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Chemical and Physicochemical Quality Parameters in Carrots Dehydrated by Power Ultrasound

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Preservation of the quality and bioactivity of carrots dehydrated by power ultrasound (US) under different experimental conditions including prior blanching has been evaluated for the first time by measuring the evolution of the Maillard reaction and the changes in soluble sugars, proteins, total polyphenols, antioxidant activity, and rehydration ability. This study also includes a comparison with a freeze-dried sample and data of commercial dehydrated carrots. The synergic effect of US and temperature (60 °C) increased the dehydration rate of carrots (90% moisture loss in only 75 min) while still providing carrots with a level of 2-furoylmethyl-amino acids significantly lower than that of dehydrated commercial samples. Whereas a decrease in the content of reducing soluble sugars was observed with processing temperature, minor carbohydrates (*scyllo*- and *myo*-inositol and sedoheptulose) were rather stable, irrespective of the US dehydration parameters. Blanching significantly improved the rehydration ability of US-dehydrated carrots without increasing the loss of soluble sugars by leaching. As supported by the similarity of most quality indicators studied in both US-treated and freeze-dried carrots, the mild processing conditions employed in US dehydration gave rise to premium quality dehydrated carrots.

KEYWORDS: Dehydrated carrot; ultrasound; Maillard reaction; 2-furoylmethyl-lysine (furosine); soluble sugars; protein; polyphenols; antioxidant activity; rehydration ability

INTRODUCTION

Since fresh vegetables are highly perishable and difficult to preserve, the market for dehydrated vegetables has noticeably increased over recent years to provide consumers with long-shelf-life foods which are easy to handle and store (*1*). Among the different dehydrated vegetables commercially available, carrots (*Daucus carota* L.) are increasingly being used in the elaboration of a number of food products (*2*).

Although different techniques have been reported in the literature for carrot dehydration, convective air drying is the process of choice for industrial applications. However, the operating conditions usually employed in convective drying (typically 40−80 °C air temperature, 0.5−5 m s^{-1} air velocity, and drying times as long as 20 h) (*3*) may produce important chemical changes in the thermolabile carrot constituents (vitamins, phenolic compounds, etc.) and in their physical properties (texture, rehydration ability, etc.), resulting in a product of considerably lower quality when compared to the raw material (*4*).

One of the most relevant chemical changes that occurs at low water activity and high-temperature conditions used for drying is the Maillard reaction (MR), which takes place between reducing carbohydrates and free amino groups of amino acids, peptides, and proteins. 2-Furoylmethyl-amino acids (2-FM-AA), obtained from the acid hydrolysis of the Amadori compounds formed at the early stages of the MR, have been recently proposed as sensitive indicators for the early detection of changes in the nutritional value and organoleptic properties of several dehydrated vegetables (*5*, *6*), their contents being dependent on the vegetable species and their processing and/or storage conditions. It has also been suggested that these derivatives should be used in combination with hydroxymethylfurfural to assess the quality of hot-air-dried carrots (*7*).

A number of references have been reported on the content of major sugars in carrots of different varieties (*8*) and/or submitted to different processing and storage conditions (*9*, *10*). However, the role of reducing sugars in the MR has been scarcely studied in dehydrated carrots (*6*). Recently, minor carbohydrates in carrots have been studied by Soria et al. (*11*) because of their remarkable role in a variety of biological functions.

Moreover, carrots are known as a good source of bioactive compounds such as natural antioxidants, including carotenoids, vitamins, phenolic compounds, and flavonoids. Changes in several of these bioactives, such as β-carotene, lycopene, etc., in carrots submitted to different drying techniques and the evolution of antioxidant capacity during the storage of selected fruits and vegetables have been previously studied (*4*, *12*, *13*).

Among the physical quality parameters, rehydration ability is one of the most relevant parameters for the acceptance of dehydrated carrots by consumers. Conditions selected for

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Table 1. Sample Codes of Carrots Dehydrated by US

	dehydration conditions	
carrot sample	air temp (°C)	drying time (min)
US20	20	120
US20BL[a]	20	120
US40	40	90
US40BL	40	90
US60	60	75
US60BL	60	75

[a] BL: carrots were blanched prior to dehydration.

pretreatment, drying, and rehydration noticeably affect the structure and composition of carrot tissues (*14*), which determine the organoleptic properties of carrots upon rehydration (*15*).

On the other hand, with the aim of preserving the quality of dehydrated vegetables, several studies have been focused on the evaluation of the most relevant parameters involved in dehydration (*16*), the improvement of existing processes (*17*), or the search for alternative or emergent technologies (*18*). Among the latter, the use of power ultrasound (US) for the dehydration of vegetables has recently emerged as a novel alternative to conventional drying processes, with the advantages of mild treatment temperatures and short drying times (*19*). Although several papers have dealt with parametric and kinetic studies on moisture loss during the ultrasonic drying of carrots (*20*, *21*), no reference has yet addressed the physical, chemical, and physicochemical changes of vegetables during US-assisted drying.

The aim of this paper is to evaluate the quality and bioactivity of carrots processed under different US operating conditions, with a view to obtain premium-quality dehydrated carrots. Our study also includes a comparison with a freeze-dried sample (used as a quality control) and data for commercial dehydrated carrots. To the best of our knowledge, this is the first time that the evolution of the MR and changes in soluble sugars, proteins, total polyphenols, antioxidant activity, and rehydration ability have been determined in US-dehydrated carrots.

