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Oxygen availability in model solutions and purées during heat treatment and the impact on vitamin C degradation

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ABSTRACT

Oxygen availability in different media during heat treatment (8 h at 80 °C) and the related vitamin C loss was assessed. Dissolved oxygen in water containing 3 mmol kg^{-1} of ascorbic acid decreased initially and seemed to be replaced by oxygen from the headspace in the course of time, as oxygen values increased again. In apple purée and carrot purée in contrast, oxygen was depleted within 60 min. Vitamin C in ultrapure water was stable even in the presence of oxygen. A trigger seemed to be crucial to launch vitamin C degradation. Fe^{3+} ions added to water, but also the media Mc Ilvaine citrate-phosphate buffer (pH 3.5) or apple purée, initiated degradation. Adding Fe^{3+} ions to apple purée did not accelerate vitamin C degradation but shifted the equilibrium between ascorbic acid and dehydroascorbic acid to the latter. Oxygen deprivation stabilized completely vitamin C, independently of the medium tested. A temperature decrease to 70 or 60 °C, in contrast, had no effect on the degradation extent of vitamin C in water containing 20 μmol kg^{-1} Fe^{3+} ions but led to complete stability in apple purée.

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1. Introduction

Vitamin C is well known for exhibiting beneficial health properties (Block, 1991; Gale, Martyn, Winter, & Cooper, 1995; Zandi et al., 2004). It is however susceptible to deterioration. In raw products, enzymes as for example polyphenol oxidase accelerate this reaction. Polyphenol oxidase catalyzes the oxidation of polyphenols which leads to consumption of ascorbic acid since it acts as reducer of generated quinones (Aka, Courtois, Louarme, Nicolas, & Billaud, 2013). At elevated temperatures, where enzymes are inactive, degradation still proceeds in significant amounts. The aerobic degradation pathway of ascorbic acid proceeds faster than the anaerobic one, which only occurs to significant amounts over 120 °C (Dhuique-Mayer et al., 2007; Oey, Verlinde, Hendrickx, & Van Loey, 2006; Verbeyst, Bogaerts, Van der Plancken, Hendrickx, & Van Loey, 2013). The availability of oxygen is thus crucial for the fate of vitamin C at an intermediate temperature range.

Oxygen is soluble in water up to 100 °C (Penicaud, Peyron, Gontard, & Guillard, 2012) Its saturation is temperature and matrix dependent. An increase of temperature, salinity or °Brix leads to a decrease of dissolved oxygen. Verbeyst et al. (2013) supposed that dissolved oxygen in strawberry and raspberry pastes in the range 80–120 °C was quickly consumed and led to the plateau they observed in terms of vitamin C degradation, even though heat treatment continued. Oxygen concentrations were however not measured simultaneously to degradation. In agar gel, a gradient of oxygen and vitamin C concentration occurs at 20 °C in the course of time with higher degradation near the surface (Penicaud, Broyart, Peyron, Gontard, & Guillard, 2011). The role of dissolved oxygen in citrus juice on vitamin C degradation at 90 °C was studied by nitrogen substitution of initial dissolved oxygen (Dhuique-Mayer et al., 2007). However, as gases' solubility is decreased by heat, it is very likely that in this study, the liberation counted also for nitrogen and thus the oxygen-to-nitrogen-ratio can be assumed to have changed in the headspace too. Hence, a lower vitamin C degradation rate which was associated to lower dissolved oxygen contents, was presumably rather a combination of headspace and dissolved oxygen depletion.

Up to now it is not known how fast oxygen is consumed at elevated temperatures and if it is replaced quickly from the headspace. In addition, other oxidizable components are present in real food products contributing to oxygen consumption. Dissolved oxygen measurements at higher temperatures were difficult to achieve in the past. Measurements at precise locations became possible due to development of oxygen sensors (Liebsch, Klimant,

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Frank, Holst, & Wolfbeis, 2000) that can be installed on recipients' walls and are specially calibrated for elevated temperatures.

Besides oxygen, other food oxidants such as Fe^{3+} ions interfere in the degradation of ascorbic acid. Fe^{3+} ions accelerate the oxidation of ascorbic acid to dehydroascorbic acid and the hydrolysis of dehydroascorbic acid to 2,3-diketogulonic acid, by which the vitamin activity is lost (Serpen & Gökmen, 2007). This seems to result from of a redox reaction with ascorbic acid being oxidized and Fe^{3+} ions being reduced to Fe^{2+} ions. Fe^{2+} ions, in turn, react in the Fenton reaction with hydrogen peroxide (Choe & Min, 2005) which is formed during the oxidation of ascorbic acid (Boatright, 2016). However, it is not known if the concomitant presence of oxygen is necessary for the Fe^{3+} ion-caused oxidation. Under anaerobic conditions and in the presence of Cu^{2+} ions, the stability of ascorbic acid can almost completely be preserved in contrast to aerobic conditions where ascorbic acid degrades rapidly (Boatright, 2016).

The aim of the present study was to reveal oxygen availabilities in different media during heat treatment at 80 °C and to understand the influence of Fe^{3+} ions, ascorbic acid concentrations and temperature. Furthermore, the linked vitamin C degradation was investigated.

