



## Effect of red sweet pepper dehydration conditions on Maillard reaction, ascorbic acid and antioxidant activity



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### ABSTRACT

In this study we evaluated the application of low pressure-controlled temperature drying in the production process of dehydrated sweet red pepper and contrast the quality of this dehydrated vegetable in terms of the concentration of some heat liable components (ascorbic acid), decrease on the antioxidant activity and formation of furosine (Amadori compound of lysine formed during the Maillard reaction). Analysis of data showed that furosine is a good indicator for controlling the dehydration process of red pepper. In addition, the stability of the antioxidant activity and proteical damage (measured as furosine formation) in red pepper is a function of the dehydration conditions: Higher temperatures exert a strong influence on the kinetics of degradation, accelerating the rate of decomposition of antioxidant compounds and the loss of proteical quality.

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### 1. Introduction

The pericarp of the pepper fruit (*Capsicum annuum* L.) accumulates considerable amounts of a wide array of phytochemicals mainly vitamins C, A and E, as well as phenolic and carotenoids compounds with well-known antioxidant properties (Deepa et al., 2007) which conferring protection against carcinogenic components and delaying the aging process (Simonne et al., 1997). Due to high content of bioactive compounds (Daood et al., 1996) the red pepper is considered as important healthy foods or food ingredients. The market for dehydrated vegetables has increased over recent years to provided products with long-shelf life foods which are easy to handle and store. Also, dehydrated vegetables are being used in health foods and formulation of nutraceutical products. Vegetables can be dried using various methods and their quality depends on many parameters such as vegetable variety, total soluble solid content of the fresh product, air humidity, size of the vegetable segments, air temperature and velocity, and the efficiency of the drying system (Sagar and Suresh Kumar, 2010). Dehydrated pepper has commonly been obtained by hot air drying, which allows rapid and massive processing, although the maintenance of nutritional and commercial quality of this pepper through the process has presented some serious problems in the past (Simal et al., 2005). However, the use of vacuum systems allows the reduction of the applied temperature and/or dehydration time, giving rise to the improvement of the overall quality of foods.

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During the drying operation, chemical modifications take place such as the Maillard reaction, which are responsible of nutritional and sensorial quality changes (Legault et al., 1954; Peleg et al., 1970). This reaction is favoured in systems with intermediate moisture content, temperatures over 50 °C, pH 5–7 and long processing times, which are the main characteristics of the dehydration process. Furosine [ $\epsilon$ -N-(2-furoylmethyl-L-lysine)], produced by acid hydrolysis of the Amadori compounds is a good heat damage index in many vegetable foods such as dehydrated garlic and onion (Cardelle-Cobas et al., 2005; Rufián-Henares et al., 2008), dehydrated fruits (Sanz et al., 2001), tomato products (Schröder and Eichner, 1996), soybeans, barley and malt (Molnár-Perl et al., 1986), potatoes, carrots and rice (Resmini and Pellegrino, 1991; Wellner et al., 2011), fruit-based infant foods or jams (Guerra-Hernández et al., 1999; Rada-Mendoza et al., 2004), roasted peanuts (Wellner et al., 2012) among others. In addition, furosine and other furoylmethyl derivatives formed from Amadori compounds are originated before the occurrence of sensory changes. Therefore, their determination provides a very sensitive indicator for early detection of quality changes caused by the Maillard reaction (Olano and Martínez-Castro, 1996) and are suitable for controlling the dehydration process because of the lower temperatures used in this process compared to more drastic ones like baking.

In a previous paper (Rufián-Henares et al., 2008) it was demonstrated the presence of furosine and hydroxymethylfurfural in different kind of vegetables dehydrated under vacuum. In addition, the usefulness of furosine as a quality indicator for controlling the dehydration process was hypothesised. Therefore, the objective of this research was to assess the influence of dehydration time

and temperature on Amadori compounds formation and complementary over the stability of the ascorbic acid and antioxidant activity in red sweet pepper. To reach this goal, the furosine content as well as the ascorbic acid content and overall antioxidant activity of red pepper was measured during the dehydration process under vacuum at different temperatures and appropriate times to reach a suitable final humidity.

## 2. Materials and methods

### 2.1. Chemicals

2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-Azobis(2-amidino-propane) dihydro-chloride (AAPH), ascorbic acid (AA) and potassium persulphate were purchased from Sigma (St. Louis, MO, USA). 2,2'-azobis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) were from Fluka Chemicals (Madrid, Spain). Iron (III) chloride, sodium phosphate monobasic, sodium chloride, hydrochloric, metaphosphoric, and sulphuric acids, methanol, acetone and ethyl ether were purchased from Panreac (Madrid, Spain).

### 2.2. Samples

Ripe fruits of red sweet pepper *Capsicum annuum longum* L. var. Lamuyo were obtained from a local store. Fresh peppers were chosen with a similar size (10–14 cm length, 9–10 cm width, 400–500 g each fruit) and red colour. Three kilograms were immediately processed and stored before dehydration. The processing consisted on the removal of seeds and stalk, being the rest chopped and homogenised with a Moulinex A320 blender (Moulinex Iberica, Barcelona, Spain). This procedure is similar to that used by the local vegetable products company which provided the dehydrated samples used in a previous paper (Rufián-Henares et al., 2008). Subsamples each of 15 g were set in round-bottom glass evaporation flasks and stored at  $-20^{\circ}\text{C}$  before dehydration.

