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Combined Effect of Storage Temperature and Water Activity on the Antiglycoxidative Properties and Color of Dehydrated Apples

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Phytochemical contents, color, and inhibition efficacy toward oxidative and glycoxidative reactions were studied in dehydrated apples following storage in the water activity range from 0.1 to 0.7 at 20, 30, and 40 °C, which can be considered as room conditions. Hunter colorimetric parameters were analyzed at different temperatures and time intervals, and nonenzymatic browning was modeled according to pseudo-zero-order kinetics. The effect of temperature on the browning rate followed the Arrhenius equation, with an activation energy of 64000 J/mol, which was not affected by the water activity level. The phytochemical contents, inhibition efficacy of protein glycation, and antioxidant properties were then analyzed in the products stored under selected “equivalent” conditions in terms of browning effects, namely, 120 days/20 °C, 50 days/30 °C, and 22 days/40 °C. After storage for 120 days/20 °C, the retention percentages of hydroxycinnamic acids, phloridzin, and epicatechin were >86%, but ascorbic acid, catechin, and procyanidins were less stable; concurrently dehydrated apples retained about 80% of the radical scavenging activity and 70% of the ability to inhibit protein glycation. Following storage at higher temperatures the expected browning effect occurred in a shorter time scale; however, the patterns of product degradation were different. A sharp increase in the degradation rates of all antioxidants, relative to browning rate, was observed at temperatures ≥30 °C, and this trend was accelerated with concurrent increase in water activity at >0.3 levels. The application of low-temperature/long-time conditions for storage of dehydrated apples corresponded to maximum retention of their efficacy to counteract oxidative and glycoxidative reactions, which have been linked to human chronic diseases.

KEYWORDS: Apple; *Malus domestica* Borkh.; protein glycation; antioxidant; browning; degradation

INTRODUCTION

In fruit production worldwide apples rank fourth (http://www.fao.org/es/ess/top/commodity.html). Consumption of apples has been linked to the prevention of coronary disease, cerebrovascular disease, lung cancer, and obstructive pulmonary disease (*1*). Apples are a good source of fiber, especially pectin, and phenolics. The main structural classes of apple phenolics include monomeric (epicatechin and catechin), dimeric, trimeric, and oligomeric flavan 3-ols, hydroxycinnamates (chlorogenic acid and an ester of *p*-coumaric acid), and dihydrochalcones (phloridzin, i.e., phloretin 2′-*O*- glucoside, and phloretin 2′-*O*- xyloglucoside). Flavonols (quercetin glycosides) are found in minor amounts and are mainly located in the skin. Anthocyanins (cyanidin glycosides) are present only in the skins of some red cultivars (*1*).

A number of studies suggest that there is a “synergism” between dietary fiber and phenolics in controlling the risk of cardiovascular disease. In fact, apple pectin has a better cholesterol-lowering effect than other pectins. Furthermore, it was found that apple pectin and the polyphenolic fraction lower plasma cholesterol and triglycerides and are more effective together than are either apple pectin alone or apple phenolics alone (*2*).

Apple pectin and phenolics could also have a role in the treatment of metabolic syndrome and diabetes. In fact, highly methoxylated apple pectin reduces blood glucose and plasma insulin in rats (*3*). On the other hand, hyperglycemia is known to cause oxidative damage through several mechanisms, including glucose autoxidation, protein glycation, and formation of advanced glycation endproducts (AGE). In consideration of the significance of glycoxidative stress to diabetic pathology, a supplementation of antioxidants of fruits, vegetables, and beverages has been suggested as a strategy for preventing hyperglycemia complications (*4*). In addition, phloridzin, the dihydrochalcone typically contained in apples and processed apple foods, has been suggested to improve diabetic symptoms in mice by suppressing glucose absorption in the small intestine through reversion of the abnormal overexpression of the sodium/glucose cotransporter gene (Sglt1) (*5*).

The beneficial effect of apples on human health should therefore be maximum with the consumption of fresh apples and apple derivatives obtained from the whole product, such as apple puree, intermediate moisture apples (water activity, a_w,

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in the range between 0.25 and 0.75), and dehydrated apple snacks (a_w below 0.25).

In industrial processing of apples, control of browning has always been a challenge. Products are thermally treated to inactivate the polyphenol oxidase (PPO) that is responsible for enzymatic browning (*6*). Nonenzymatic degradation occurs in heat-stabilized products. The Maillard reaction, which begins with amino−carbonyl interactions producing glycosylated proteins, is the major cause of nonenzymatic browning (*7*). Some antioxidants are involved in the Maillard reaction, being degraded (*8*). On the other hand, some products of this reaction possess potent antioxidant activity (*8*). Therefore, the Maillard reaction could also affect the antioxidant contents and antiglycation properties of apples. Kinetic models for nonenzymatic browning of apple purees and juices have been devised (*9*−*11*). However, although browning is accelerated in low-moisture foods (*12*), little is known of its kinetics in dehydrated apples and its effects on the potential antiglycation properties of the product.

