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Rate of Antioxidant Degradation and Color Variations in Dehydrated Apples as Related to Water Activity

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Dehydrated apples were studied to evaluate the effects of water activity on the stability of their antioxidants and color. Apples were freeze-dried, ground, then equilibrated, and stored at eight water activity levels, ranging from 0.058 to 0.747, at 40 °C. Their contents of hydroxycinnamic acids, dihydrochalcones, catechin, epicatechin, polymeric flavan-3-ols, and hydroxymethylfurfural, their antioxidant activity values, and their Hunter colorimetric parameters were analyzed at different storage times. Antioxidant degradation followed pseudo-first-order kinetics and was accelerated by increasing the water activity. The order of antioxidant stability in the products at water activity levels below 0.316 was catechin, epicatechin, and ascorbic acid < total procyanidins < dihydrochalcones and *p*-coumaric acid < chlorogenic acid; however, in the products at water activity levels above 0.316, the degradation of all antioxidants was very fast. The hydroxymethylfurfural formation rate increased exponentially during storage, especially at high water activity levels. The antioxidant activity of the dehydrated apples decreased during storage, consistent with antioxidant loss. The variations of the colorimetric parameters, namely, lightness (L^*), redness (a^*), and yellowness (b^*), followed pseudo-zero-order kinetics and were accelerated by increasing water activity. All analytical indices indicated that the dehydrated apples were stable at water activity levels below 0.316, with the degradation rate accelerating upon exposure to higher relative humidities. Above 0.316, a small increase in water activity of the product would sharply increase the degradation rate constants for both antioxidant and color variations.

KEYWORDS: Apple; *Malus domestica*; antioxidant; water activity; degradation; color

INTRODUCTION

Scientific evidence accumulated over the past few years points to relevant health properties of apples (*Malus domestica* L.). An *in vivo* study on humans revealed that the consumption of apple juice inhibits low-density lipoprotein (LDL) oxidation by 9−34% (*1*). Extracts from raw apples strongly inhibit colon and liver cancer cell proliferation *in vitro*. These properties have been attributed to apple phenolic compounds (*2*, *3*), which are absorbed, metabolized, and excreted by humans (*4*).

The main structural classes of apple phenolics include flavan-3-ols, hydroxycinnamates, dihydrochalcones, flavonols and anthocyanins (*5*). The flavan-3-ols are present in monomeric (epicatechin and catechin), dimeric, trimeric, and oligomeric forms named procyanidins. The average degree of polymerization in apples varies from 4 to 7.1 (*6*, *7*). The hydroxycinnamic acids are mainly represented by chlorogenic acid; another compound of the same class is an ester of *p*-coumaric acid. Dihydrochalcones are mainly represented by phloridzin (phloretin 2′-*O*-glucoside) and phloretin 2′-*O*-xyloglucoside. Flavanols, such as quercetin glycosides, are found in minor amounts and are mainly located in the skin. Anthocyanins, such as cyanidin glycosides, are only present in the skins of some red cultivars (*5*).

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Apples rank fourth among the most important fruits worldwide (http://www.fao.org/es/ess/top/commodity.html). The largest apple producer in the world is China, followed by the U.S. and then Turkey, Iran, Italy, France, Poland, and Russia. Besides fresh apples, dry and intermediate-moisture apples are of particular interest for the food industry. In fact, progressive reduction of water activity (a_w) decreases the rates of microbial growth, microbial production of toxins, and enzyme activities. For most foods, the limits below which microorganisms cannot grow or produce toxins is in the a_w range between 0.6 and 0.7. Upon a further decrease in the a_w level, water also becomes unavailable as a solvent to support enzymatic and chemical reactions. The maximum food stability occurs at the monomolecular moisture content, in which a monolayer of water is strongly bound to specific sites on the food solids (*8*). In addition to increased stability, dry and intermediate-moisture products possess different sensory attributes than the fresh fruits. For dry snack food (a_w below 0.25), crispness is a desirable sensory property, and its loss, because of the absorption of water, limits consumer acceptability. Apple chips show optimal crispness, measured by both mechanical tests and sensory evaluation, at a_w levels below 0.2 (*9*). For intermediate-moisture foods (a_w in the range between 0.25 and 0.75), food texture is firm and flexible (*8*) and water acts as the main solvent of the volatile compounds, thus influencing flavor retention/release (*10*).

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In intermediate- and high-moisture apples, increasing a_w from 0.3 to 0.85 gradually solubilizes the volatile compounds, resulting in increased aroma and flavor. At a_w levels above 0.85, the volatiles in the water phase progressively decrease (*11*).

