Design and Exploitation of a New Experimental Device to Forecast the Degradation of Nutritional Quality and the Inactivation of Microorganisms in Canned Vegetables.

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Design and exploitation of a new experimental device to forecast the degradation of nutritional quality and the inactivation of microorganisms in canned vegetables

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ABSTRACT

Sterilization is the most relevant technique to guaranty safety of shelf-stable food products at ambient temperature. However, these thermal processes are often optimised to microbiological aspect and consequently are not without consequences on quality. Only few studies are focussed on the simultaneous management of these two aspects. Ascorbic acid is of interest due to its vulnerability and health benefits. Its degradation at sterilization temperature (> 100 °C) is poorly documented. The aim of this work is to model thermal processes in a risk/benefit approach, taking in account the nutritional aspect. Its final objective is to design new thermal processes which will be efficient to inactivate heat-resistant microorganisms, but not too drastic in order to maintain the maximum quality. Using a new instrumented reactor (thermoresistometer Mastia®), the behaviors of ascorbic acid food model solution at pH = 3.5, in aerobic and anaerobic conditions, was measured. The obtained data have shown that degradation of ascorbic acid does not follow classical kinetics in conditions of appertization, it follows 0.5-order kinetics in aerobic conditions and zero-order kinetics in anaerobic conditions.

Keywords: Thermoresistometer; benefit-risk; modelling; heat-resistance; nutritional qualities

INTRODUCTION

Thermal treatment is the most popular and efficient method to inactivate microorganisms in canned vegetables in order to make them stable (Blasco, Esteve et al. 2004, Peng, Mah et al. 2012, Zimmermann, Longhi et al. 2014). Currently, there is an increased demand of nutritive foods, thereby, there were many attempts to maximise nutrients retention during industrial process as well as during transport and storage (Sapei and Hwa 2014). To be efficient, a thermal treatment must ensure that the product has been exposed at sufficiently high temperature for a sufficient time in order to destroy an appropriate number of targeted microorganisms (Zimmermann, Longhi et al. 2014). Nevertheless, thermal treatment can generate undesirable chemical reactions like vitamin degradation, which can lead to products having less nutritive interest. In the literature, only few studies are focussed on the management of both the chemical and microbiological aspects, *a priori* antagonistic. These two aspects were studied in this work by choosing two entities which allowed us to take in account the benefit/risk balance. Ascorbic acid was chosen for the chemical aspect due to its health benefits on the one hand (Arya, Mahajan et al. 1998, Furusawa 2001, Castro, Teixeira et al. 2004, Derossi, De Pilli et al. 2010, Kokkinidou, Floros et al. 2014) and for its thermolability on the other hand (Lin and Agalloco 1979, Torregrosa, Esteve et al. 2006, Mesías-García, Guerra-Hernández et al. 2010, Hsu, Tsai et al. 2012, Bosch, Cilla et al. 2013, Sapei and Hwa 2014).

Ascorbic acid degradation at sterilization temperatures is poorly known, probably because of the difficulty to work at temperature higher than 100 °C. Using a new experimental device, the thermoresistometer Mastia®, we studied the degradation of ascorbic acid at sterilization temperature in model solution. The thermoresistometer Mastia® is used in microbiology to determine heat resistances of microorganisms (Condón, Arrizubieta et al. 1993, Palop, Marco et al. 1997, Palop, Sala et al. 1997, Palop, Raso et al. 1999). This is the first study in which this device is used in chemistry in order to determine heat resistances of chemical compounds. This appliance has proven its potential in a lot of studies of heat resistances of