MATERIALS AND METHODS

US-Dehydrated Carrot Samples. 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 dehydration. Experiments were carried out using a prototype of air-borne ultrasonic dehydration (*19*). The experimental setup mainly consists of (i) a hot-air generator, (ii) a stepped-plate power ultrasonic transducer with the corresponding electronic generator, (iii) a flat plate parallel to the ultrasonic radiator acting both as a reflector for the formation of a standing wave and as a sample holder, where suction is applied to remove the moisture, and (iv) a static pressure system to get good mechanical coupling between the carrot sample (16 slices: 24 mm diameter, 4 mm thickness) and the vibrating plate of the transducer.

In all experiments, ultrasonic parameters other than air temperature (20, 40, and 60 °C) and drying time (75, 90, and 120 min) were kept constant: ultrasonic frequency, 20 kHz; power level, 100 W cm^{-2}; air speed, 1.2 m s^{-1}; suction pump, 120 mbar; and contact pressure, 1.6 kg cm^{-2}. To evaluate the effect of sample pretreatment on dehydration, carrots were blanched in boiling water for 1 min (ratio sample:water was 1:30) and were also processed under the different US experimental conditions detailed above (**Table 1**). Two replicates per set of dehydration conditions were carried out. For determinations other than the rehydration ability, samples were freeze-dried and finely ground using a thermostated laboratory grinder (IKA A10, Jankie & Kunkel).

Other Dehydrated Carrot Samples. Six commercial dehydrated carrots (COMM1−6) from three different suppliers in Spain and a laboratory freeze-dried (FD) carrot were also analyzed. Samples were kept at −20 °C until analysis.

Carrot Characterization. The dry matter (DM) content was determined gravimetrically by drying the samples until constant weight (*22*). Total nitrogen (TN) was determined by the Kjeldahl method (*23*), and the protein level was calculated using 6.25 as conversion factor (TN × 6.25). All determinations were carried out in duplicate.

HPLC Analysis of 2-Furoylmethyl-amino Acid (2-FM-AA) Derivatives. The determination of 2-FM-AA was carried out by ion-pair RP-HPLC analysis (*24*) using a C_8 column (250 mm ×4.6 mm i.d.) (Alltech, Lexington, KY) thermostated at 37 °C. A linear binary gradient (A, 4 mL L^{-1} acetic acid; B, 3 g L^{-1} KCl in A) at a flow rate of 1.2 mL min^{-1} was used. The elution program was as follows: 100% A (from 0 to 12 min), 50% A (from 20 to 22.5 min), and 100% A (from 24.5 to 30 min). A variable-wavelength detector was set at 280 nm (LCD Analytical SM 4000).

Samples (0.25 g) were hydrolyzed under inert conditions (helium) with 4 mL of 8 M HCl at 110 °C for 23 h in a screw-capped Pyrex vial with PTFE-faced septa. The hydrolysate was filtered through a medium-grade paper filter (Whatman no. 40). Next, 0.5 mL of the filtrate was applied to a Sep-Pack C_{18} cartridge (Millipore, MA), prewetted with 5 mL of methanol and 10 mL of water, and then eluted with 3 mL of 3 M HCl; 50 μL was injected.

The identification of 2-FM-AA derivatives other than furosine was first carried out by comparing the retention times with data previously obtained for standards synthesized in our laboratory and analyzed under identical experimental conditions (*25*). The identity of 2-FM derivatives was further confirmed by HPLC-MS following the method described by del Castillo et al. (*26*). Analyses were carried out at room temperature on a Hewlett-Packard 1100 liquid chromatograph coupled to a quadrupole HP-1100 mass detector (both from Hewlett-Packard, Palo Alto, CA), working in electrospray ionization mode, under atmospheric pressure and positive polarity (API-ES positive). The mobile phase was acetic acid in Milli-Q water (20 mL L^{-1}), and elution was under isocratic conditions at a flow rate of 0.7 mL min^{-1}. Mass spectrometer values of needle potential, gas temperature, drying gas, and nebulizer pressure were 4000 V, 350 °C, 11 L min^{-1}, and 55 psi, respectively. The scan range was 100−900 uma, and the fragmentator potential was 60 V.

Quantitation was performed by the external standard method, using a commercial standard of 2-FM-lysine (furosine) (Neosystem Laboratoire, Strasbourg, France). All the analyses were performed in duplicate, and the data shown in this paper are the mean values expressed as mg/100 g of protein.

GC Analysis of Carbohydrates. Carrot soluble sugars were extracted in duplicate according to the method described by García-Baños et al. (*27*). First, 0.1 g of carrot sample was weighed into a 25-mL volumetric flask and extracted at room temperature with 5 mL of Milli-Q water for 20 min with constant stirring. The volume was made up to 25 mL with pure ethanol to obtain a final 80% ethanolic solution. Samples were then centrifuged at 9600*g* and 10 °C for 10 min. Precipitates were submitted to a second extraction with 25 mL of 80% ethanol to obtain recovery values close to 100%. One milliliter of supernatant was mixed with 0.2 mL of an ethanolic solution of phenyl-β-D-glucoside (1 mg mL^{-1}; Sigma Chemical Co., St. Louis, MO) used as internal standard. The mixture was evaporated under vacuum at 40 °C.

GC analyses were performed with an Agilent Technologies 7890A gas chromatograph equipped with a flame ionization detector (FID), using nitrogen as carrier gas. The trimethylsilyl oxime (TMSO) derivatives, prepared as described by Sanz et al. (*28*), were separated using an HP-5 MS fused-silica capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness) coated with 5% phenylmethylsilicone (J&W Scientific, Folsom, CA). The carrier gas flow rate was 1 mL min^{-1}. The oven temperature was held at 200 °C for 11 min, raised to 270 °C at a heating rate of 15 °C min^{-1}, raised again to 300 at 3 °C min^{-1}, and finally raised to 325 at 15 °C min^{-1}. The injector and detector temperatures were 280 and 325 °C, respectively. Injections were made in the split mode (1:40).