On the other hand, oxidation and non-enzymatic browning reactions are the major causes of degradation of dried and intermediate-moisture foods (*8*, *12*−*14*). Therefore, knowledge on the rate of antioxidant degradation and color changes in dry and intermediate-moisture apples would be helpful to predict the potential of these products as sources of dietary phytochemicals.

The present study was focused on dehydrated apples ($0.058 \leq a_w \leq 0.747$), with the aim of evaluating the kinetics of degradation for ascorbic acid, phenolics, and antioxidant activity and the browning rate as a function of a_w.

MATERIALS AND METHODS

Chemicals. The salts used to prepare saturated solutions (namely, LiBr, $ZnBr_2$, LiCl, KF, $MgCl_2$, NaBr, KI, and NaCl), the 2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl radical (DPPH), the reference antioxidant 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), and the standards of ascorbic acid, chlorogenic acid, phloridzin, *p*-coumaric acid, and hydroxymethylfurfural (HMF) were purchased from Sigma-Aldrich (Milan, Italy). Standards of epicatechin and catechin were purchased from Extrasynthese (Lyon, France).

Apple Dehydration. Two lots of apples (8 kg) (var. Golden Delicious) were obtained from a local market. Fruits were sorted, cut into eight slices, and peeled, and the core was removed. The apple slices were then dehydrated by freeze-drying in a Lyoflex Edwards (Crawley, U.K.) apparatus.

Storage Study. Freeze-dried apples were ground into powders with a model K 3000 Braun Multisystem blender (Braun Electronic, Kronberg, Germany) and sieved (800 μm). The powders were first weighed in Petri dishes (8.5 cm diameter, 0.141 g of apple product per cm^2). The dishes were then placed into airtight plastic boxes on wire-mesh racks situated above saturated salt solutions. The boxes were stored at 40 °C in a thermostated cabinet. To equilibrate products at different a_w levels, the following saturated salt solutions were used: LiBr (a_w at 40 °C = 0.0580 ± 0.0039), $ZnBr_2$ (a_w at 40 °C = 0.0754 ± 0.0025), LiCl (a_w at 40 °C = 0.1121 ± 0.0021), KF (a_w at 40 °C = 0.2268 ± 0.0081), $MgCl_2$ (a_w at 40 °C = 0.3160 ± 0.0013), NaBr (a_w at 40 °C = 0.5317 ± 0.0041), KI (a_w at 40 °C = 0.6609 ± 0.0023), and NaCl (a_w at 40 °C = 0.7468 ± 0.0013). The observed a_w values of the saturated solutions were consistent with those reported previously (*14*). The equilibrium moisture content was reached within 2−3 days. Two lots of dehydrated apples were incubated at each a_w. Samples were analyzed periodically for 76 days.

Moisture Content and a_w. Moisture contents of apple products were determined using a vacuum oven at 70 °C and 50 Torr for 18 h. The a_w of apple products and saturated salt solutions was measured by a dew-point hygrometer (Aqualab, Decagon Devices, WA). Triplicate determinations were made for each sample.

Soluble Solids, pH, and Titratable Acidity. Dehydrated apples were mixed with water (0.5 g of powder in 20 mL), and the mixtures were equilibrated at 20 °C. Soluble solids were measured at 20 °C using a RFM 340 refractometer (Bellingham and Stanley Ltd., Tunbridge Wells, U.K.) and expressed as °Brix (grams of sucrose per 100 g of dry product). The pH was determined with a model 62 pH meter (Radiometer Copenhagen, Denmark). Titratable acidity was determined by titration with 0.1 M NaOH to pH 8.1. Results were expressed as grams of malic acid equivalent per 100 g of dry product. Duplicate determinations were made for each sample.

Color. A SL-2000 Chromameter (Labo Scientifica, Parma, Italy) was used, which provided the Hunter L^*, a^*, and b^* colorimetric coordinates. L^* is an index of lightness, which is assigned by considering each color as equivalent to a member of a gray scale, between black ($L^* = 0$) and white ($L^* = 100$). The colorimetric parameter a^* takes positive values for reddish colors and negative values for greenish ones; therefore, $+a^*$ and $-a^*$ are referred to as redness and greenness indices, respectively. The colorimetric parameter b^* takes positive values for yellowish colors and negative values for bluish colors; therefore, $+b^*$ and $-b^*$ are referred to as yellowness and blueness indices, respectively. The chromameter was calibrated with a white standard. Triplicate determinations were made for each sample.

