Quality parameters in convective dehydrated carrots blanched by ultrasound and conventional treatment

Running title: **Quality parameters in blanched dehydrated carrots**

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ABBREVIATIONS

MR: Maillard reaction
2-FM-AA: 2-furoyl-methyl amino acids
2-FM-Lys: furosine
US: high intensity ultrasound
TPC: total phenolic content
GC-FID: gas chromatography-flame ionization detector
RP-HPLC: reverse phase-high performance liquid chromatography
SDS-PAGE: Sodium dodecyl sulfate - polyacrylamide gel electrophoresis
RR: rehydration ratio

ABSTRACT

The effect of previous ultrasound and conventional treatments on drying and quality parameters (furosine -as indicator of lysine participation in the Maillard reaction-, carbohydrates, total polyphenols, protein profile, rehydration ratio, microstructure changes) of convective dehydrated carrots has been assessed. The most striking feature was the influence of blanching on the subsequent furosine formation during drying, probably due to changes in the protein structure. The highest values of furosine were found in carrots conventionally blanched with water at 95 ºC for 5 min. However, samples previously treated by ultrasound presented intermediate values of furosine and carbohydrates as compared to the conventionally blanched samples. Dried carrots previously subjected to ultrasound blanching preserved their TPC and showed rehydration properties, which were even better than those of the freeze-dried control sample. The results obtained here underline the usefulness of furosine as an indicator of the damage suffered by carrots during their blanching and subsequent drying.

KEYWORDS: carrot, blanching, ultrasound, convective dehydration, quality parameters, polyphenols, carbohydrates, furosine, protein, rehydration ratio, microstructure.

1. Introduction

As pointed out by different epidemiological studies, the risk of suffering several degenerative pathologies, such as cancer and cardiovascular diseases, can be decreased with a high intake of vegetables (Liu, Manson, Lee, Cole, Hennekens, Willet & Buring, 2000; Riboli & Norat, 2003). In this sense, their high contents of β-carotene, vitamins C, B1, B2, B6 and B12, folic acid, potassium, magnesium and pectin make carrots (*Daucus carota* L.) one of the healthiest vegetables (Erenturk & Erenturk, 2007). However, as with the rest of vegetables, carrots are highly seasonal and abundantly available at particular times of the year. For extending the availability of this root, several preservation processes have been assayed. Among them, drying is one of the most important since it not only significantly extends vegetable shelf-life but also diversifies the offer of foods for consumers (Lewicki, 1998).

The most common dehydration technique used in the vegetable industry is hot air drying under forced convection since it offers the advantages of low complexity and cost (Garcia-Noguera, Oliveira, Gallao, Weller, Rodrigues & Fernandes, 2010). Several studies have been performed on the drying of carrots; modelling of the process was one of the most important aspects studied (Erenturk & Erenturk, 2007; Mulet, Berna & Roselló, 1989). However, convective drying can also give rise to significant chemical changes (non-enzymatic browning, among others), which may affect the quality of the product. Most of the browning occurring during drying and subsequent storage is via the Maillard reaction (MR) (Mcbean, Joslyn & Nury, 1971). In this sense, the usefulness has been recently demonstrated of 2-furoyl-methyl amino acid derivatives (2-FM-AA) and, particularly of furosine (2-furoyl-methyl-Lys), as sensitive indicators for early detection of MR advance in carrots subjected to drying before important changes in nutritive value can be produced (Rufián-Henares, García-Villanova & Guerra-

Hernández, 2008; Soria, Olano, Frías, Peñas & Villamiel, 2009a; Soria, Corzo-Martínez, Montilla, Riera, Gamboa-Santos & Villamiel, 2010; Wellner, Huettl & Henle, 2011).

Moreover, the microstructure of vegetables might also be damaged during drying. Thus, the loss of integrity of the cell membranes, loss of turgor and deterioration of cell wall structure might result in significant shrinkage and loss of the rehydration potential of dehydrated vegetables (Lewicki, 1998).

The quality of dried products is not only affected by the drying conditions but also by other operations such as the pre-treatment of the material (Negi & Roy, 2001). Blanching can reduce the initial number of microorganisms, inactivate enzymes, remove gases from surface and intercellular spaces to prevent oxidation and reduce drying time (Rahman & Perera, 1999). Typically, blanching is carried out by treating the vegetable with steam or hot water for 1-10 min at 75-95 °C; the time/temperature combination selected is dependent on the type of vegetable. In the case of carrots, low-temperature/long-time and high-temperature/short-time blanching methods have been applied (Sanjuán, Hernando, Lluch & Mulet, 2005; Shivhare, Gupta, Basu & Raghavan, 2009).

In addition, other methodologies such as high-intensity ultrasound (US) have emerged as an alternative pre-treatment, increasing the mass transfer rate during drying. A number of works have been carried out on the application of US before conventional drying and as a medium to assist osmotic dehydration of vegetables and fruits (Jambrak, Mason, Paniwnyk & Lelas, 2007; Opalic, Domitran, Komes, Belscak, Horzic & Karlovic, 2009; Azoubel, Melo-Baima, Rocha-Amorim & Oliveira, 2010; Fernandes, Rodrigues, Law & Mujumdar, 2011; Rawson, Tiwari, Tuohy, O'Donnell & Brunton, 2011). Most of these works have been carried out in ultrasonic baths at mild

temperatures and have been mainly focused on the kinetic of moisture loss during drying: US: showed a noticeable reduction in the overall drying time and gave rise to a variable loss of total sugars. In carrots, our research group (Gamboa-Santos, Montilla, Soria & Villamiel, 2012a; Gamboa-Santos, Soria, Pérez-Mateos, Carrasco, Montilla & Villamiel, 2013a), has studied the inactivation of peroxidase (POD) and pectin methyl esterase (PME), the losses of soluble compounds by leaching and the sensorial properties of dehydrated carrots blanched conventionally or by US (in a bath or with probe treatments). In the present paper, the effect of different blanching (US and conventional) processes on the kinetic of drying and quality of carrots dehydrated in a convective drying prototype system has been investigated, paying special attention to the influence of blanching on the MR evolution during the subsequent drying process. In addition, other complementary quality parameters such as total polyphenols, carbohydrates, proteins, rehydration capacity and microstructural changes have been studied.