### 2.3. Dehydration process

The dehydration, up to a 4.5 g water/100 g sample, was performed at laboratory scale with a rotary evaporator Büchi R210 (Büchi Labortechnik AG, Flawil, Switzerland). Samples were dehydrated in duplicate at low pressure and controlled temperature ( $60\text{--}90^{\circ}\text{C}$ ) for 7.5, 10.0, 12.5, 15.0, 17.5, 20.0, 22.5, 25.0, 30.0 and 45 min. Dehydrated samples were allowed to cool at room temperature and then stored at  $-20^{\circ}\text{C}$ .

### 2.4. Furosine assay

Furosine determination was performed following the method described by Resmini and Pellegrino (1991). Samples containing 6.5 mg of protein per ml of 7.95 mol/l HCl were hydrolysed at  $120^{\circ}\text{C}$  for 23 h in a Pyrex screw-cap vial with PTFE-faced septa. High-purity  $\text{N}_2$  gas bubbled through the solution for 2 min. The hydrolysate was filtered with a medium-grade paper filter. A 0.5 ml portion of the filtrate was applied to a Sep-pack  $\text{C}_{18}$  cartridge (Waters, Milford, MA, USA) prewetted with 5 ml of methanol and 10 ml of deionised water and was then eluted with 3 ml of 3 mol/l HCl. Fifty microlitres of the solution were analysed by ion-pair RP-HPLC, which consisted on a Waters model 600 quaternary gradient bomb (Milford, MA, USA) and a UV/VIS detector model 200 from Konik (Barcelona, Spain) set at 280 nm. The analytical column was a  $\text{C}_8$  column (250 mm  $\times$  4.6 mm i.d.) from Altech (Furosine dedicated) thermostated at  $32^{\circ}\text{C}$ . Quantisation was performed by the external standard method. A standard stock

solution containing 744 mg/ml of furosine (NeoMPS, Strasbourg, France) in 0.1 M HCl was used to prepare the working standard solution. The calibration was realised by adding increasing quantities of furosine standard, within the expected concentration range, to a raw pepper hydrolysed sample. These hydrolysed were filtered and applied to a Sep-pack  $\text{C}_{18}$  cartridge. The curve was constructed in units of area against micrograms of added furosine. The equations for the curves were  $Y = 6.25 \times 10^6 X + 5283$  for low concentrations, between 0.00135 and 0.0636  $\mu\text{g}/50 \mu\text{l}$  of eluate injected ( $r^2 = 0.994$ ) and  $Y = 6.76 \times 10^6 X + 57532$  for high concentrations, between 0.0636 and 0.5510  $\mu\text{g}/50 \mu\text{l}$  of eluate injected ( $r^2 = 0.993$ ) where Y is the peak area and X the  $\mu\text{g}$  of furosine.

The analysis was performed in duplicate and the data are the mean values expressed as milligrams per 100 g of protein.

### 2.5. Ascorbic acid assay

The analysis of ascorbic acid was performed following the method of Giménez et al. (2002) with slight modifications. Portions of 25 mg of dehydrated sample were mixed with 2.5 ml of a 10% (w/v) metaphosphoric acid solution, and then diluted to a final volume of 25 ml in an amber glass volumetric flask with bi-distilled de-ionised water. The sample was centrifuged at 16,500 g/15 min (Hettich Universal 32, Hettich GmbH, Tuttlingen, Germany). Two millilitres of clear supernatant were filtered with 0.2  $\mu\text{m}$  nylon filters (Millipore, Bedford, MA, USA) and then submitted to HPLC analysis. Twenty microlitres of the above mentioned solution were analysed by RP-HPLC, which consisted on a binary LC pump model Prostar 250 (Varian Inc., Palo Alto, CA, USA), an UV/Vis detector and an autosampler. The chromatographic column was a Novapak  $\text{C}_{18}$  (250 mm  $\times$  4.6 mm i.d.; 4  $\mu\text{m}$ ) from Waters (Milford, MA, USA) thermostated at  $25^{\circ}\text{C}$ . Ascorbic acid was released under isocratic conditions using bi-distilled de-ionised water acidified with sulphuric acid to pH 2.2 as solvent at a flow rate of 0.6 ml/min. A calibration curve was established by measuring ascorbic acid peak areas over an eightfold concentration range (from 0.8 to 153.6 mg/l). The analysis was performed in duplicate and the data are the mean values expressed as milligrams per gram of dry weight.

### 2.6. Antioxidant assays

The extracts used for the antioxidant activity were obtained following the procedure described by Mínguez-Mosquera and Hornero-Méndez (1993). Samples of 25 mg were extracted with 5 ml of acetone:water (60:40) using an ultrasonic liquid processor Vibra.Cell VC365 (Sonic & Materials, Danbury, CT, USA) set at 90 of intensity (0–100 scale, 20 kHz) during 8 min. Samples were sonicated in an ice-water bath in order to avoid excessive heating and degradation of carotenoids. After sonication, samples were centrifuged at 16,500 g/5 min (Hettich Universal 32, Hettich GmbH, Tuttlingen, Germany) and the supernatant removed. The process was repeated twice performing the extraction with a vortex during 15 s and a subsequent centrifugation. A final extraction with 10 ml of acetone:water was done, being the four extracts combined, filled up to 25 ml and stored until analysis.

#### 2.6.1. DPPH assay

The antiradical activity of different samples was estimated according to the procedure reported by Yen and Chen (1995). A 500  $\mu\text{l}$  aliquot of the extract or trolox standard was added to 1 ml of methanolic solution of DPPH· (74 mg/l). A daily-prepared solution of DPPH· gave a final absorption at 520 nm of 1.8 AU. Mixture was shaken and allowed to stand for 1 h at room temperature, and then absorption was measured at 520 nm in a Lambda 25 spectrophotometer (Perkin Elmer, Waltham, MA, USA). The

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