Extraction Procedures. Three extraction procedures were applied on dehydrated apples as follows: aliquots of powders (0.5 g) were added to either 10 mL of methanol (*15*), 10 mL of acetone/water (70:30, v/v) (*16*), or 5 mL of 6% metaphosphoric acid containing 1 g/L sodium metabisulphite (*17*). The mixture was vortexed for 2 min and centrifuged (10000*g* for 10 min at 15 °C). The supernatant was filtered through Whatman No. 4 filter paper. Duplicate extractions were made for each sample.

High-Performance Liquid Chromatography (HPLC) Equipment. The HPLC equipment consisted of a model 600 HPLC pump coupled with a Waters model 2996 photodiode array detector, operated by Empower software (Waters, Vimodrone, Italy).

UV−Vis Spectrophotometer. UV−vis measurements were performed on a Jasco UVDEC-610 spectrophotometer (Jasco Europe, Cremella, LC, Italy).

Ascorbic Acid. The ascorbic acid contents of sample extracts in 6% metaphosphoric acid containing 1 g/L sodium metabisulphite were analyzed according to the HPLC procedure of Mannino and Pagliarini (*18*). A Bio-Rad fruit quality analysis column (300 × 7.8 mm inner diameter) was used. Chromatographic separation was performed with 1 mM H_2SO_4 as an eluent under isocratic conditions, with a 1 mL/min flow rate, at room temperature. Ascorbic acid was quantified at 245 nm using a calibration curve built with a pure standard. Concentrations were expressed as milligrams per kilogram of dry product. Duplicate extracts were analyzed for each sample.

Phenolic Compounds and HMF. The phenolic and HMF contents of sample extracts in methanol were analyzed according to the HPLC procedure by Tomas-Barberan et al. (*19*), with modifications. A 250 × 4.6 mm inner diameter, 5 μm, Symmetry reverse-phase C-18 column (Waters, Vimodrone, Italy) equipped with a Symmetry C-18 precolumn was used. Formic acid (5%) was added to both methanol and water before preparing the following mobile phase: (A) water/methanol (95:5, v/v), (B) water/methanol (88:12, v/v), (C) water/methanol (20:80, v/v), and (D) methanol. The following gradient elution was used: 0−5 min, 100% A; 5−10 min linear gradient to reach 100% B; 10−13 min, 100% B; 13−35 min linear gradient to reach 75% B and 25% C; 35−50 min linear gradient to reach 50% B and 50% C; 50−52 min linear gradient to reach 100% C; 52−57 min, 100% C; 57−60 min, 100% D. The injection volume was 20 μL. The flow rate was 1 mL/min.

Standards of chlorogenic acid, epicatechin, catechin, phloridzin, and HMF were used to identify peaks by retention times and UV−vis spectra and to build calibration curves for quantification. Epicatechin, catechin, phloridzin, and HMF were quantified at 280 nm. Chlorogenic acid was quantified at 330 nm. A peak was tentatively assigned to phloretin 2′-*O*-xyloglucoside based on its UV−vis spectrum and literature data and quantified at 280 nm using the calibration curve built with phloridzin. The presence of a *p*-coumaric acid ester was revealed by alkaline hydrolysis according to Nardini et al. (*20*). Briefly, 0.5 mL of the methanolic extract was added to 4.5 mL of 2 N NaOH. The mixture was then incubated at 30 °C for 30 min and neutralized with 0.9 mL of concentrated HCl prior to HPLC analysis. *p*-Coumaric acid ester was quantified at 311 nm using a calibration curve built with *p*-coumaric acid. Concentrations of phenolic compounds were expressed as milligrams per kilogram of dry product. Duplicate extracts were analyzed for each sample.

Total Procyanidins. The procyanidin contents of apple extracts in acetone/water (70:30, v/v) were analyzed. For sample preparation, an aliquot of extract (0.25 mL) was dried with nitrogen and redissolved in 1 mL of 0.1 M phosphate buffer at pH 7.0. Then, the sample was applied to a 500 mg Sep-pak C18 Cartridge (Waters, Vimodrone, Italy) and eluted with 1 mL of methanol (*17*). For the vanillin assay, the reaction medium comprised 0.5 mL of sample (after Sep-pak purification) or standard in methanol, 1.25 mL of 1% vanillin in methanol, and 1.25 mL of 9 N H_2SO_4 in methanol (*21*). The mixture was incubated at 25 °C until the time necessary to reach maximum absorbance at 500 nm. The maximum absorbance at 500 nm was used to quantify procyanidin content using a calibration curve built with catechin. Procyanidin content was expressed as milligrams of catechin equivalents per kilogram of dry product. Duplicate extracts were analyzed for each sample.