Data acquisition and integration were performed using Agilent ChemStation Rev. B.03.01 software (Wilmington, DE). The identification of TMSO derivatives of carbohydrates was carried out by comparing the experimental retention indices with those of standards previously derivatized. Quantitative data (mg g^{-1} DM) were calculated from FID peak areas. Standard solutions of fructose, glucose, sucrose, *scyllo*-, and *myo*-inositol (all of them from Sigma Chemical Co.) over the expected concentration range in carrot extracts were prepared to calculate the

response factor relative to phenyl-β-D-glucoside. In the absence of any commercial standard, the concentration of sedoheptulose was estimated assuming a response factor equal to 1.

Preparation of Protein Isolates and Analysis by SDS−PAGE. Powdered dehydrated carrot samples (100 mg) were mixed with 2 mL of Milli-Q water containing 1% sodium metabisulfite (Merck, Darmstadt, Germany) and stirred thoroughly for 2 h. The mixed slurry was centrifuged at 3000*g* for 15 min, and the supernatant was analyzed by SDS−PAGE.

For SDS−PAGE analysis, 32.5 μL of sample supernatant was added to 12.5 μL of 4X NuPAGE LDS sample buffer (Invitrogen, Carlsbad, CA) and 5 μL of 0.5 M dithiothreitol (DTT, Sigma-Aldrich) and heated at 70 °C for 10 min. Samples (20 μL) were loaded on a 12% polyacrylamide NuPAGE Novex Bis-Tris precast gel, and a continuous MES SDS running buffer was used. Gels were run for 41 min at 120 mA/gel and 200 V and stained using the Colloidal Blue Staining Kit (Invitrogen). The molecular weight of proteins was estimated by using a mixture of standard proteins with relative molecular weights ranging from 2.5 to 200 kDa (Invitrogen): myosin, 200 kDa; β-galactosidase, 116.3 kDa; phosphorylase B, 97.4 kDa; BSA, 66 kDa; glutamic dehydrogenase, 55.4 kDa; lactate dehydrogenase, 36.5 kDa; carbonic anhydrase, 31 kDa; trypsin inhibitor, 21.5 kDa; lysozyme, 14.4 kDa; aprotinin, 6 kDa; insulin B chain, 3.5 kDa; and insulin A chain, 2.5 kDa.

Measurement of Total Phenolic Content (TPC) by Folin−Ciocalteau Method. Methanolic extracts were prepared by adding 2.5 mL of HPLC grade methanol to 0.1 g of dehydrated carrot powders and homogenizing for 1 min at 24 000 rpm using an Ultra-Turrax T-25 homogenizer (IKA Labortechnik, Janke & Kunkel, Saufen, Germany). The samples were stirred for 20 min at 750 rpm using a Thermomixer (Eppendorf, Germany) and centrifuged for 15 min at 2000*g*. Supernatants were then filtered through PVDF Acrodisc syringe filters (0.45 μm, Sigma-Aldrich) for subsequent analysis.

TPC in carrots was determined using Folin−Ciocalteau reagent (2N, Sigma) according to the methods of Singelton et al. (*29*) and Patras et al. (*30*) with slight modifications. First, 100 μL of filtered methanolic extract, 100 μL of MeOH, and 100 μL of Folin−Ciocalteau reagent were vortexed in a 1.5 mL eppendorf flask. After 5 min, 700 μL of 75 g L^{-1} Na_2CO_3 was added, and the samples were vortexed briefly. The eppendorfs were then left in the dark for 20 min at room temperature. Following this, the samples were centrifuged at 13 000 rpm for 3 min. The absorbance of the sample was read at 735 nm using aqueous gallic acid (Sigma-Aldrich), 10−400 mg L^{-1}, as standard. Results were expressed as milligrams of gallic acid equivalent (GAE)/g DM.

Antioxidant Activity by the Oxygen Radical Absorbance Capacity (ORAC) Assay. Twenty-five mg of dehydrated carrot powders accurately weighed were mixed with 1 mL of acetone/water (50:50, v/v) extraction solvent. The mixture was shaken at room temperature for 1 h. The extracts were centrifuged at 14000 rpm for 15 min, and the supernatant was ready for analysis after appropriate dilution with 75 mM potassium phosphate buffer solution (pH 7.4) (Sigma-Aldrich) (*30*).

The ORAC assay using fluorescein as fluorescent probe was based on that proposed by Ou et al. (*31*) and modified by Dávalos et al. (*32*). Trolox, a water-soluble analogue of vitamin E, was used as a control standard. The reaction was carried out at 37 °C in 75 mM phosphate buffer (pH 7.4) with a blank sample (no antioxidant) in parallel, and the final assay mixture (200 μL) contained fluorescein (116.6 nM), AAPH (48 mM), and antioxidant (10−80 μM Trolox or sample). All standards used for ORAC assay were purchased from Sigma-Aldrich.