2. Materials and methods

2.1. Sample preparation

Fresh carrots (*Daucus carota* L. var. Nantesa) were purchased from a local market in Madrid (Spain) and stored in the dark at 4 °C for a maximum period of 5 days until processing. Carrots were washed in tap water and then were cut into 24 mm diameter slices and 4 mm thick or as minced carrots (1–2 mm).

2.2. Processing

In a previous paper (Gamboa-Santos et al., 2012a), a wide range of blanching conditions by conventional or US treatments were assayed. Among them, we selected

for the present paper those providing a high enzymatic inactivation of POD and a relatively low loss by leaching.

Table 1 summarises the codes and blanching conditions of the samples under analysis in the present paper. In the US assays, an ultrasonic system (450 Digital Sonifier, Branson Ultrasonics Corporation, Danbury, CT, USA) equipped with a temperature sensor and a 13 mm diameter tip directly attached to a disruptor horn (20 kHz, 400 W full power) was used. For steam blanching treatments, an autoclave (CERTOCLAV CV-EL GS, Austria) was used. The carrot-distilled water ratio (40 g: 200 mL) was the same for all carrot pre-treatments assayed.

Blanched carrots were subsequently dried by convection in a tray dryer (SBANC, Edibon Technical Teaching Units, Spain) at a temperature of 46 °C and an air rate of 4.8 m/s. These operating conditions had previously been optimized by Gamboa-Santos, Soria, Fornari, Villamiel and Montilla (2013b) on the basis of the drying kinetic and the levels of quality parameters such as the 2-FM-AA determined in carrots subjected to different convective drying conditions. For comparative purposes, a previously freeze-dried (FD) sliced raw carrot was used as a control.

2.3. Analytical determinations

2.3.1. Characterization of samples

Water activity (a_w) was determined at 25 °C using a Novasina a_w Sprint TH-500 (Pfäffikon, Switzerland) system previously calibrated with saturated solutions of different salts. Total nitrogen (TN) was determined by means of the Kjeldahl method, and the protein level was calculated using 6.25 as conversion factor (TN × 6.25) (Helrich, 1990a). The dry matter (DM) content was determined gravimetrically by

drying the samples to constant weight (Helrich, 1990b). All determinations were carried out in duplicate, and the results expressed as mean values.

2.3.2. Extraction and analysis of total phenolic content (TPC)

Aliquots (0.1 g) of dried carrot samples were homogenized in 2.5 mL of HPLC grade methanol by using an Ultra Turrax (IKA Labortechnik, Janke & Kunkel, Staufen, Germany) operating at 24000 rpm for 1 min. During the extraction, the temperature was controlled by using an ice-water bath. Homogenates were stirred (750 rpm) for 20 min at room temperature using a Thermomixer (Eppendorf, Germany) and centrifuged at 2000*g* for 15 min. Supernatants were filtered through PVDF Acrodisc syringe filters (0.45 μm, Sigma-Aldrich) for subsequent analysis.

TPC content of carrot extracts was colorimetrically determined using Folin–Ciocalteu reagent (2 N, Sigma), as described by Singleton, Orthofer and Lamuela-Raventos (1999), with slight modifications. The filtered methanolic solution (100 μL), added with 100 μL of MeOH, 100 μL of Folin-Ciocalteu reagent and 700 μL of 75 g/L Na_2CO_3 was vortexed briefly. The samples were left in the dark for 20 min at room temperature. Following this, the samples were centrifuged at 13000 rpm for 3 min. The absorbance of the sample was read at 735 nm in a spectrophotometer (Power Wave XS Microplate, BIO-TEK) using the KC Junior Data Reduction software. Aqueous solutions of gallic acid (Sigma-Aldrich) in the range 10-400 mg/L were used to prepare the calibration curve. Results (average for n = 3 replicates) were expressed as milligrams of gallic acid equivalent (GAE)/g DM of carrots.

2.3.3. GC analysis of soluble carbohydrates

Soluble carbohydrates were determined by GC-FID following the method of Soria et al. (2010). Samples were ground to powders using a laboratory mill IKA A-10 (IKA Labortechnik, Staufen, Germany) and aliquots of 30 mg were weighed into a polyethylene tube and extracted at room temperature with 2 mL of Milli-Q water under constant stirring for 20 min. Next, 8 mL of absolute ethanol were added, followed by 0.2 mL of an ethanolic solution 10 mg/mL of phenyl-β-D-glucoside (Sigma-Aldrich Chemical, St. Louis, Missouri, USA) used as internal standard. After stirring for 10 min, samples were centrifuged at 10 °C and 9600*g* for 10 min and the supernatant was collected. Precipitates were subjected to a second extraction with 10 mL of 80% ethanol under the same conditions to obtain recovery values close to 100%. Finally, an aliquot (2 mL) of supernatant was evaporated under vacuum at 40 °C and derivatised.

The dried mixtures were treated with hydroxylamine chloride (2.5%) in pyridine (200 µL) and kept at 70 °C for 30 min. Subsequently, samples were persilylated by addition of 200 µL of hexamethyldisilazane and 20 µL of trifluoroacetic acid, followed by heating at 50 °C for 30 min. Reaction mixtures were centrifuged at 8800*g* for 2 min and supernatants containing the derivatised sugars were injected into the GC or stored at 4 °C until analysis.