Table 1. Physicochemical Characterization of Two Lots of Dehydrated Apples

quality index	lot 1	lot 2
titratable acidity (g of malic acid/100 g of dw)	2.57 ± 0.01	2.41 ± 0.01
pH	3.69 ± 0.13	3.70 ± 0.16
soluble solids (g of sucrose/100 g of dw)	70 ± 6	70 ± 6
Antioxidant Contents (mg/kg of dw)		
ascorbic acid	58 ± 5	24 ± 1
chlorogenic acid	784 ± 79	676 ± 66
p-coumaric acid derivative	51 ± 2	53 ± 2
epicatechin	343 ± 1	293 ± 1
catechin	43 ± 4	50 ± 5
phloridzin	69 ± 10	53 ± 1
phloretin 2′-*O*-xyloglucoside	47 ± 1	41 ± 2
total procyanidins	1745 ± 210	1784 ± 171
DPPH Scavenging Activity (mmol of TE/kg of dw)		
methanol extract	12.2 ± 0.9	11.2 ± 0.4
acetone/water (70:30, v/v) extract	14.9 ± 0.1	16.5 ± 0.5
Colorimetric Parameters		
L^*	78.4 ± 0.4	70.5 ± 0.3
a^*	−0.30 ± 0.2	0.64 ± 0.2
b^*	24.7 ± 0.3	17.8 ± 0.4

DPPH Scavenging Test. This assay was performed as described previously (*22*). Briefly, 1 mL of different dilutions of both the sample extracts in methanol or the sample extracts in acetone/water (70:30, v/v) was added to 2.5 mL of a 6.35×10^{-5} M methanolic solution of DPPH. The decrease in absorbance at 515 nm was determined after 30 min of incubation at room temperature (when a constant value was reached). The percent decrease of DPPH concentration was calculated with respect to the initial value. A dose−response curve was constructed, and the amount of sample required to lower the initial DPPH concentration by 50%, I_{50}, was interpolated. Trolox was used as a reference antioxidant. The antioxidant activity of apple powders was expressed as Trolox equivalents (TE). TE is the ratio of the I_{50} of Trolox (nanomoles) to the I_{50} of the sample [milligrams, dry weight (dw)]. Duplicate extracts were analyzed for each sample.

Statistical Analysis. The quality indices used to characterize two dehydrated apple lots were evaluated in duplicate or triplicate, as specified before, on independent sample aliquots. The kinetic constants represent the average values obtained from two lots of dehydrated apples. Data regressions were conducted using the Statgraphics 5.1 software (STCC, Inc., Rockville, MD).

RESULTS AND DISCUSSION

Initial Characterization of the Dehydrated Apples. The characterization of dehydrated apple lots at the time of production is shown in **Table 1**. The values for soluble solids content, titratable acidity, and pH were consistent with those found in fresh full-ripe fruits (*23*). Polyphenol contents of dehydrated apples were similar to those reported previously for fresh apple pulp (*23*, *24*). A comparison between the ascorbic acid content of the dehydrated apples to literature data is not feasible, because this compound in apples varies within a wide range of 28−550 mg/kg dw (*16*, *17*).

Effect of a_w on Antioxidant Degradation. Dehydrated apples were equilibrated and stored at eight a_w levels ranging from 0.058 to 0.747, at 40 °C. To model the variation in antioxidant content during storage, a regression analysis of the concentrations versus storage time was carried out for the various antioxidants at each a_w level. Antioxidant contents decreased following pseudo-first-order kinetics. The pseudo-first-order rate constants for antioxidant degradation and half-life values are shown in **Tables 2** and **3**. At the lowest a_w (0.058), catechin, epicatechin, and ascorbic acid contents decreased, whereas the contents of all other antioxidants remained unchanged. Catechin degraded below the detection limit within 6 days; therefore, for this compound, it was not possible to compute the degradation rate. Half-life values for ascorbic acid and epicatechin were 49 and 83 days, respectively.

In the a_w range of 0.075−0.316, all antioxidants degraded, except for chlorogenic acid, which was stable. In fact, with increasing a_w from 0.075 to 0.316, the half-life values of ascorbic acid and epicatechin decreased from about 40 to about 14 days and the half-life values of total procyanidins decreased from 101 to 38 days. In the same a_w range, dihydrochalcones and the *p*-coumaric acid derivative had a half-life value of about 200 days.