A microassay based on the use of black 96-well microplates (96F untreated, Nunc, Denmark) was used for fluorescence measurements. The plate was automatically shaken before the first reading, and the fluorescence was recorded every minute for 98 min after addition of AAPH. A Polarstar Galaxy plate reader (BMG Labtechnologies GmbH, Offenburg, Germany) with 485-P excitation and 520-P emission filters was used, controlled by the Fluostar Galaxy software version 4.11-0. AAPH and Trolox solutions were prepared daily, and fluorescein was diluted from a stock solution (1.17 mM) in 75 mM phosphate buffer (pH 7.4). All reaction mixtures were prepared in duplicate, and the analysis for each sample was carried out in triplicate. Fluorescence measurements were normalized to the curve of the blank. From the normalized curves, the area under the fluorescence decay curve (AUC) was calculated as

$$\mathrm{AUC} = 1 + \sum_{i=1}^{i=98} f_i/f_0 \quad (1)$$

where f_0 is the initial fluorescence reading at 0 min and f_i is the fluorescence reading at time *i*. The net AUC corresponding to a sample was calculated as

$$\text{net AUC} = \mathrm{AUC}_{\text{antioxidant}} - \mathrm{AUC}_{\text{blank}} \quad (2)$$

The regression equation between net AUC and antioxidant concentration was calculated. The slope of the equation was used to calculate the ORAC value by using the Trolox curve obtained for each assay. Final ORAC values were expressed as μmol of Trolox equivalent (TE)/g DM.

Rehydration Ability. *General Procedure*. Carrot slices were rehydrated by immersion in distilled water (solid-to-liquid ratio 1:50) at room temperature for 24 h. After blotting with tissue paper to remove any superficial water, rehydrated carrots were weighed. Each rehydration experiment was performed in triplicate, and no correction was made for lost solids.

The rehydration ratio (RR) (*33*) was calculated as

$$\mathrm{RR} = \frac{m_r}{m_d} \quad (3)$$

where m_r is the mass of the rehydrated sample (g) and m_d is the weight (g) of the dehydrated carrot.

Leaching Loss. Lost solids during rehydration were determined according to the AOAC method (*22*). The soak water was placed in a preweighed evaporating beaker and dried in a conventional oven at 105 °C for 24 h. The residue was weighed, and the percentage of leached solids (LL, %) with respect to the initial weight of dehydrated carrot was calculated.

Diameter Change. The change in the carrot diameter during the rehydration process was measured using vernier callipers (Mitutoyo Corp., Japan) and calculated according to Bhattacharya (*34*):

$$d\,(\%) = \frac{d_r - d_d}{d_d} \quad (4)$$

where *d* is the carrot diameter increase during the rehydration process (%), d_r is the diameter of the rehydrated sample (mm), and d_d is the diameter of the initial dehydrated sample (mm).

Statistical Analysis. Data were subjected to one-way analysis of variance (Tukey HSD Multiple Range Test) by applying the Statgraphic 4.0 program (Statistical Graphics Corp., Rockville, MD) for Windows. The significance of differences was defined as $P < 0.05$.

RESULTS AND DISCUSSION

Carrot Dehydration. Figure 1 shows the drying curves obtained in the dehydration of carrots by US under different operating conditions. As observed, moisture loss values higher than 85%, which ensure the microbiological stability of dehydrated carrots, were obtained for the different conditions tested. In agreement with the results previously reported by other authors (*19*, *20*), the synergic effect of US and temperature increased the dehydration rate of carrots, with moisture loss rates up to 90% in 75 min for sample US60BL. Ultrasound technology produces a series of effects (microagitation, creation of microscopic channels, and cavitation of water molecules) which make the moisture removal easier and allow dehydration to be carried out at milder temperatures (60 °C or less) (*35*), this being particularly useful for preserving the bioactivity of heat-sensitive carrot constituents.

Blanching also showed a positive effect on the dehydration rate of US-processed carrots at any set of operating conditions. It is well-known that high-temperature and short-time blanching has a beneficial effect not only of inactivation of enzymes, which, if untreated, could be active at least during the early stages of drying, but also of shortening drying times (*36*).

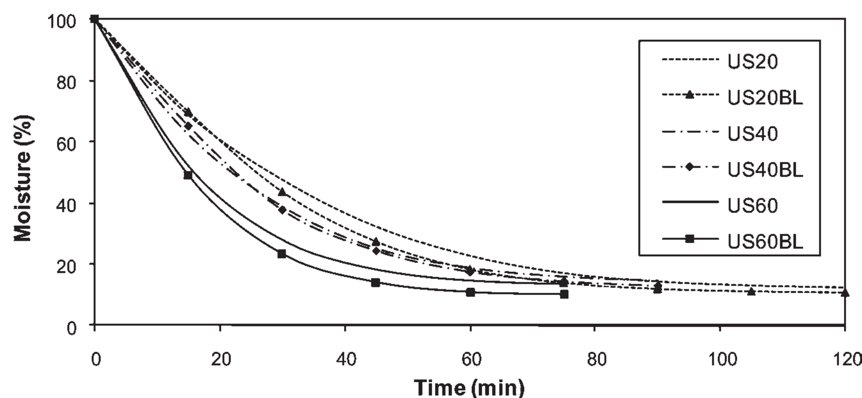

Figure 1. Drying curves of carrots dehydrated by US under different operating conditions. For nomenclature of samples, see **Table 1**.

