. In order to understand chemical modifications inside foods, it is important to study the reactivity of food compounds in conditions that mimics the internal part of food ingredients.

Vitamins and amino acids are particularly important because of their key role for good health.

Here is reported the study of two water-soluble vitamins, more precisely two organic acids, ascorbic acid and nicotinic acid. Ascorbic acid is reported to be sensitive to heat and readily oxidisable. It is involved in many biological processes in the human body such as the synthesis of collagen and of carnitine, and it is the most abundant vitamin in plant tissues (fruits and vegetables)[1,4]. Unlike ascorbic acid, nicotinic acid is described as stable to heat, light, oxidation and acid and basic conditions [5]. It acts as a co-enzyme in human metabolism and it is the most abundant vitamin in animal tissues [6]. The reactivity of a non-essential amino acid, L-cysteine, and an essential amino acid, L-methionine, were also investigated since they are reported as sensitive to oxidation and respectively a source of sulfur in human metabolism or building blocks of proteins [7,8].

In order to identify the chemical modifications of these compounds in food, the intracellular system was first modeled by heating these organic compounds alone in water at 100 °C for up to 96 hours. Water was chosen as the solvent of reaction since in food these bioactive compounds are often in the aqueous medium of the cytosol. The temperature of 100 °C was chosen because foods are mainly constituted of water, so that the inner temperature of food is generally lower than 100 °C at atmospheric pressure during home conventional heating for food production [9]. The time-course evolution of ascorbic acid in water was not much described in the food science literature. Serpen and Gökmen reported, as a control, the heating of ascorbic acid in water at 90 °C for 6 hours [10]. The loss of ascorbic acid was analyzed by high pressure liquid chromatography (HPLC) and was found to be about 72 % starting from an initial concentration of 200 mg/L. In their study on the browning phenomena of model solutions of ethanolic beverages [11], Chuang and others reported, for a standard, the heating of a solution of ascorbic acid in water (pH = 3) at 45 °C. After two days and analysis by HPLC, there is a loss of 26 % of ascorbic acid from an initial concentration of the solution of 0.5 mg/mL. Ascorbic acid could not be detected after 10 days of storage. A thorough review of the literature showed there was no study on the time-course evolution of nicotinic acid, L-cysteine or L-methionine during a thermal treatment in food or in aqueous media.

High Pressure Liquid Chromatography is the main analytical method used for the quantification of vitamins and amino acids. However, this method needs extraction steps which can be incomplete, and the development of analytical protocols can be time-consuming. In contrast, in situ quantitative proton nuclear magnetic resonance spectroscopy (*is* q ^{1}H NMR) can be helpful because it avoids any extraction process or sample preparation, since the reaction solvent is the same as the solvent used for NMR analysis. Moreover, it is a non-destructive, fast and sustainable method with a possibility of low variation coefficient. [12]

We reported here the time-course evolution of two water-soluble vitamins (L-ascorbic acid and nicotinic acid), and two amino acids (L-cysteine and L-methionine) in water at 100 °C up to five days..

Materials and Methods

Products

D_2O (99.9 %) and 3-(trimethylsilyl)propionic-2,2,3,3-d4 acid, sodium salt (TSP-d_4; 98 %) were obtained from Aldrich (Steinheim, Germany). Ascorbic acid RP normapur was