At a_w levels above 0.316, antioxidant degradation rates increased further. Above this a_w level, it was not possible to compute the degradation rate for ascorbic acid. The half-life value of epicatechin was 9 days for the product at a_w of 0.532 and was not computable for the product at a_w of 0.747. By increasing a_w from 0.532 to 0.747, half-life values decreased from 24 to 17 days for total procyanidins, from 37 to 10 days for phloretin 2′-*O*-xyloglucoside, from 103 to 21 days for phloridzin, from 92 to 51 days for the *p*-coumaric acid derivative, and from 139 to 44 days for chlorogenic acid.

The main cause for antioxidant loss in dehydrated apples could be oxidation, which is known to occur in dehydrated foods (*8*). However, it is worth noting that antioxidant loss also occurs in apple purée stored in closed jars: after 6 months of storage at 30 °C, procyanidin, chlorogenic acid, and phloretin 2′-*O*-glucoside contents decrease up to 49.5, 26, and 18%, respectively (*7*). In this study, the rate constants for antioxidant degradation were plotted against a_w (Figure**1**). In foods containing polyunsaturated fats, a U-shaped curve describes the dependence of the oxidation rate on a_w, indicating that the maximum oxidation rate occurs at both very low and very high a_w levels (*8*). Dehydrated apples were most stable at low a_w levels, probably because of their low fat content. In fact, the degradation rates of the most unstable antioxidants, such as ascorbic acid and flavan-3-ols, increased linearly with increasing a_w. The degradation rates of the relatively more stable antioxidants, namely, dihydrochalcones, chlorogenic acid, and *p*-coumaric acid derivative were markedly accelerated at a_w above 0.316.

Effect of a_w on HMF Formation. During food processing and storage, HMF can be formed by both hexose degradation and the Maillard reaction, which involves reducing sugars and the free amino groups of amino acids. These reactions cause development of brown color, which is undesirable for apple products. In fact, evaluation of HMF was proposed as an index of the severity of heating applied to apple juices during processing and as an index of quality deterioration of apple products during storage (*25*). In this study, HMF was not detectable in freeze-dried apples at the time of production. In the dehydrated apple products stored at 40 °C, HMF content increased exponentially with increasing storage time, especially at high a_w levels (**Figure 2**). For instance, after 60 days of storage at 40 °C, HMF content was 29 mg/kg fresh weight (fw) (corresponding to 30 mg/kg dw) in the products at a_w levels in the range of 0.058−0.112; over the same storage time and temperature, HMF contents were 66, 105, 145, and 155 mg/kg fw (corresponding to 70, 120, 145, and 155 mg/kg dw) in the products at a_w levels of 0.227, 0.532, 0.661, and 0.747, respectively. In juice concentrates (65−75 °Bx) made with the same apple variety as considered in this study, HMF contents are in the range of 160−338 mg/kg fw after 60 days of storage at 37 °C (*25*). HMF content up to 220 mg/kg dw is found in commercial apple pomace, whereas the HMF content of commercial apple juices is about 0.9 mg/L (*26*), which approximately corresponds to 9 mg/kg dw. Therefore, in general, HMF

Table 2. Rate Constants and Half-Life Values for the Degradation of Flavan-3-ols and Ascorbic Acid in Dehydrated Apples Stored at Various a_w Levels, at 40 °C, as Calculated Assuming Pseudo-First-Order Kinetics: $\ln(C) = \ln(C_0) + kt$[a]

	epicatechin		total procyanidins		ascorbic acid	
a_w	$k \pm$ SE (day^{-1})	$t_{1/2}$ (day)	$k \pm$ SE (day^{-1})	$t_{1/2}$ (day)	$k \pm$ SE (day^{-1})	$t_{1/2}$ (day)
0.058	−0.0083 ± 0.0006	83	nv		−0.0142 ± 0.0017	49
0.075	−0.0158 ± 0.0013	43	−0.0068 ± 0.002	101	−0.0193 ± 0.0020	36
0.112	−0.0158 ± 0.0013	43	−0.0086 ± 0.002	81	−0.0193 ± 0.0010	36
0.227	−0.0395 ± 0.0009	18	−0.0125 ± 0.0017	55	−0.0398 ± 0.0023	17
0.316	−0.0489 ± 0.0012	14	−0.0183 ± 0.0014	38	−0.0545 ± 0.0023	13
0.532	−0.0802 ± 0.0002	9	−0.0287 ± 0.0017	24	nd	
0.661	−0.1140 ± 0.0001	6	−0.0293 ± 0.0015	24	nd	
0.747	nd	nd	−0.0416 ± 0.0054	17	nd	

[a] $R^2 > 0.94$ for epicatechin and ascorbic acid degradations, and $R^2 > 0.76$ for total procyanidin degradation. $p < 0.01$. nv = no variation with respect to the value at time 0. nd = not detectable because the compound diminished below the limit of detection within a few days.