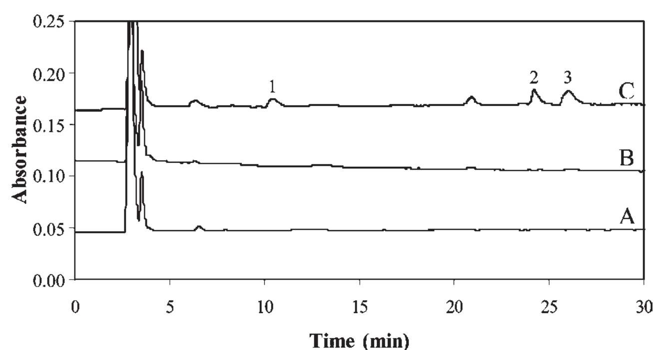

Figure 2. RP-HPLC-UV chromatogram of 2-furoylmethyl-amino acids in acid hydrolysates of (A) freeze-dried carrot, (B) US dehydrated carrot (US60BL), and (C) commercial dehydrated carrot (COMM2). (1) 2-FM-Ala, (2) 2-FM-GABA, and (3) 2-FM-Lys +2-FM-Arg.

Regarding precision, relative standard deviation values in the range 0.1−0.3% show the excellent reproducibility of the US dehydration process irrespective of the experimental conditions tested here.

Maillard Reaction Evolution. Figure 2 shows, as an example, the RP-HPLC chromatographic profile of 2-FM-AA obtained for the acid hydrolysates of three of the dehydrated carrots under analysis: FD, US60BL, and COMM2. Identification of 2-FM-AA of Lys, Arg, γ-aminobutyric acid (GABA), and Ala was confirmed by coinjection of the corresponding standards synthesized in our laboratory and by LC-MS analysis.

2-FM-Lys +2-FM-Arg (peak 3 in **Figure 2**) were detected only in carrots dehydrated by power US at 60 °C (**Table 2**). Samples subjected to blanching before US processing presented a slightly higher level of 2-FM-Lys+2-FM-Arg as compared to samples with no pretreatment, probably due to a higher dehydration rate of these samples (**Figure 1**). Similarly to the freeze-dried carrots, no formation of 2-FM-Lys+2-FM-Arg in carrots processed by US at 20 and 40 °C was detected. Levels of this quality marker in carrots dehydrated by US at 60 °C were significantly lower than those of commercial dehydrated carrots analyzed here and data previously reported by Soria et al. (*7*) for industrially dried carrots (average of 589 mg/100 g of protein). Levels of 403 mg of 2-FM-Lys/100 g of protein have also been reported by Rufián-Henares et al. (*6*) in a carrot-based product dehydrated under mild temperature conditions (30 °C for 180 h). The content of 2-FM-Ala and 2-FM-GABA (traces in US-dehydrated samples) was always lower than that of 2-FM-Lys +2-FM-Arg, supporting thus the usefulness of the latter joint marker as a sensitive quality indicator to control the early stages of MR in carrots subjected to dehydration.

Table 2. Quantitative Analysis of 2-Furoylmethyl-amino Acids (2-FM-AA) in Dehydrated Carrot Samples (Mean of Two Replicates ± SD)

	2-FM-AA (mg/100 g of protein)		
carrot sample	2-FM-Lys +2-FM-Arg	2-FM-GABA	2-FM-Ala
FD[a]	ND[b]a	ND a	ND a
FDBL[c]	ND a	ND a	ND a
US60	23 ± 1 a	tr[b]a	ND a
US60BL	39 ± 1 a	tr a	ND a
COMM1	848 ± 49 b	312 ± 10 b	98 ± 6 b
COMM2	447 ± 11 c	279 ± 16 bc	216 ± 34 c
COMM3	426 ± 18 c	228 ± 19 c	119 ± 10 b
COMM4	819 ± 102 b	599 ± 65 d	618 ± 82 d
COMM5	416 ± 18 c	312 ± 6 b	154 ± 3 bc
COMM6	358 ± 14 c	152 ± 6 e	134 ± 1 bc

[a] FD: Freeze-dried carrot. [b] ND: not detected; tr: traces. [c] BL: carrots blanched prior to dehydration. Samples with the same lower-case letter a−e within the same column showed no statistically significant differences for their mean values at the 95.0% confidence level.

Carbohydrate Analysis. Table 3 lists the concentration of major and minor soluble carbohydrates determined in carrots experimentally dehydrated by US or freeze-drying and in six commercial dehydrated carrots. In agreement with the evolution of the MR early stages, the content of reducing sugars (fructose and glucose) showed the highest change for blanched samples processed by US at 60 °C, this decrease (54%) being particularly noticeable for glucose due to its higher involvement in MR. A very low decrease in the content of glucose and fructose was observed in samples US20 and US40, as compared to data for US60. These results seem to indicate the slight effect of US processing on reducing carbohydrates at low temperature. Hardly any change was found for sucrose and minor carbohydrates in carrots processed by power US at different operating conditions.

Regarding the effect of blanching, no significant differences associated with the loss of sugars by lixiviation were found for freeze-dried samples FD and FDBL. However, the probably higher porosity of blanched samples, which could favor the diffusion of water and the most soluble carbohydrates to the surface, might contribute, among others, to their loss during US dehydration.