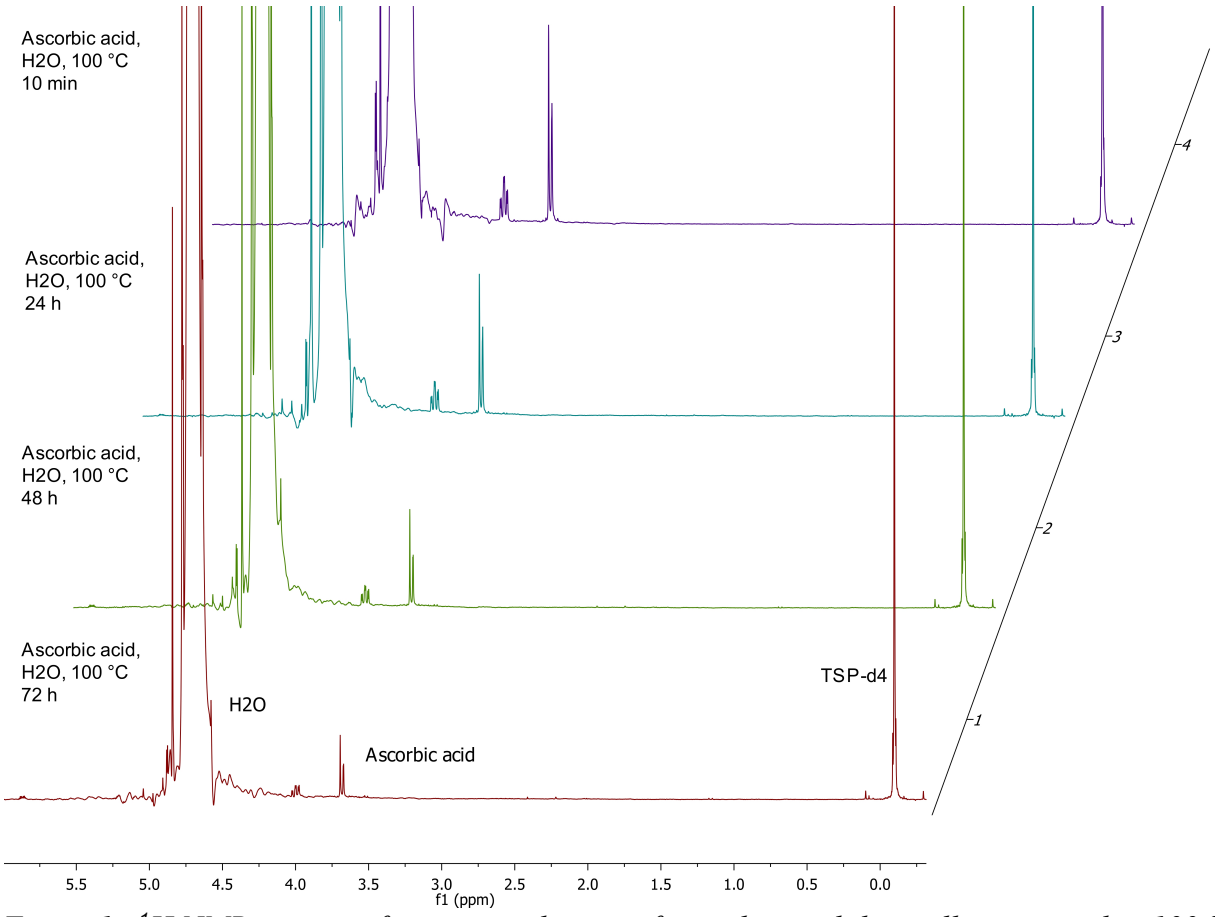

*Figure 1. ^1H NMR spectra of aqueous solutions of ascorbic acid thermally processed at 100 °C for 10 min (*top*), 24 h and 48 h (*middle*) and 72 h (*bottom*) at mass concentration C_m = 0.014 g/g. The amount of ascorbic acid decreased over five days. After 96 h, there was 18 % of ascorbic acid left in the reaction mixture, which corresponds to 2.2 mg of vitamin at 96 h over 12.5 mg at 10 min of reaction (Figure 2, (A)). We identified furfural using 1D and 2D ^1H NMR spectra since its resonances are in the aromatic zone and isolated from the other resonances.*

obtained from Prolabo, L-cysteine 97 % from Aldrich, L-methionine from Aldrich 98 %, nicotinic acid 99 % from Alfa Aesar. All samples and solutions, at each step of the analytical process, were weighed three times, using a 0.1 mg precision balance (Mettler Toledo AG 153).

NMR analysis

For NMR analysis, the *is* q NMR method was used [13]. A superconducting Ultrashield 300 MHz (7.05 T) 54 mm magnet NMR spectrometer BZH 30/300/70 E Bruker Biospin (Wissembourg, France) was used. All NMR spectra were recorded at 298 K with 32 scans of 32 K data points; they were acquired with a spectral width of 6 kHz and an acquisition time of 2.65 s. A recycle delay of 8 s per scan was set up for ascorbic acid and 18 s for L-cysteine, L-methionine and nicotinic acid per scan in order to allow complete relaxation and absolute quantification; the pulse angle was 90 °.

The aqueous solutions to be analyzed (0.7 g) were put directly in NMR tubes with a sealed capillary tube containing an aqueous solution