various microorganisms (like spore-formers bacteria), both in model solution and in various matrices, during isothermal or non-isothermal treatments and for more than 20 years (Condón, Arrizubieta et al. 1993, Garza, Teixidó et al. 1994, Raso, Palop et al. 1995). It is nowadays already used and it has undergone several improvements to be more and more efficient and robust (Esteban, Conesa et al. 2015, Gayán, Serrano et al. 2015, Gironés-Vilaplana, Huertas et al. 2016, Maté, Periago et al. 2016). The accuracy of the thermoresistometer allows determining *D*-values at the nearest thousandth of a minute (Condón, Arrizubieta et al. 1993), especially by its fast homogenization. This advice was chosen in order to go beyond the state-of-the-art, that is to say work at temperature higher than 100 °C under pressure in order to study the degradation kinetics of ascorbic acid, under aerobic and anaerobic conditions. The potential of the thermoresistometer must be validated for chemical studies.

MATERIALS & METHODS

Reagents. All the reagents used were purchased from Fisher Chemical and are analytical grade or better.

Preparation of ascorbic acid solution. A 5.0000 g accurately weighed portion of L-ascorbic acid was dissolved in 25 mL of 2.31 % of acetic acid solution at pH = 2.6 in a 25 mL volumetric flask. The mixture is then stirred until complete dissolution of ascorbic acid. 1.8 mL of this solution was injected inside the vessel of the thermoresistometer, in order to have an initial concentration of ascorbic acid in the heating media at 90 mg/100 mL.

Model solution. Citrate-phosphate McIlvaine buffer (pH = 3.5) was used as food model solution. McIlvaine buffer was prepared using a 0.1 M monohydrate citric acid solution and a 0.2 M disodium phosphate solution (McIlvaine 1921). The buffer was stored at 4 °C until used.

High-Performance Liquid Chromatography – Analysis of ascorbic acid. We used a 1260 Agilent infinity LC chromatograph with a 1290 Agilent diode-array detector. A 150 x 3.0 mm Synergi Polar RP-C18 column (Phenomenex) was used with a precolumn and both have a particle size of 4 µm. The mobile phase was a 2.31 % acetic acid solution as eluent A and acetonitrile as eluent B. The flow rate was fixed at 0.8 mL.min^{-1}, the column temperature was set at 30 °C and the injection volume was 5 µL. A gradient program was performed as follows: the initial conditions were 96 % A / 4 % B; 0-5 min, 96 % A / 4 % B; 5-10min, 80 % A / 20 % B; 10-15 min, 96 % A / 4 % B. The data acquisition was assessed at 245 nm, corresponding to the maximum absorbance of ascorbic acid in UV-visible spectrometry (Jaffe 1984). This analysis method was inspired by Louarme and Billaud (Louarme and Billaud 2012). Quantification of ascorbic acid was carried out by external standard method with a calibration curve, which is the mean of five calibration curves established by five standard solution of ascorbic acid injected in HPLC by the same method.

Determination of heat resistances by the thermoresistometer Mastia®. Thermal treatments were carried out in a thermoresistometer TR-SC Mastia® (figure 1). It's operation system is well described by Conesa et al. (Conesa, Andreu et al. 2009). This experimental device allows working on a temperature range from 20 °C to 150 °C under pressure, using different gases like air or nitrogen, having a constant and regulated stirring, sampling or injecting at any time during the heat treatment, even under pressure, without perturbation of the experimental media, working at important volumes (maximum 400 mL), and simulating isothermal and non-isothermal heat treatments.
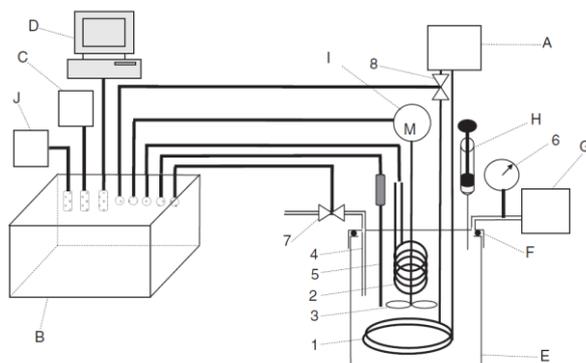
Figure 1. Diagram of the Thermoresistometer Mastia