Table 3. Rate Constants and Half-Life Values for the Degradation of Hydroxycinnamic Acids and Dihydrochalcones in Dehydrated Apples Stored at Various a_w Levels, at 40 °C, as Calculated Assuming Pseudo-First-Order Kinetics: $\ln(C) = \ln(C_0) + kt$[a]

	chlorogenic acid		*p*-coumaric acid derivative		phloridzin		phloretin 2′-*O*-xyloglc	
a_w	$k \pm$ SE (day^{-1})	$t_{1/2}$ (day)	$k \pm$ SE (day^{-1})	$t_{1/2}$ (day)	$k \pm$ SE (day^{-1})	$t_{1/2}$ (day)	$k \pm$ SE (day^{-1})	$t_{1/2}$ (day)
0.058	nv		nv		nv		nv	
0.075	nv		−0.0034 ± 0.0005	203	−0.0037 ± 0.0008	187	−0.0035 ± 0.0003	198
0.112	nv		−0.0034 ± 0.0005	203	−0.0037 ± 0.0008	187	−0.0035 ± 0.0003	198
0.227	nv		−0.0034 ± 0.0005	203	−0.0037 ± 0.0008	187	−0.0035 ± 0.0003	198
0.316	nv		−0.0034 ± 0.0005	203	−0.0037 ± 0.0008	187	−0.0035 ± 0.0003	198
0.532	−0.0050 ± 0.0007	139	−0.0075 ± 0.001	92	−0.0067 ± 0.0016	103	−0.0185 ± 0.0029	37
0.661	−0.0066 ± 0.0015	105	−0.0100 ± 0.002	69	−0.0093 ± 0.0014	75	−0.0225 ± 0.0040	31
0.747	−0.0159 ± 0.0020	44	−0.0136 ± 0.0016	51	−0.0334 ± 0.0054	21	−0.0717 ± 0.0045	10

[a] $R^2 > 0.92$. $p < 0.01$. nv = no variation with respect to the value at time 0.

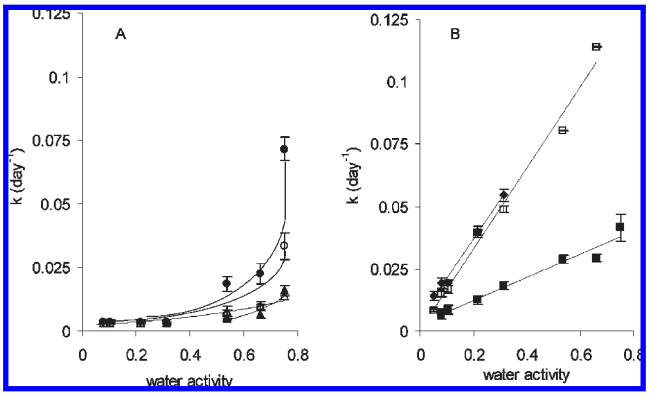

Figure 1. Pseudo-first-order rate constants (absolute values) for antioxidant degradation in dehydrated apples as a function of a_w, at 40 °C. (A) Phloridzin (○), phloretin 2′-*O*-glucoside (●), chlorogenic acid (▲), and *p*-coumaric acid derivative (△). (B) Ascorbic acid (◆), epicactechin (□), and total procyanidins (■).

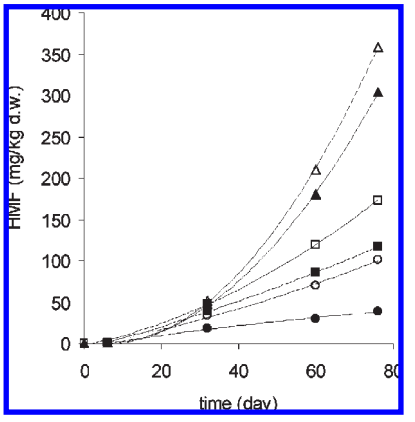

Figure 2. Time course for HMF formation in dehydrated apples with a_w levels of 0.058−0.112 (●), 0.227 (○), 0.316 (■), 0.532 (□), 0.661 (▲), and 0.747 (△), at 40 °C.

contents of dehydrated apples were lower than those of apple juice concentrates and apple pomace but higher that that of apple juices.