The trimethylsilyloximes of carbohydrates were quantitatively analysed (n = 3) in an Agilent Technologies 7890A gas chromatograph (Agilent Technologies, Santa Clara, California, USA) equipped with an HP-5MS capillary column (30 m length x 0.25 mm i.d. x 0.25 µm film thickness) (J & W Scientific, Folsom, California, USA). Nitrogen at a flow rate of 1 mL/min was used as carrier gas. The oven temperature was held at 200 °C for 11 min, raised to 270 °C at a heating rate of 15 °C/min and raised again to 315 °C at 3 °C/min. Temperatures of the injector and the flame ionization

detector were 280 °C and 315 °C, respectively. Injections were carried out in split mode (1:30). Data acquisition and integration were performed using Agilent ChemStation Rev. B.03.01 software (Wilmington, DE, USA). Solutions containing fructose, glucose, *myo*-inositol and sucrose were prepared over the expected concentration range in carrot samples to calculate the response factor of each of these sugars relative to the internal standard.

Confirmation of identities was done based on experimental data for standards (linear retention indices and mass spectra) and data from literature (Soria, Sanz, & Villamiel, 2009b). GC-MS analyses of derivatised samples were carried out using a 7890A gas chromatograph coupled to a 5975C quadrupole mass detector (both from Agilent Technologies, Palo Alto, CA, USA). Chromatographic conditions other than carrier gas (He) were similar to those previously mentioned for GC-FID analysis. The mass spectrometer was operated in electron impact mode at 70 eV, scanning the 35-700 *m/z* range. Acquisition was done using HP ChemStation software (Agilent Technologies).

2.3.4. Furosine determination

Samples of dehydrated carrots (0.25 g) were thermally hydrolysed under inert conditions (helium) with 4 mL of 8 N HCl at 110 °C for 23 h in a screw-capped Pyrex vial with PTFE-faced septa. The hydrolysed samples were filtered through a Whatman no. 40 paper filter and 0.5 mL of the filtrate was applied to a Sep-Pack C_{18} cartridge (Millipore) prewetted with 5 mL of methanol and 10 mL of water and then eluted with 3 mL of 3 N HCl.

Determination of 2-FM-Lys was carried out by ion-pair RP-HPLC analysis (Resmini & Pellegrino, 1991), using a C_8 column (250 mm × 4.6 mm i.d.) (Alltech,

231 Lexington, KY) thermostated at 37 °C, with a linear binary gradient composed of phase
232 A (4 mL/L acetic acid) and phase B (3 g/L KCl in phase A solution). The elution
233 program was as follows: 0-12 min: 100% A; 20-22.5 min: 50% A; 24.5-30 min: 100%
234 A. The flow rate was 1.2 mL/min and injection (50 µL) was carried out using a manual
235 Rheodyne valve. Detection was done in a variable-wavelength detector (LCD
236 Analytical SM 4000) set at 280 nm.
237 Quantitation was performed by the external standard method, using a
238 commercial standard of furosine (Neosystem Laboratoire, Strasbourg, France). All
239 analyses were done in triplicate and mean values expressed as milligrams per 100 g of
240 protein.
241
242 2.3.5. Protein profile by sodium dodecyl sulphate - polyacrylamide gel electrophoresis
243 (SDS-PAGE)
244 Powdered dehydrated carrot samples (100 mg) were mixed with 2 mL of 1%
245 sodium metabisulfite (Merck, Darmstadt, Germany) aqueous solution. Next, samples
246 were stirred thoroughly for 2 h and centrifugated at 3000*g* for 15 min. The supernatants
247 were analysed by SDS-PAGE.
248 Protein analysis was carried out by adding 32.5 µL of sample supernatant to 12.5
249 µL of 4X NuPAGE LSD sample buffer (Invitrogen, Carlsbad, California, USA)
250 provided with 5µL of 0.5 mol/L dithiothreitol (Sigma-Aldrich). Samples were heated at
251 70 °C for 10 min and 20 µL were loaded on a 12% polyacrylamide NuPAGENoveBis-
252 Tris precast gel (Invitrogen). Gels were run for 41 min at 120 mA per gel and 200 V
253 with a continuous MES SDS running buffer (Invitrogen) and were stained using the
254 Colloidal Blue Staining Kit (Invitrogen). A mixture of standard proteins with molecular
255 weights ranging from 2.5 to 200 kDa (Invitrogen) was used to estimate the molecular

256 weight of carrot proteins. Myosin, 200 kDa; β-galactosidase, 116.3 kDa; phosphorylase
257 B, 97.4 kDa; bovine serum albumin, 66 kDa; glutamic dehydrogenase, 55.4 kDa; lactate
258 dehydrogenase, 36.5 kDa; carbonic anhydrase, 31 kDa; trypsin inhibitor, 21.5 kDa;
259 lysozyme, 14.4 kDa; aprotinin, 6 kDa; insulin B chain, 3.5 kDa and insulin A chain, 2.5
260 kDa were chosen as standards.
261
262 2.3.6. Rehydration ratio (RR)
263 Rehydration of dehydrated carrot samples was performed according to Soria et
264 al. (2010). Dried samples were rehydrated by immersion in distilled water (solid: liquid
265 ratio of 1:50) at ambient temperature for 24 hours. Carrots were placed on paper towels
266 to remove the surface water and then weighed. Each rehydration experiment was
267 performed in triplicate and RR was calculated as:
268 $RR = m_r/m_d$ (1)
269 Where m_r and m_d are the weights of rehydrated and dehydrated carrot, respectively.
270
271 2.3.7. Scanning Electron Microscopy (SEM)
272 The surface microstructure of dehydrated or control samples was observed by
273 Scanning Electron Microscopy. Prior to SEM observations, the samples were coated
274 with gold: palladium 80:20 in a sputter coater SC7460 Polaron (Quorum Technologies,
275 Newhaven, U.K.), at 5-10 mA and 800 V plasma current in order to stabilize the
276 structure. Then they were viewed with a Philips XL 30 ESEM Electron Microscope at
277 an accelerating voltage of 25 kV. Duplicate specimens were viewed at different
278 magnifications (200, 400, 800 and 1500) and images of representative areas were saved
279 for further analysis.
280

2.4. Statistical analysis

To study the effect of temperature and air rate on the quality parameters determined, one-way analyses of variance (ANOVA) were carried out using Statgraphics (version 5.1; StatPoint, Inc., Warrenton, VI, USA). Individual treatments were compared using the least significant difference test (LSD, 95%).