The thermoresistometer TR-SC possesses a 2 kW heating electric element (2) and a cool ring (1) in which one cold water (2 °C) provided by the cooling system (A) is circulating. This advice is implemented with a programmable logic control (PLC) (B), which powers heating electric element and the cooling system. The PLC is connected to a tactile screen (C) allowing communication with to the PLC, and a computer (D) provided with a software enable to program or/and register temperature profiles (Conesa, Andreu et al. 2009). The working media is put in a classical stainless vessel or a Teflon™-coated stainless vessel (8.5 x 12 cm outer diameter) respectively for microbiological or chemical studies (E), which is screwed on the thermoresistometer cap with an O-ring (F). This cap has a stirring shaft with a propeller (3) which is powered by the stirring motor (I), and eight ports with screw cap; one is holding the pressure source (G), another is the injection port and contains a gas chromatography septum, a third one holds the sampling tube (4), another holds the thermocouple (5), two ports maintain the electric element and two others maintain the cooling ring.

The vessel can be pressurized by a circuit including a manometer (6) connected to the pressure source (G). Pressurization is needed to allow extraction of samples and to avoid boiling at temperatures higher than 100 °C in aqueous solutions. A specific Hamilton-type syringe (H) was used to injected samples in the vessel in order to overcome the pressure.

For all experiments, the pressure inside the vessel was set at 0.2 MPa. The temperature range studied is from 95 °C to 115 °C. Aerobic conditions were performed by using air as headspace gas. Anaerobic conditions were performed by removal of oxygen from the media by heating to 95 °C during 30 minutes and bubbling nitrogen *in situ* during 30 minutes at 20 °C through the sampling tube, and by using nitrogen as headspace gas. Each thermal treatment lasted 320 minutes.

Analysis of data. All experiments (full kinetics) were repeated at least two times and each point was sampled twice; the results are reported as average.

RESULTS & DISCUSSION

Modulating conditions for ascorbic acid degradation

As oxygen is a major co-factor in ascorbic acid degradation, a comparison of aerobic and anaerobic conditions was a primordial test of the thermoresistometer's potential. To obtain anaerobic conditions, the media was first degassed by heating followed by nitrogen bubbling.
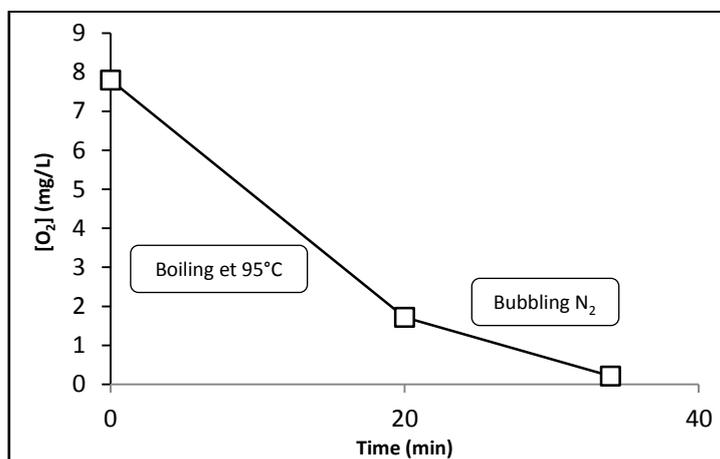

Figure 2. Evolution of initial oxygen concentration after different steps of deareation