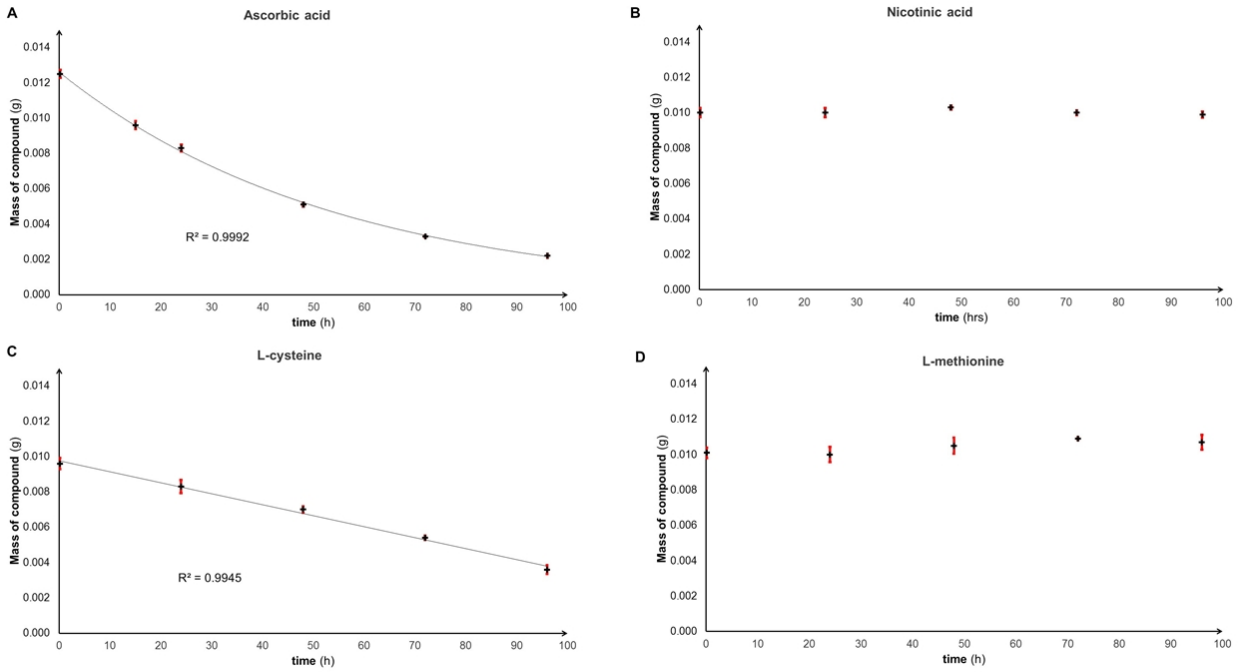

Figure 2. Time-course degradation in water at 100 °C of (A) ascorbic acid, (B) nicotinic acid, (C) L-cysteine and (D) L-methionine. Values are means for three replicates except for the point at 96 h for L-methionine which was done in duplication. The vertical bars show the standard deviation.

of TSP-d_4 in D2O (5 % of TSP). TSP-d_4 was used as internal reference and its chemical shift was set up at 0 ppm.

Spectra were acquired under an automation procedure (automatic shimming and automatic sample loading). They were Fourier-transformed with 0.3 Hz line broadening, phase and baseline were corrected using XWIN NMR 3.5 software (Bruker Biospin, Rheinstellen, Germany). The resonances in all spectra were integrated using NMR Notebook 2.7 (build 0.11, Illkirch-Graffenstaden, France) software.

For the interpretations of NMR spectra, chemical shifts (δ) are expressed in parts per million. The analysis of each sample was performed using TSP-d_4 as internal lock. For each compound, one resonance was integrated for the quantification study. We chose well separated resonance isolated from the resonance of water. Ascorbic acid was characterized using the multiplet at 3.77-3.75 ppm. L-cysteine was characterized using the multiplet at 3.17-3.01 ppm. L-methionine was characterized using the triplet at 2.65 ppm. Nicotinic acid was characterized using the multiplet at 8.11-8.06 ppm.

For quantification, the area of the TSP-d_4 resonance was used as a reference of area fixed to 1 (arbitrary units, a.u.). For each compound of interest, calibration curves were established from a mean of three calibration curves. Each calibration curve was obtained from four solutions containing a known mass of the compound of interest. Degradation curves of the processed compounds were performed using the equation of the calibration curve. All calculations were carried out using the software Maple 18 (Maplesoft, Waterloo Maple Inc, Ontario, Canada). Regression coefficient R^2 and graphs were calculated using the software Microsoft Excel 2013.

Thermal treatment of compounds

MilliQ water (14 g) was put in a 25 mL two

necked-round-bottomed flask fitted with a reflux condenser. The water was refluxed using a heater block Drysyn and 200 mg of compound were added to the boiled water. The two necked-round-bottomed flask was surrounded by aluminum foil in order to avoid heat losses and limit photochemical reactions. The reaction was refluxed up to 96 h depending of the stability of the compounds. Samples of the reaction mixture was taken, via a syringe B-Braun injekt-F fitted with a needle B-Braun Sterican, at different times according to the reaction degradation profile (0.17 h, 15 h, 24 h, 48 h, 72 h, 96 h). The first sample was carried out at 0.17 h to ensure the homogeneity of the solution. We considered that there was no degradation of compounds at 0.17 h to get the 100 % of mass of compound in the reaction mixture given the slow degradation of compounds. Samples were cooled down to room temperature before NMR analysis.
Each reaction was carried out three times to obtain statistical values.

Aknowledgments:
We thank Pr Arnaud Haudrechy for fruitful scientific discussion concerning the interpretation of results, and particularly we praise his expertise about solvent effects.

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