3. Results and discussion

3.1. Dehydration of blanched carrot samples

Fig. 1 depicts the drying curves obtained in the dehydration of minced and sliced carrots by convection after different blanching treatments (see Table 1). As can be observed, curves with different slopes were obtained depending on the blanching applied and the geometry of samples; minced carrots presented higher slope values (0.059-0.221) than sliced carrots (0.040-0.082). This fact could be due to the higher values of initial moisture of minced (7.6-24.0 kg H_2O/kg DM) as compared to sliced carrots (6.9-13.3 kg H_2O/kg DM) and/or the higher specific surface of minced carrots. Thus, for boiling blanched samples, with similar initial moisture, minced carrot samples were dehydrated more quickly than sliced ones. Moreover, carrots blanched by conventional treatments at 60 °C for 40 min presented the highest slope value and the highest initial moisture content.

In relation to the final product, dried samples showed DM contents in ranges from 88.5-93.1% and 85.0-88.7%, respectively, for minced and sliced carrots. All these values were very close to those considered as microbiologically safe for dried products (85%) (Belitz, Grosch & Schieberle, 2009). Determination was also made of a_w and the values obtained were within the interval from 0.238-0.375. As is known, foods with a_w values near 0.3 are stable against non-enzymatic browning, microorganism development

and enzymatic activities during their adequate storage (Labuza, 1971). In addition, samples that after blanching presented some residual activity of POD (subjected to steam blanching, hot water at 60 °C for 40 min and to US blanching at 60 °C for 10 min and at 70 °C for 15 min; Gamboa-Santos et al., 2012a) were evaluated after drying and, in all cases, no residual activity of this enzyme was found. Thus, regardless of the blanching treatment applied, all the dried carrots under study showed great stability, which might guarantee their safe consumption over the course of their shelf-life.

The dehydration of samples pre-treated with US originated final products with intermediate slopes, as shown in Fig. 1. Other authors have found that different fruits (Malay apple, melon, pineapple) subjected to US pre-treatment dried faster during the air-drying stage compared to fresh fruit with no pre-treatment. This could be explained in that US pre-treatment might increase the effective water diffusivity in the fruit, thereby reducing the dehydration time (Fernandes et al., 2011; Mothibe, Zhang, Nsor-atindana & Wang, 2011).

3.2. Chemical changes during drying of carrot samples

TPC values of samples dried by convection after several blanching procedures (Table 2) were within the 1.312-1.524 mg GAE/g DM range. These results were similar to those published by Soria et al. (2010) for sliced carrots of the same size and blanched with boiling water for 1 min and further dehydrated by ultrasound-assisted convective drying. A slight decrease, only significant for several samples, was observed in the dried carrots previously blanched by conventional heat treatments as compared to the control sample. It has been described that changes in physical properties of carrots processed under different drying conditions can modify the extractability of bioactive compounds (Gorinstein et al., 2009). Thus, the freeze-drying process might alter tissue

structure and make the extraction of flavonoids easier (Pérez-Gregorio, Regueiro, González-Barreiro, Rial-Otero & Simal-Gándara, 2011). It is also noteworthy that samples subjected to a previous blanching by US presented similar TPC values to those of FD carrot samples. This could be due to the fact that US treatment can give rise to pores in the vegetal tissue and, consequently, improve the extraction of polyphenols during sample preparation. In spite of the small differences observed, in general, it is possible to say that hardly any change in the TPC content, and indirectly in their antioxidant activity, was measured in the samples analysed. Previous papers have demonstrated a high correlation between TPC and antioxidant activity measured by the ORAC method and that dehydration might be considered a good method for preserving the content of these compounds (Rababah, Ereifej & Howard, 2005; Soria et al., 2010).

Other changes that can take place during dehydration of vegetables are the losses of carbohydrates due to thermal treatment and/or leaching during blanching (Rodríguez-Sevilla, Villanueva-Suárez & Redondo-Cuenca, 1999; Wennberg, Ekwall, Olsson & Nyman, 2006). Table 3 shows the carbohydrate content of dried carrots previously subjected to the various blanching procedures assayed. Fructose, glucose and sucrose were the major carbohydrates determined in all the samples analysed; sedoheptulose, *scyllo*- and *myo*-inositol were also present as minor carbohydrates in all these samples. In general, carbohydrate content was in good agreement with data previously reported for raw and processed carrots (Soria et al., 2009b; 2010; Gamboa-Santos et al., 2012a).

As observed in Table 3, the concentration of carbohydrates in dried carrot samples previously steam blanched (D-CS-2-M and D-CS-2-S) showed no significant differences with respect to FD sample. However, in the other type of samples, significant ($P<0.05$) losses (10.2-49.9% total carbohydrates) were detected in relation to the same control sample. When considering the same blanching conditions, sliced

carrots preserved carbohydrate content better, probably due to the lower specific surface as compared to minced ones. The lowest amount of carbohydrates was detected in dried samples subjected to a previous conventional blanching at 95 and 60 °C. With respect to dried samples pre-treated by US, the carbohydrate content was close to that of some conventional blanching treatments.

Regardless of the geometry of the sample, the loss of fructose and glucose during blanching was higher than that of sucrose, probably due to the higher solubility of monosaccharides as compared to sucrose. A certain loss of reducing carbohydrates (fructose and glucose) could also be suspected as a result of their involvement in the MR. However, when comparing the results obtained after drying of samples with those previously reported by Gamboa-Santos et al. (2012a) for carrots subjected to identical blanching conditions, it can be concluded that the major losses of carbohydrates (considering the overall process) take place by lixiviation during blanching. Thus, the operating conditions used here for convective drying (46 °C, 4.8 m/s) seem not to be strong enough to give rise to appreciable changes in the carbohydrate fraction.

As MR mainly takes place under the moisture conditions achieved during the drying process, MR assessment was also carried out in the carrot samples under study by means of the determination of 2-FM-Lys (Table 2). Although, as previously indicated, hardly any change was observed in the fraction of reducing carbohydrates during the dehydration process; considerable formation of this compound was found in the dehydrated carrots subjected to different blanching treatments. As only traces were detected in blanched samples (Gamboa-Santos et al., 2012a), and all carrot samples were dried under the same operating conditions, the evolution of MR in dried samples can be solely attributable to the drying process.