Effect of a_w on Antioxidant Activity. The antioxidant (DPPH scavenging) activity evaluations of the dehydrated apple products were performed in both acetone/water (70:30, v/v) and methanol extracts. The antioxidant activity values in the freeze-dried apples were higher in the acetone/water (70:30, v/v) than methanol extracts (**Table 1**). The procyanidin fraction is more soluble in acetone/water (70:30, v/v) than in methanol (*16*). This latter solvent also solubilized Maillard reaction products. In fact, the values of absorbance at 420 nm of the acetone/water (70:30, v/v) extracts increased progressively with increasing the a^* values of the apple products (data not shown).

The changes in the overall antioxidant properties of foods during processing can be attributed to the sum of the different and sometimes opposite events, such as the loss of naturally occurring antioxidants and the formation of novel compounds having pro-oxidant activity or the formation of novel compounds having antioxidant activity (i.e., Maillard reaction products) (*27*). Dependent upon the relative rates of these reactions, the overall antioxidant activity can decrease, increase, or remain unchanged during food processing. In dehydrated apple products stored at 40 °C, the antioxidant activity decreased by following pseudo-first-order kinetics (**Table 4**). In previous studies, dried apples and apple purées were found to have a lower antioxidant activity with respect to the corresponding fresh fruits (*28*) and the antioxidant activity of apple purée decreased during storage (*7*). In this study, the antioxidant activity

decreased consistently with antioxidant loss. The decrease was more pronounced in the methanol extracts than in the acetone/water (70:30, v/v) extracts, probably because of solubilization of Maillard reaction products by this latter solvent, which partially balanced antioxidant loss.

Effect of a_w on Color Changes. Color of foods is one of the main attributes affecting consumer preference (*29*). As expected from the observed HMF formation, the colorimetric parameters of the dehydrated apples changed during storage at 40 °C: the redness index (a^*) increased progressively in the products at a_w levels in the range of 0.058−0.747, while the yellowness (b^*) and lightness (L^*) indices remained unchanged in the products at a_w levels below 0.227 and decreased in those at a_w levels above 0.227 (**Table 5**). The variation of the colorimetric parameters fitted pseudo-zero-order kinetics. The same kinetic model fits the color changes in apple juice concentrates stored between 5 and 37 °C (*25*). On the other hand, for concentrated apple juices submitted to more severe heat treatments, such as heating in the temperature range of 60−90 °C for up to 600 h, the best fitting was found using the logistic model, which incorporates the maximum or plateau value reached at extending heating times (*29*). The rate constants for colorimetric parameter variations were plotted against a_w (**Figure 3**). The color changes were greatly accelerated in the products at a_w levels above 0.316, as also observed for the antioxidant degradation.

Overall Quality of Dehydrated Apples. On the basis of the results obtained, all analytical indices indicated that the critical a_w above which degradation phenomena were accelerated was 0.316. Above this latter a_w level, if there is any exposure to high environmental relative humidity, a small increase in a_w of the product would sharply increase the degradation rate constants for both antioxidant and color variations. Differential scanning calorimetry and rheological evaluations reveal that water diffusion properties and changes in consistency, volume, and shape of low-moisture apple products are greatly enhanced in the a_w range of 0.40−0.50 (*30*). An increase in a_w can enhance the rate of oxidation by increasing mobility of reactants and bringing catalysts into solution. As the apple solid matrix swells, new surfaces for oxidation are exposed (*8*). Physical/structural changes of apple tissue could also enhance the exposed surface, thus promoting degradation reactions (*14*). In addition, a possible involvement of antioxidants in the Maillard reaction (*27*) could explain the accelerated degradation of the relatively more stable antioxidants that was observed above a_w of 0.316. Accordingly, for most foods, the rate of the Maillard reaction increases from the dry state, starting at a critical a_w, which is in the range of 0.2−0.3, to a maximum at a_w in the range of 0.5−0.8. Below that range, the reaction rate is slow because of the low mobility of water; above that range, the reaction rate slows down probably because of the dilution of the reactants (*8*). In this study, the individual apple antioxidants showed different stabilities. The order of antioxidant stability in the products at a_w levels below 0.316 was catechin, epicatechin, and ascorbic acid < total procyanidins < dihydrochalcones and *p*-coumaric acid < chlorogenic acid; however, in the products at a_w levels above 0.532, the degradation of all antioxidants was very fast. The reasons could be that different compounds have different