Major sugars showed a wide variability in commercial dehydrated carrots, the content here determined for samples COMM1−6 falling in the range previously reported for other dehydrated carrots (*7*, *9*, *11*). Inositols, such as *scyllo*- and *myo*-inositol, naturally present in several food products of vegetable origin, have been reported to be stable during the different stages of convective air-drying of carrots (*7*) and in orange juice subjected to different storage and processing conditions (*37*). In agreement with this,

Table 3. Quantitative Analysis of Carbohydrates in Dehydrated Carrot Samples under Analysis (Mean of Two Replicates ± SD)

	carbohydrates (mg g^{-1} DM) ± SD					
carrot sample	fructose	glucose	sucrose	sedoheptulose	*scyllo*-inositol	*myo*-inositol
FD[a]	79.7 ± 0.6 a	76.7 ± 4.3 a	493.6 ± 11.1 a	16.7 ± 0.8 ab	2.2 ± 0.1 abc	2.9 ± 0.2 a
FDBL[b]	75.1 ± 1.5 b	74.4 ± 1.6 a	465.5 ± 16.2 ab	15.6 ± 1.3 abc	1.9 ± 0.1 abd	2.6 ± 0.01 a
US20	74.2 ± 2.2 bc	62.9 ± 3.3 ab	470.5 ± 21.2 ab	19.1 ± 0.8 b	2.7 ± 0.2 ce	3.5 ± 0.6 ab
US20BL	51.2 ± 10.5 d	41.0 ± 9.9 cde	422.6 ± 11.1 bc	16.4 ± 0.3 abc	2.0 ± 0.2 abcd	3.1 ± 0.3 ab
US40	73.8 ± 1.4 abc	62.5 ± 3.7 ab	458.9 ± 17.3 ab	13.7 ± 0.3 abcde	1.5 ± 0.3 df	2.6 ± 0.1 a
US40BL	49.9 ± 2.0 d	39.1 ± 1.1 cde	443.1 ± 17.4 ab	15.3 ± 0.9 abc	1.2 ± 0.07 f	2.5 ± 0.1 a
US60	51.7 ± 3.1 cd	45.7 ± 1.7 bde	489.6 ± 8.7 a	17.6 ± 0.7 ab	2.2 ± 0.2 abc	3.4 ± 0.2 ab
US60BL	45.6 ± 12.5 de	34.1 ± 8.3 f	436.8 ± 5.6 b	14.9 ± 3.4 abce	2.2 ± 0.1 abc	3.5 ± 0.5 ab
COMM1	47.8 ± 2.8 de	39.9 ± 2.4 cde	347.9 ± 8.9 de	8.8 ± 0.4 df	1.7 ± 0.2 adf	3.0 ± 0.2 a
COMM2	41.3 ± 0.2 de	21.9 ± 0.2 cf	347.2 ± 0.3 de	7.5 ± 0.1 f	2.2 ± 0.1 abc	4.0 ± 0.02 bc
COMM3	47.3 ± 1.9 de	35.5 ± 2.4 cdf	375.2 ± 12.0 ce	10.0 ± 0.7 def	2.4 ± 0.2 bc	4.8 ± 0.2 c
COMM4	85.2 ± 4.5 a	58.3 ± 4.1 abe	314.0 ± 13.6 d	27.1 ± 0.7 g	3.2 ± 0.2 e	4.0 ± 0.1 bc
COMM5	57.1 ± 0.9 bcd	48.6 ± 1.5 bde	434.2 ± 13.0 b	11.4 ± 0.02 cdef	2.6 ± 0.05 ce	4.7 ± 0.1 c
COMM6	27.2 ± 0.3 e	16.3 ± 1.4 f	447.1 ± 5.1 ab	8.7 ± 0.3 df	3.0 ± 0.1 e	5.0 ± 0.04 c

[a] FD: Freeze-dried. [b] BL: carrots blanched prior to dehydration. Samples with the same lower-case letter a−f within the same column showed no statistically significant differences for their mean values at the 95.0% confidence level.

results listed in **Table 3** show the low variability in the concentration of these two compounds irrespective of the processing conditions (US, freeze-drying) or the commercial sample considered (COMM1−6). However, a wider range of variation was found for sedoheptulose, a minor carbohydrate described for the first time in carrots by Soria et al. (*11*). Whereas the average concentration of sedoheptulose in US-dehydrated carrots (16 mg g^{-1} DM) matched well that of freeze-dried carrots (16.7 mg g^{-1} DM), it was higher than that of most commercial carrots (8−11 mg g^{-1} DM except for COMM4, showing 27 mg g^{-1} DM). The different variety or degree of ripeness of carrots subjected to dehydration could be responsible for the differences observed. Losses of minor carbohydrates due to blanching were lower than those of the more water-soluble major sugars.

SDS−PAGE Analysis of Carrot Proteins. As a consequence of the dehydration process, cross-linking and aggregation of proteins may occur, modifying their structure and, consequently, their functionality. However, and to the best of our knowledge, no studies on the evaluation of the changes in the structure of carrot proteins following dehydration by power US have been carried out so far. With this purpose, the electrophoretic profiles of FD, COMM4, COMM6, US60, and COMM1 samples were obtained by SDS−PAGE under reducing conditions. FD carrots showed four major bands with molecular weights of ∼18, 36.5, 41.2, and 55.4 kDa (**Figure 3**, lane 1). All commercial samples (**Figure 3**, lanes 2, 3 and 5) analyzed showed different patterns of bands as compared to FD. According to the results derived from the analysis of the acid hydrolysates of the Amadori compounds (**Table 2**), bands observed in commercial samples could be attributed to unfolding, cross-linking, and aggregation of proteins taking place during the advanced stages of the MR. This is particularly noticeable in sample COMM4 (**Figure 3**, lane 2), which showed a variety of bands with slower electrophoretic mobility and different molecular weight, indicative of the formation of a wide range of glycated species of proteins.

In contrast, a pattern similar to that of FD was observed for US60 (**Figure 3**, lane 4), indicating that ultrasonic drying does not cause important structural changes in carrot proteins, as supported by the limited extent of the MR in US60 when compared to the commercial samples analyzed (**Table 2**).