The highest concentrations of furosine were determined in carrots previously blanched at 95 °C for 5 min, whereas the samples with the lowest evolution of MR were those previously blanched by steam, boiling water and hot water at 60 °C. Carrots treated by US before drying presented intermediate values of this quality marker. Considering the effect of geometry, in general, no clear conclusion can be established since, under the same processing (blanching plus drying) conditions, no significant differences were found between minced and sliced samples.

The amounts of furosine found in the samples analysed here were, in general, lower than those reported by other authors for carrots dried under convection (Rufián-Henares et al., 2008; Soria et al., 2009a; Soria et al., 2010; Wellner et al., 2011). This could probably be due either to the more intense processing conditions used in previous studies or to the different variety of carrot processed. To the best of our knowledge, no data have been previously reported on the effect of different blanching procedures on the further evolution of MR during drying.

According to these data, it is presumable that some modification during the previous blanching could affect the structure of the protein the free amino groups of which could be more or less available to react with the carbonyl group of the reducing carbohydrates during drying. Thus, the highest values of furosine for D-C95-5-M and D-C95-5-S could be explained assuming that, under these blanching conditions, a certain unfolding of protein by heat treatment takes place and this unfolding makes the reaction with carbohydrates more favourable. This is also supported by the significant losses of carbohydrates detected (Table 3). Furthermore, and according to several authors (Yoo & Lee, 1993; Leslie, Israeli, Lighthart, Crowe & Crowe, 1995), the stability of proteins can be increased by the carbohydrate concentration. Thus, an increase in hydrophobic interactions and hydrophilic properties, due to the formation of

405 protein-sugar complexes, can stabilize the three dimensional structure of proteins,
406 keeping or protecting its functionality. On the other hand, the samples subjected to US
407 blanching showed relatively high furosine values. In this case, since the temperatures of
408 the treatments were low (up to 60 and 70 °C), the main influence was probably the
409 physical effect of US related to the opening of hydrophilic parts of amino acids, as
410 shown by Krešić, Lelas, Jambrack, Herceg and Brncic (2008). During US treatment of
411 soy protein isolate, an increase in levels of free amino groups was also observed by Mu,
412 Zhao, Yang, Zhao, Cui and Zhao (2010), who attributed this result to an unfolding of
413 protein and breaking of peptide bonds by hydrolysis.

414 To gain more insight into possible changes associated with carrot processing, an
415 SDS-PAGE analysis of the protein fraction of carrots under study was carried out (Fig.
416 2). As observed, most of the samples presented similar electrophoretic bands to those of
417 the protein profile of the freeze-dried carrots previously reported by Soria et al. (2010).
418 However, lanes 5 and 9, corresponding to D-C95-5-M and D-C95-5-S samples,
419 respectively, presented a different pattern with a non-defined protein profile. In this
420 case, a variety of bands with slower electrophoretic mobility and different molecular
421 weight were detected, indicating that, in addition to a possible unfolding, cross-linking
422 and aggregation of proteins also took place. The previously mentioned high furosine
423 content of both samples (D-C95-5-M and D-C95-5-S) (Table 2) also confirms that
424 blanching carried out under these conditions could have changed the structure of
425 proteins to promote, at a higher extent over other blanching conditions, the evolution of
426 MR during drying.
427
428
429

3.3. Physical changes during drying of carrot samples

Although rehydration cannot be considered as a reversible process to dehydration, since blanching and drying can provoke tissue disruption that gives rise to a certain hysteresis during rehydration (Lewicki, 1998), this property is highly correlated with consumers' acceptance of dried products.

Carrot samples processed in this study were evaluated for their rehydration ability after drying and the results are shown in Fig. 3. The RR values ranged from 4.2 to 14.8. Carrots blanched with steam and boiling water presented RR values close to 5, significantly lower than that of the FD sample. Giri & Prasad (2009) also found higher RR values in freeze-dried mushrooms (4.3) than in the same type of vegetable dried by convection (2.5); however, in both cases no pre-treatment was previously applied. Soria et al. (2010) reported RR values within the range of 5.7-7.2 for commercially dehydrated carrots and 6.7 for laboratory freeze-dried samples previously blanched by boiling water for 1 min. Similar values were obtained by Gamboa-Santos, Soria, Villamiel and Montilla (2012b) in carrot samples industrially processed by hot-air after a previous blanching (with water spray or microdroplets) at 98 °C for 20 min. In this study, the highest RR values were found in dried samples blanched at 95 °C for 5 min and at 60 °C for 40 min, in agreement with their highest initial content of moisture, as shown in Fig. 1.

The RR of dried samples blanched by US, particularly that of the D-USP70-15-S sample, were significantly ($P<0.05$) higher than those of D-CS-2 and D-CB-1 carrots. In a study on accelerated drying of mushrooms, Brussels sprouts and cauliflower by power US, Jambrak et al. (2007) found intermediate rehydration properties of dried samples (60 °C, 0.3 m/s) previously treated by US with a probe (20 kHz) or bath (40 kHz), as

compared to freeze-dried samples and dried samples previously blanched at 80 °C for 3 min.

Some authors have postulated that when PME activity is present, cell walls become harder, avoiding ulterior thermal damage and this could imply a decrease in the rehydration level (Heredia-León et al., 2004). However, when considering samples processed in this paper, carrots D-C60-40-M, D-USP60-10-M and D-USP70-15-S presented high values of RR and, coincidently, these carrot samples showed a certain residual activity of PME after blanching (Gamboa-Santos et al., 2012a). On the contrary, samples blanched with steam, boiling water and water at 95 °C did not present any PME residual activity and their RR after drying was highly variable, as indicated in Fig. 3. Therefore, within the range of experimental conditions studied here, there was no apparent correlation between PME activity and the rehydration properties. These results could probably be due to the fact that the residual PME activity of samples D-C60-40-M, D-USP60-10-M and D-USP70-15-S after blanching could have disappeared during the drying process. Therefore, other effects such as the physical changes on microstructure could be the main factor affecting RR.