2. Material and methods

2.1. Chemicals and food matrices

2,2′-Bipyridyl, ascorbic acid, trichloroacetic acid, DL-dithiothreitol, Na_2HPO_4, $NaH_2PO_4xH_20$, *N*-Ethylmaleimide and citric acid monohydrate were from Sigma-Aldrich (Deisenhofen, Germany). *Ortho*-phosphoric acid 85%, Iron(III)chloride hexahydrate from VWR (Leuven, Belgium) and ethanol from by Fisher Scientific (Fair Lwan, NJ, USA).

Apple purée (brand: HIPP) and carrot purée (brand: POUCE) without added vitamin C were purchased in a local supermarket in Avignon/France.

2.2. Supplementation and heat treatment

Ascorbic acid supplementation was carried out in corning tubes. After addition of ascorbic acid, samples were vortexed thoroughly, then transferred to a beaker and preheated to the respective temperature of interest while stirring. When temperature was achieved, the sample was immediately conveyed to a double walled system (Société Legallais, Montferrier-sur-Lez, France, Fig. 1) which was filled up to a height of 1 cm. The inner diameter of the vessel was 4.5 cm and the depth of the inner volume 2.7 cm. The double walled glass vessel was connected to a water bath (ED-19 Julabo, Seelbach, Germany) to maintain temperature of the medium. To avoid water evaporation, a plastic cap supported by silicon fat was put onto the top. As a consequence, the headspace became vapor saturated. Aliquots were withdrawn after the preheating step, corresponding to the initial value, and at the end of the heat treatment, and immediately deep-frozen (≤ -18 °C).

When the effect of oxygen deprivation was evaluated, nitrogen was bubbled into a stirred and heated solution. Subsequently, the solution was transferred to a 12.5 cm glass tube (diameter 1 cm) which was filled up to 4.5 cm under nitrogen flow. The tube was closed with a cap including a septum and allowing thus to maintain anaerobic conditions. The heat treatment was carried out by putting the glass tubes in a block heater (Stuart; Roissy Charles de Gaulle, France).

2.3. Oxygen measurement

Planar oxygen sensors (Presens GmbH, Regensburg, Germany) with a diameter of 5 mm and calibrated up to a temperature of 80 °C were stuck with silicon glue at the inner bottom, at a height of 1 cm and in the headspace region of the double walled glass vessel (Fig. 1). Oxygen was measured from the outside of the vessel via a polymer optical fiber connected to a Fibox 4 Transmitter (Presens GmbH, Regensburg, Germany). Temperature in the vessel was measured by a sensor Pt100 (Presens GmbH, Regensburg, Germany) which was also linked to the Transmitter.

2.4. Vitamin C analysis

Vitamin C was quantified spectrophotometrically (Stevens, Buret, Garchery, Carretero, & Causse, 2006). Approximately 500 mg aliquots were taken and absorption was measured at 525 nm on a spectrophotometer (Safas Xenius, Monaco).

3. Results

3.1. Treatment in water

Ultrapure water served as reference medium. After transferring the preheated solution to the double walled system, oxygen contents reached equilibrium in approximately 15 min (Fig. 2A), and then remained stable at 0.07 mmol L^{-1} in all three locations until the end of the measurement time. The oxygen content is in the range reported by Penicaud et al. (2012) for ultrapure water at 80 °C. Oxygen equilibrium in the headspace and the medium was attained at the same pace.

In water containing 3 mmol kg^{-1} of ascorbic acid, oxygen contents dropped as in ultrapure water at the beginning of measurements (Fig. 2B). The decrease in the liquid was however higher than in the headspace, which can be ascribed to oxygen consumption by ascorbic acid. Oxygen was not entirely consumed even though ascorbic acid was in molar excess compared to oxygen (Fig. 2B). Since oxygen concentrations increased again, oxygen consumption and diffusion from the headspace into the medium seemed to compete and to lead to a dynamic equilibrium. In addition, as the oxygen consumption rate at the bottom and the surface of the medium was not significantly different, oxygen diffusion appeared to be a continuous and fast process at 80 °C. The headspace oxygen level was however not reached again in the medium within the 8 h.

Furthermore, in spite of oxygen consumption and increase of dehydroascorbic acid, the overall vitamin C content did not decrease (Fig. 2). Buettner (1988) observed also high stability of ascorbic acid when the medium was completely deprived of metals.

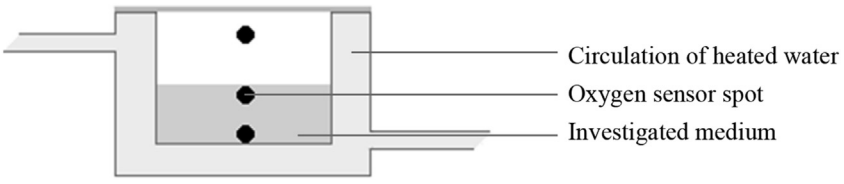

Fig. 1. Double-walled vessel containing medium and three oxygen sensor spots.

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