Total Phenolic Content and Antioxidant Activity. As previously described, the antioxidant activity of carrots is due to different compounds, including β-carotene, vitamin C, polyphenols, etc. Solvent extraction is usually employed for isolation of antioxidants, and both extraction yield and activity of extracts

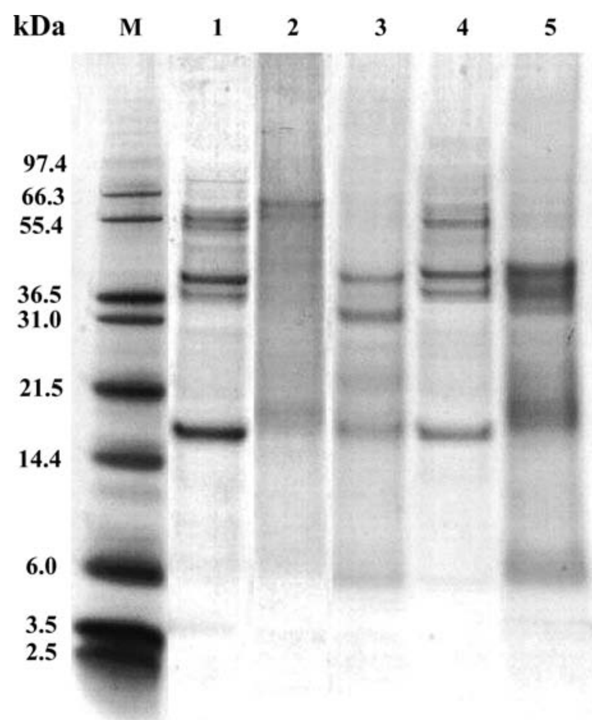

Figure 3. SDS−PAGE analysis of dehydrated carrots: (1) FD, (2) COMM4, (3) COMM6, (4) US60, and (5) COMM1. (M) Markers of molecular weight.

are strongly dependent on the solvent, due to the different antioxidant properties of compounds with different polarity extracted (*38*). Therefore, two carrot extracts in solvents of different polarity were prepared as described under Materials and Methods.

First, the total phenolic content (TPC) of carrot methanolic extracts was determined by the Folin−Ciocalteau method (**Table 4**). Similar results were obtained for all the laboratory-dried carrots, irrespective of the dehydration technique (US or freeze-drying). The longer processing time in US40 and higher processing temperature in US60 as compared to US20 could be responsible for the slight decrease in polyphenol content of these samples. A similar effect has been reported by Chantaro et al. (*39*) for other thermolabile antioxidants, such as β-carotene in carrot peels subjected to drying at 60 and 70 °C (shorter drying times at

Table 4. Total Phenolic Content (TPC) and Antioxidant Activity by ORAC Assay of Dehydrated Carrots under Analysis (Mean of Two Replicates ± SD)

carrot sample	TPC (mg GAE/g DM)	ORAC (μmol TE/g DM)
FD[a]	1.365 ± 0.081 a	31.22 ± 0.606 a
US20	1.366 ± 0.046 a	21.82 ± 0.075 c
US20BL[b]	1.331 ± 0.078 ac	24.49 ± 0.030 b
US40	1.111 ± 0.023 b	19.09 ± 0.016 d
US40BL	1.101 ± 0.011 b	24.33 ± 0.189 b
US60	1.235 ± 0.039 abc	24.43 ± 0.548 b
US60BL	1.252 ± 0.080 abc	25.41 ± 0.385 b
COMM1	1.628 ± 0.056 d	29.57 ± 0.460 a
COMM2	2.885 ± 0.049 e	56.38 ± 0.955 e
COMM3	1.931 ± 0.011 f	45.97 ± 0.893 f
COMM4	3.246 ± 0.065 g	53.91 ± 0.572 e
COMM5	1.651 ± 0.006 d	36.52 ± 0.743 g
COMM6	1.680 ± 0.072 d	38.40 ± 0.374 g

[a] FD: freeze-dried. [b] BL: carrots blanched prior to dehydration. Samples with the same lower-case letter a−g within the same column showed no statistically significant differences for their mean values at the 95.0% confidence level.

Table 5. Rehydration Ratio (RR), Leaching Loss (LL, %) and Diameter Change (*d*, %) after Rehydration of Carrot Samples under Analysis (Mean of Three Replicates ± SD)

carrot sample	RR	LL (%)	*d* (%)
FD[a]	6.36 ± 0.02 abc	44.96 ± 2.06 ab	
FDBL[b]	6.71 ± 0.95 bcd	50.49 ± 1.40 abcd	
US20	4.66 ± 0.20 e	51.26 ± 1.09 abcd	24.67 ± 2.87 a
US20BL	8.00 ± 0.09 d	44.49 ± 1.58 ab	41.89 ± 2.05 b
US40	4.87 ± 0.11 ae	52.79 ± 0.49 bcd	28.22 ± 0.03 ac
US40BL	7.96 ± 0.26 cd	46.94 ± 5.21 ab	45.87 ± 4.46 b
US60	4.92 ± 0.16 ae	55.30 ± 0.44 cd	21.00 ± 1.84 a
US60BL	8.04 ± 0.50 d	46.60 ± 2.62 abc	39.70 ± 1.75 bc
COMM1	6.22 ± 0.20 ab	55.87 ± 2.68 bcd	
COMM2	5.69 ± 0.16 abe	55.34 ± 2.52 d	
COMM3	7.20 ± 0.31 bcd	43.86 ± 1.50 a	
COMM4	6.25 ± 0.83 abe	49.49 ± 5.52 abcd	
COMM5	5.97 ± 0.14 abe	53.01 ± 0.69 bcd	
COMM6	6.47 ± 0.01 abcd	51.30 ± 2.96 abcd	

[a] FD: Freeze-dried. [b] BL: carrots blanched prior to dehydration. Samples with the same lower-case letter a−e within the same column showed no statistically significant differences for their mean values at the 95.0% confidence level.

higher temperatures decrease the degradation reaction). Changes in physical properties such as texture, matrix softening, etc. in carrots processed at different drying conditions may also affect the extractability of antioxidants and, therefore, their bioactivity (*40*).