The microstructure analysed by SEM of the FD and convective dried samples after blanching by conventional and US treatments is shown in Fig. 4. As can be observed, FD carrots show a perfect organization of the vegetal tissue. Cells are polyhedrical, similar sized and uniformly distributed through the matrix. This is due to the fact that during water sublimation in freeze drying, hardly any change is produced and this contributes to a great extent to preserving the original organization of the cellular parenchyma. However, in the case of samples thermally processed, the cell walls are more or less twisted, depending on the severity of the treatment. Particularly under the most severe conditions (95 °C, 5 min), the original cellular structure is

noticeably transformed and a structural collapse is provoked, probably due to the degradation of pectinacious material during processing and the appearance of intercellular voids. According to microstructural observations, Sanjuán et al. (2005) indicated that conventional blanching of carrots at 95 °C for 1 min tends to cause separation along their cell walls, forming voids among the phloem parenchyma cells. These voids would be filled with water during rehydration, thus showing the slightly higher rehydration properties. Similar results were reported for carrot samples treated at 105 °C for 10 min, steamed-blanched carrots and slightly cooked carrots (Fuchigami, Miyazaki & Hyacumoto, 1995; Kidmose & Martens, 1999; Rastogi, Nguyen & Balasubramaniam, 2008). Thus, the highest RR found in D-C95-5-M, D-C95-5-S and D-C60-40-M carrot samples could be due to their loss of structure, which can facilitate water diffusion during rehydration.

Samples blanched by US (D-USP70-15 and D-USP60-10) also presented a noticeably modified cellular structure; however, in this case, the mechanism involved is related to the creation of a porous material that facilitates water movement due to expansions and compressions ("sponge effect") (Ortuño, Pérez-Munuera, Puig, Riera & García-Pérez, 2010). According to Fernandes et al. (2011), the cavitation and micro-streaming provoked by US can contribute to the formation of microscopic channels in the vegetal tissues. All of this could justify the high RR values found for these carrot samples (Fig. 3). Garcia-Noguera et al. (2010) reported a breakdown of tissue structure in strawberries pre-treated in an ultrasonic bath at 30 ºC for 60 min, whereas other authors did not find important differences in Malay apples processed under similar conditions (Oliveira, Gallao, Rodrigues & Fernandes, 2011).

4. Conclusions

It can be concluded that the drying process and quality parameters of convectively dehydrated carrots are highly dependent on the blanching type and conditions. Samples conventionally processed under long time-low temperature (LTLT) conditions (60 °C, 40 min) or under the most severe conditions (95 °C, 5 min) were dehydrated faster and showed the highest rehydration ratio and loss of carbohydrates. The highest advance of the Maillard reaction was observed in carrot samples subjected to blanching at 95 °C for 5 min, as evidenced by its furosine content and by the changes in its protein pattern determined by SDS-PAGE. However, samples conventionally blanched with boiling water or by steam presented a lower rate of drying and lower losses of carbohydrates and formation of furosine. Samples processed by US showed an intermediate dehydration rate and TPC levels and rehydration properties similar to those of the control sample. To the best of our knowledge, this is the first time that the effect of blanching on the subsequent evolution of MR during drying of vegetables has been assayed. The results obtained in the present paper underline the usefulness of furosine as a marker of carrot processing, particularly if avoiding losses of nutritive value due to the participation of lysine in the MR is intended.

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Figure captions:

Fig. 1. Drying curves obtained in the dehydration by convection at 46 °C and at a drying rate of 4.8 m/s of minced and sliced carrots subjected to different blanching treatments (Table 1).

Fig. 2. SDS-PAGE analysis of protein fraction of dehydrated carrots subjected to different blanching treatments. (1) Markers of molecular weight, (2) FD (control), (3) D-C60-40-M, (4) D-CB-1-M, (5) D-C95-5-M, (6) D-USP60-10-M, (7) D-CS-2-S, (8) D-CB-1-S, (9) D-C95-5-S, (10) D-USP70-15-S.

Fig. 3. Rehydration ratio (RR) of carrot samples under analysis (Table 1). Mean of 3 replicates and standard deviation in error bars. Samples with the same letter (a-g) showed no statistically significant differences for their mean values at the 95% confidence level.

Fig. 4. Electron microphotographs of dried carrots (400X). a: FD (control); b: D-CS-2-M; c: D-CB-1-M; d: D-CB-1-S; e: D-C95-5-S; f: D-C60-40-M; g: D-USP70-15-S; h: D-USP60-10-M.

Table 1 Processing conditions used during conventional/ultrasound blanching of carrots and further drying by convection at 46 °C and at a drying rate of 4.8 m/s.

Sample code	Carrot geometry	Blanching conditions	Drying time (h)
D-CS-2-M	Minced	Steam (98 ºC, 2 min)	7
D-CS-2-S	Sliced	Steam (98 ºC, 2 min)	9
D-CB-1-M	Minced	Boiling water (98 ºC, 1 min)	7
D-CB-1-S	Sliced	Boiling water (98 ºC, 1 min)	9
D-C95-5-M	Minced	Hot water (95 ºC, 5 min)	7
D-C95-5-S	Sliced	Hot water (95 ºC, 5 min)	9
D-C60-40-M	Minced	Hot water (60 ºC, 40 min)	7
D-USP60-10-M	Minced	US probe (up to 60 ºC, 10 min)	7
D-USP70-15-S	Sliced	US probe (up to 70 ºC, 15 min)	9

741 **Table 2** Total Phenolic Content (TPC) and furosine amount (mg/100 g protein)
742 determined in dehydrated carrot samples previously subjected to different blanching
743 treatments (mean of three replicates ± standard deviation).
744