Table 4. Rate Constants and Half-Life Values for the Decrease of DPPH Radical Scavenging Activity of the Methanol and Acetone/Water (70:30, v/v) Extracts of Dehydrated Apples Stored at Various a_w Levels, at 40 °C, as Calculated Assuming Pseudo-First-Order Kinetics: $\ln(C) = \ln(C_0) + kt$[a]

	antioxidant activity			
	methanol extract		acetone/water extract	
a_w	$k \pm$ SE (day^{-1})	$t_{1/2}$ (day)	$k \pm$ SE (day^{-1})	$t_{1/2}$ (day)
0.058	−0.0091 ± 0.0012	76	nv	
0.075	−0.0091 ± 0.0012	76	nv	
0.112	−0.0091 ± 0.0012	76	nv	
0.227	−0.0143 ± 0.0003	48	nv	
0.316	−0.0143 ± 0.0003	48	−0.0027 ± 0.0004	256
0.532	−0.0202 ± 0.0007	34	−0.0079 ± 0.0009	88
0.661	−0.0202 ± 0.0007	34	−0.0080 ± 0.0009	88
0.747	−0.031 ± 0.005	22	−0.0171 ± 0.0028	41

[a] $R^2 > 0.90$. $p < 0.01$. nv = no variation with respect to the value at time 0.

Table 5. Rate Constants for the Variation of the Colorimetric Parameters Lightness (L^*), Redness (a^*), and Yellowness (b^*) in Dehydrated Apples Stored at Various a_w Levels, at 40 °C, as Calculated Assuming Pseudo-Zero-Order Kinetics: $C = C_0 + kt$[a]

	L^*	a^*	b^*
a_w	$k \pm$ SE (CU day^{-1})	$k \pm$ SE (CU day^{-1})	$k \pm$ SE (CU day^{-1})
0.058	nv	0.0212 ± 0.0017	nv
0.075	nv	0.0366 ± 0.0012	nv
0.112	nv	0.0377 ± 0.0019	nv
0.227	−0.0854 ± 0.0117	0.0818 ± 0.0039	−0.0331 ± 0.0035
0.316	−0.0999 ± 0.0112	0.0913 ± 0.0043	−0.0249 ± 0.0032
0.532	−0.356 ± 0.0234	0.243 ± 0.014	−0.1458 ± 0.0101
0.661	−0.6311 ± 0.0332	0.271 ± 0.024	−0.2615 ± 0.031
0.747	−1.13 ± 0.1199	0.49 ± 0.0531	−0.5403 ± 0.0891

[a] $R^2 > 0.90$ for increases in a^*, and $R^2 > 0.81$ for decreases in b^* and L^*. $p < 0.01$. nv = no variation with respect to the value at time 0. CU = colorimetric units.

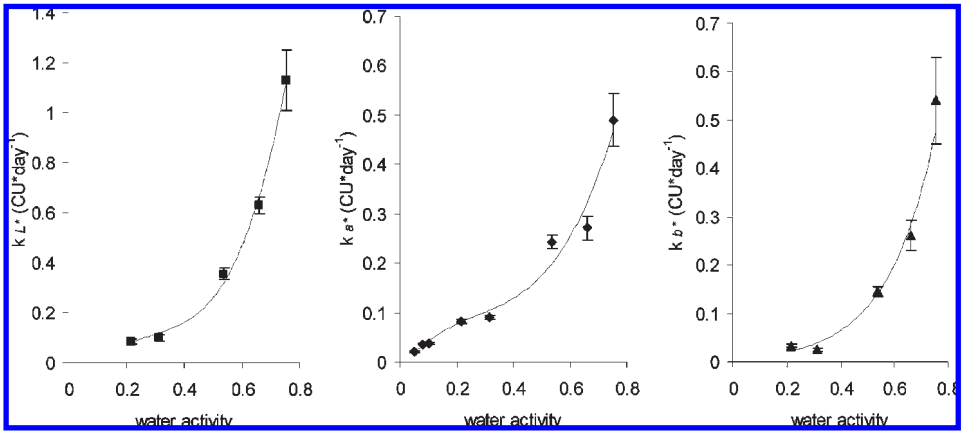

Figure 3. Pseudo-zero-order rate constants (absolute values) for the variation of the colorimetric parameters L^* (▲), a^* (◆), and b^* (■) in dehydrated apples as a function of a_w, at 40 °C. CU = colorimetric units.

reactivities toward oxygen and different roles in the Maillard reaction and, in dehydrated systems, become mobilized at different levels of a_w (*8*).

The following conclusions can be drawn regarding antioxidant and color stability of dehydrated apples: (a) dry apples (a_w below 0.316) were relatively stable during storage and are an optimal source of apple antioxidants, as long as proper packaging material is used to avoid environmental moisture absorption, and (b) effective factors that can enhance the stability of intermediate-moisture apples (those with a_w above 0.316) should be further studied to allow for the optimization of quality retention during storage.

ABBREVIATIONS USED

a_w, water activity; DPPH, 2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl; HMF, hydroxymethylfurfural.

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