This efficiently allowed establishing anaerobic conditions, followed by exclusive use of N_2 for pressurising the thermoresistometer vessel.
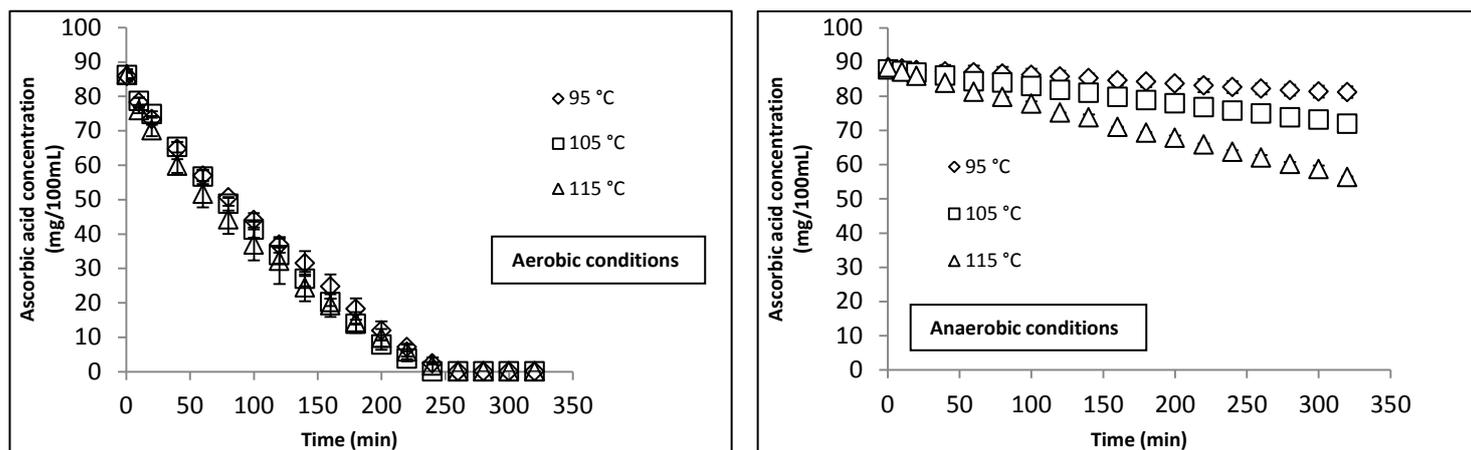
Figure 3. Comparison of ascorbic acid degradation under aerobic and anaerobic conditions at 95 °C (◊), 105 °C (□) and 115 °C (Δ)

In aerobic conditions, the temperature had a limited effect on the ascorbic acid degradation in the chosen range of temperature (95°C to 115°C). In fact, the degradation curves were superimposed regardless of temperature. Though ascorbic acid is reputed to be a fragile molecule, total degradation of an initial 90 mg/100 mL solution required times > 3h in our conditions. Further, the ascorbic acid concentration was observed to follow a linear or almost linear decrease with time.

In anaerobic conditions all the ascorbic acid was not degraded even after 320 minutes of treatment and regardless of the temperature. Moreover, the degradation of ascorbic acid was only of around 8 % at 95 °C, around 18 % at 105 °C and around 36 % at 115 °C after 320 minutes. Contrary to aerobic conditions, there was a clear effect of temperature in anaerobic conditions. The degradation levels used were too limited to confidently assess the reaction order, but linear losses were observed within the durations used.

CONCLUSION

Degradation of ascorbic acid in aerobic and anaerobic conditions at high temperature (> 100 °C) could be compared. Slower degradation was obtained in anaerobic conditions. In aerobic conditions, the kinetics did not follow the pseudo-first order commonly reported in the literature. This might be linked to oxygen availability which was reduced due to the high temperatures that limit its diffusion from headspace to the heating media.

We have confirmed that using the thermoresistometer Mastia® for chemical studies can be relevant to override the limits about temperature or oxygen availability. This device allowed us to work properly under high temperature firstly and under anaerobic conditions secondly. The possibility to sample at any time of an experiment without perturbing the heating was a great advantage. Moreover, the repeatability was excellent: we have determined low standard deviations between the two replications for each experiment. The potential of the thermoresistometer Mastia® has to be exploited in order to perform both chemical and microbiological studies in a benefit/risk approach.

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