Carrot samples	TPC (mg GAE/g DM)	Furosine (mg/100 g protein)
FD (control)	1.541 ± 0.021bc[a]	-
D-CS-2-M	1.367 ± 0.025ab	159.1 ± 2.3a
D-CS-2-S	1.329 ± 0.023a	152.1 ± 0.0a
D-CB-1-M	1.373 ± 0.068 ab	139.5 ± 8.6a
D-CB-1-S	1.342 ± 0.140a	117.58 ± 3.4a
D-C95-5-M	1.312 ± 0.001a	660.7 ± 15.3b
D-C95-5-S	1.382 ± 0.026ab	681.5 ± 26.6b
D-C60-40-M	1.352 ± 0.054a	104.3 ± 6.6a
D-USP60-10-M	1.434 ± 0.055c	274.4 ± 26.8c
D-USP70-15-S	1.524 ± 0.028abc	342.7 ± 13.3d

745 [a]Samples with the same letter (a-d) within the same column showed no statistically
746 significant differences for their mean values at the 95% confidence level.
747

748 **Table 3** Quantitative analysis of carbohydrates in dehydrated carrots under analysis (mean of three replicates ± standard deviation).

Samples	Carbohydrates (mg/g DM ± SD)						
	Fructose	Glucose	Sucrose	*Scyllo*-inositol	*Myo*-inositol	Sedoheptulose	Total
FD	67.27±2.68a[a]	73.99±2.94a	449.50±5.5a	1.48±0.02a	4.76±0.14a	2.56±0.02[a]	608.63±11.56a
D-CS-2-M	67.26±0.00a	73.97±0.00a	449.05±0.02a	1.48±0.00a	4.76±0.00a	2.56±0.00a	603.80±0.01a (0.8%)[b]
D-CS-2-S	67.19±0.00a	73.90±0.02a	448.65±0.00a	1.48±0.00a	4.74±0.00a	2.55±0.00a	603.14±0.01a (0.9%)
D-CB-1-M	41.15±0.30c	46.05±0.16e	377.58±0.99d	1.03±0.04d	3.30±0.08c	1.89±0.02d	495.00±1.68d (18.7%)
D-CB-1-S	57.39±0.07b	62.63±0.02b	409.36±1.83b	1.22±0.01b	3.97±0.01b	2.09±0.02b	546.24±2.5b (10.2%)
D-C95-5-M	31.13±0.51d	34.81±0.23d	283.20±2.15e	0.86±0.11e	2.53±0.04e	1.32±0.01e	304.91±2.72e (49.9%)
D-C95-5-S	39.00±1.16c	41.80±1.31c	349.53±2.48c	1.00±0.02cd	3.20±0.06c	1.51±0.04c	401.57±4.00c (34.0%)
D-C60-40-M	34.37±0.62c,d	42.00±3.19c	311.33±11.68e	0.57±0.06f	2.67±0.04d,e	1.46±0.01c	392.9±17.56c (35.5%)
D-US60-10-M	39.27±1.08c	40.88±3.28c	343.04±23.77c	0.91±0.06ce	2.92±0.25d	1.66±0.19f	435.29±29.45f (28.5%)
D-US70-15-S	31.76±0.88d	35.42±1.18d	333.88±1.20c	0.86±0.04e	2.81±0.04d	1.47±0.02c	404.46±1.64c (33.5%)

749
750 [a] Samples with the same letter (a-f) within the same column showed no statistically significant differences for their mean values at the 95% confidence level.
751 [b] In brackets, losses of total carbohydrates by lixiviation during blanching with respect to FD sample (control).
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753

754 **Fig. 1.** Gamboa-Santos et al.

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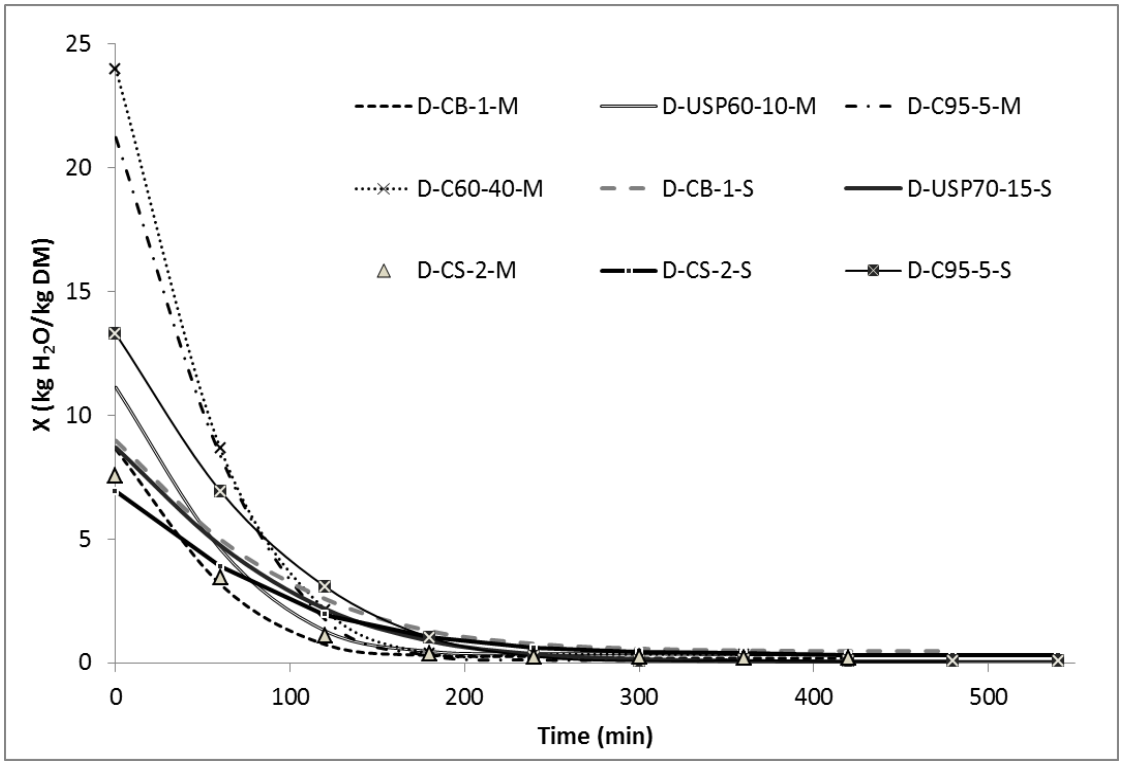