Antioxidant activity was determined by ORAC assay in acetone/water extracts. Drying temperature in the 20−60 °C range did not significantly affect the results of this assay, all of them being slightly lower than that of the FD sample. The high correlation (R^2 = 0.8662) between TPC and ORAC measurements for all the samples under analysis suggests that the presence of phenolic compounds largely accounted for their antioxidant capacity. Similar results were reported by Zhang and Hamauzu (*41*), who found that antioxidant and radical scavenging activities in different carrot tissues decreased in the same order as the phenolic content.

Results of the ORAC assay listed in **Table 4** for both commercial and laboratory-dehydrated samples fall well in the range reported for freeze-dried carrots collected from various United States marketplaces at different harvesting seasons (25−99 μM TE g^{-1} DM) (*42*). As for phenolic compounds, the differences observed between commercial and laboratory-dried carrots are supposed to originate mainly from the dependence of carrot bioactivity on its variety, geographical origin, harvest time, and processing conditions (*8*, *40*). Although the higher antioxidant activity of commercial samples could also be related to the higher MR evolution (**Table 2**), which might probably give rise to the formation of advanced glycation end products (AGEs) with antioxidant activity (*43*), it is not possible to confirm this possibility due to the lack of information on the processing conditions of these samples. The effect of storage, which is deemed to play an important role in the antioxidant capacity and phenolics of different fresh fruits and vegetables, might also contribute to these differences (*44*).

Contradictory results have been reported on the effect of a previous blanching on the preservation of the bioactive compounds and the antioxidant activity of different vegetables (*39*, *40*). In this study, while blanching showed no influence on the TPC of the samples undergoing US drying under identical operating conditions, the antioxidant potential was slightly increased with sample pretreatment before drying for samples processed at 20 and 40 °C.

Rehydration Ability. A significant improvement in the rehydration ability of carrots processed by US was observed for blanched samples (**Table 5**). It has been reported that loosening of the cellular network and separation along the middle lamella observed after blanching result in a softening of the carrot tissue. Moreover, a reduced cohesiveness of the matrix improves water absorption and yields better rehydrated products (*36*). Blanching has also been reported to modify the structural characteristics of fiber, hence facilitating the water uptake of carrot peels (*39*).

Rehydration ratios in the range 5.69−7.20 were found for samples COMM1−6; this means that the structural damage and cell shrinkage occurred during the drying process of these samples were higher than those of the blanched carrots submitted to US dehydration (RR $\approx$ 8). The development of greater internal stresses and the creation of pores which facilitate the water uptake contributed to the higher RR observed for US-dehydrated carrots. Blanched carrots dehydrated by US also showed a better rehydration ability than the freeze-dried carrots analyzed here (considered as a reference of high-quality dehydrated carrots) and those previously submitted to citric acid or NaCl treatment to improve their rehydration properties, with RR in the range of 3−7 (*45*, *46*).

As a consequence of blanching, the loss of soluble solids and the solubilization of structure polymers such as protopectin may take place (*39*). Average leaching losses of 50% were found for both US dehydrated and commercial carrots. No significant improvement in the leaching loss (%) was found for samples submitted to blanching prior to US dehydration.

In addition, the visual appearance of dehydrated products after rehydration is of prime importance for the consumers' acceptance of the product. Therefore, the change in diameter after rehydration was measured for carrots submitted to US dehydration (**Table 5**). As expected, a high correlation was found between the results of RR and *d* (%), with blanched samples showing a similar appearance to that of raw carrots (**Figure 4**).

The preservation of the quality and bioactivity of carrots dehydrated by power ultrasound (US) has been evaluated for the first time by measuring the evolution of the MR and the changes in soluble sugars, proteins, total polyphenols, antioxidant activity, and rehydration ability. The effect of conventional blanching (high temperature, short time) prior to US dehydration of carrots has also been evaluated.

Power US not only improves the rate of dehydration as compared to conventional processes, but the milder processing conditions used in US drying also limit the MR extent in dehydrated carrots. Minor changes in reducing sugars, total phenolic content, antioxidant activity, and similarity of protein profiles for

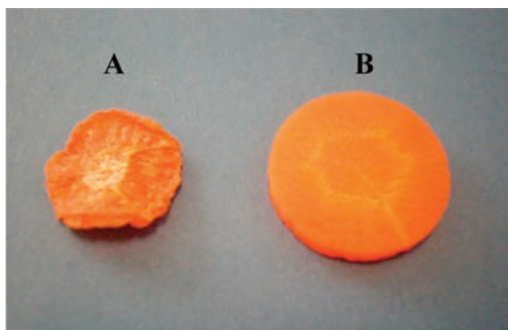

Figure 4. Rehydration of ultrasound-assisted hot air-dried carrot. Visual aspect before (A) and after (B) rehydration.

both freeze-dried and ultrasonically dried samples, together with improved rehydration properties of blanched carrots, also support US as an alternative to freeze-drying for obtaining dehydrated carrots of premium quality. Further studies should be carried out to evaluate the potential of US as a profitable alternative to freeze-drying for obtaining dehydrated carrots with enhanced quality.

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