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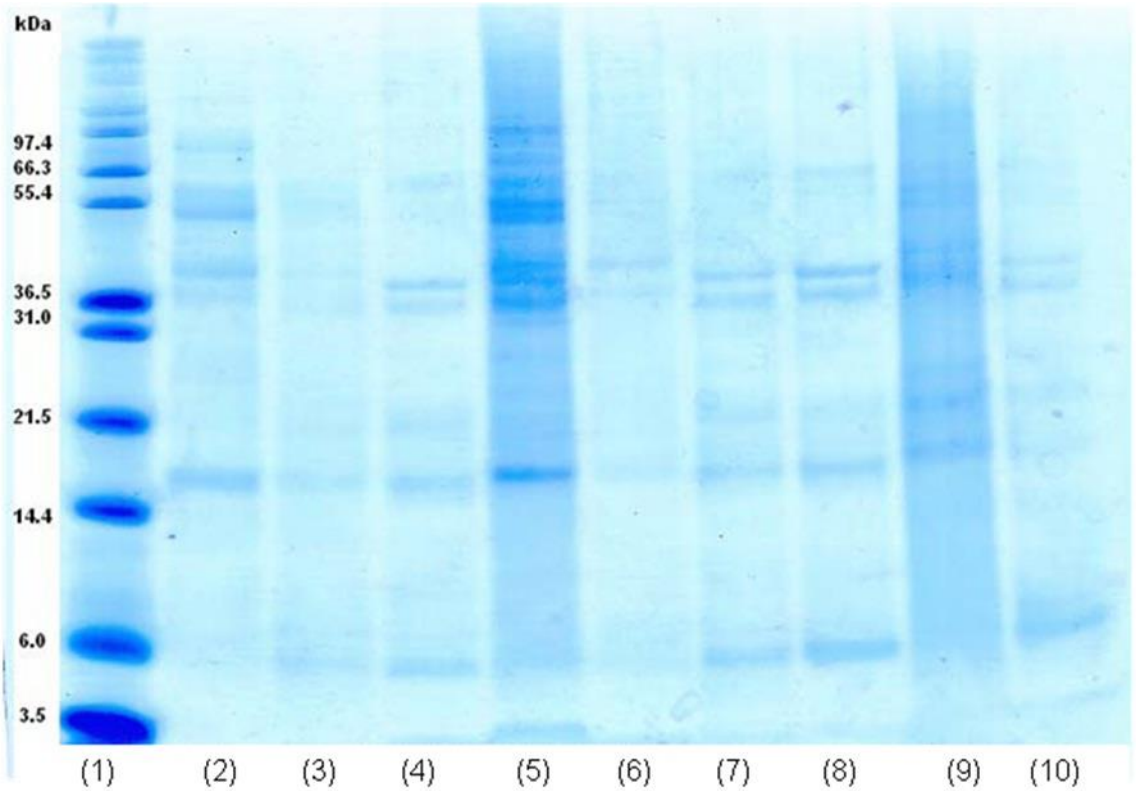
Fig. 2. Gamboa-Santos et al.



766 **Fig. 3.** Gamboa-Santos et al.

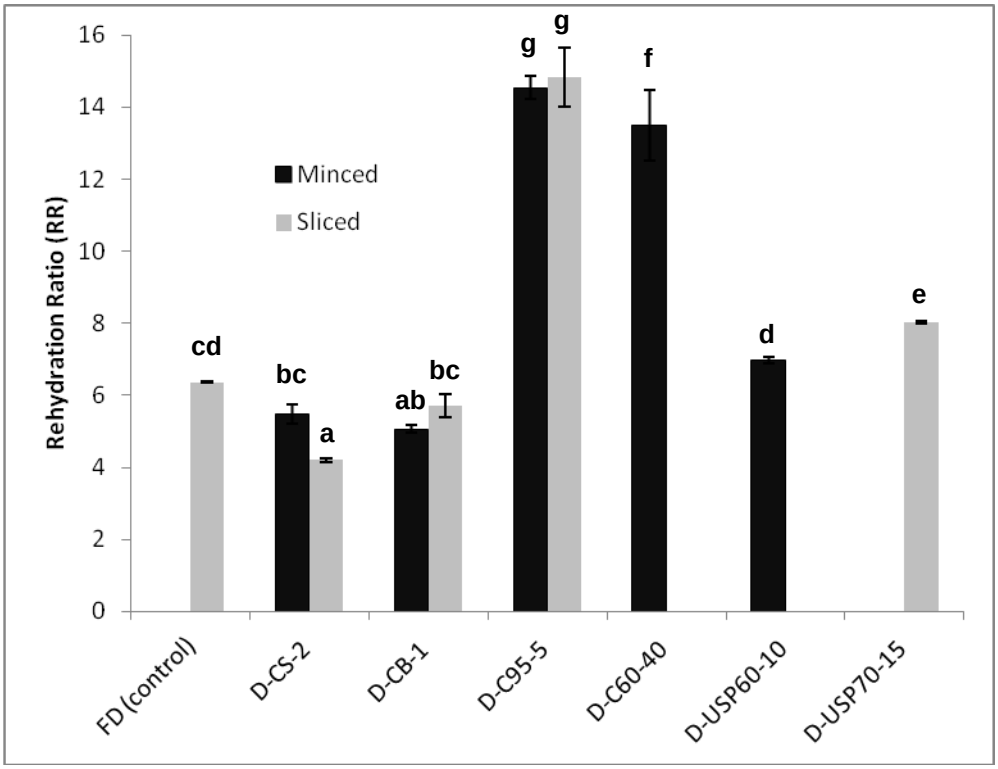


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Fig. 4. Gamboa-Santos et al.