The aims of this study were (a) to model browning kinetics in dehydrated apples ($0.1 \le a_w \le 0.7$) as a function of temperature (20−40 °C) and (b) to evaluate the relationships between browning and changes in antioxidant contents and potential anti-AGE formation properties.

The inhibitory properties against glycoxidative reactions were evaluated in vitro by following AGE formation in a model system involving bovine serum albumin (BSA) as a target and fructose. For comparison, the radical scavenging activity on the synthetic 2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl radical (DPPH) was also evaluated.

MATERIALS AND METHODS

Chemicals. The salts used to prepare saturated solutions (namely, LiCl, CH_3COOK, $MgCl_2$, NaBr, and NaCl), DPPH, the reference antioxidant 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), the Folin−Ciocalteu reagent, BSA, fructose, Chelex resin, sodium azide, and standards of ascorbic acid, gallic acid, chlorogenic acid, phloridzin, *p*-coumaric acid, and procyanidin B2 were purchased from Sigma Aldrich (Milan, Italy). Standards of epicatechin and catechin were purchased from Extrasynthese (Lyon, France).

Apple Dehydration. Apples (8 kg) (*Malus domestica* Borkh. cultivar 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 placed into a wire mesh basket and blanched in a deionized water bath at 100 °C for 4 min. The basket was rapidly removed from the boiling water, cooled by immersion in a 4 °C water bath, and drained. Then the apple slices were blended to a puree consistency in a K 3000 Braun Multisystem blender (Braun, Kronberg, Germany). Apple puree was 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) and sieved (800 μm). The powders were first weighed into Petri dishes (8.5 cm diameter, 0.141 g of apple product/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 20, 30, or 40 °C in thermostated cabinets. To equilibrate products at different a_w levels, the following saturated salt solutions were used: LiCl (a_w at 20−40 °C = 0.11 ± 0.01), CH_3COOK (a_w at 20−40 °C = 0.22 ± 0.01), $MgCl_2$ (a_w at 20−40 °C = 0.32 ± 0.01), NaBr (a_w at 20−40 °C = 0.56 ± 0.03), and NaCl (a_w at 20−40 °C = 0.75 ± 0.01). The observed a_w values of the saturated solutions were consistent with those reported previously (*13*). The equilibrium moisture content was reached within 2−3 days. Two experiments were carried out. In the first step, dehydrated apples were incubated at each a_w in duplicate Petri dishes for 120 days at 20 °C, for 60 days at 30 °C, and for 40 days at 40 °C. Color was evaluated periodically, and a model for its variation was devised. In the second step, dehydrated apples were incubated in duplicate Petri dishes at the same a_w levels as indicated before, under three selected time/temperature combinations, namely, 120 days/20 °C, 50 days/30 °C, and 22 days/40 °C, which were expected to produce the same browning effect on the basis of the results obtained by the first step. Color evaluation and chemical characterization of these samples were performed at the beginning of incubation and after storage.

Kinetics of Color Variation. The kinetics of a^* variations was fitted to a pseudo-zero-order equation as a primary kinetic model, that is

$$a^* = a^*_0 \pm k_T t \quad (1)$$

where a^* (colorimetric unit, CU) is the colorimetric parameter measured at time t (days), a^*_0 is the initial value of the colorimetric parameter, and k_T is the rate constant (CU·day^{-1}) at the fixed temperature T.

The rate constants for the variation of the redness index a^* were then used to evaluate the activation energy for color variation, by applying the Arrhenius equation as a secondary kinetic model

$$\ln k_{T1} - \ln k_{T2} = E_a R^{-1}(1/T_2 - 1/T_1) \quad (2)$$

where T_1 and T_2 are two temperatures (K), k_{T1} and k_{T2} are the rate constants at the temperatures T_1 and T_2, respectively (CU·day^{-1}), E_a is the activation energy (J·mol^{-1}), and R is the universal gas constant (8.314 J·mol^{-1}·K^{-1}).

Equations 1 and 2 were combined to calculate the equivalent times (t_{T1} and t_{T2}) causing the same browning effect at the temperatures T_1 and T_2, as follows:

$$\ln t_{T2} - \ln t_{T1} = E_a R^{-1}(1/T_2 - 1/T_1) \quad (3)$$

Moisture Content, a_w, Soluble Solids, pH, and Titratable Acidity. Moisture contents a_w, soluble solids, pH, and titratable acidity of apple products were determined as described previously (*14*).

Color. An 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 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 or 10 mL of acetone/water (70:30, v/v) or 5 mL of 6% metaphosphoric acid containing 1 g/L of sodium metabisulfite (*14*). 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.

Ascorbic Acid and Phenolics by HPLC. The ascorbic acid contents of sample extracts in 6% metaphosphoric acid containing 1 g/L of sodium metabisulfite were analyzed as described previously (*14*). 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.

The phenolic contents of sample extracts in methanol were analyzed according to the HPLC procedure of Tomas-Barberan et al. (*15*). Standards of chlorogenic acid, epicatechin, catechin, procyanidin B2, and phloridzin were used to identify peaks by retention times and UV−vis spectra and to build calibration curves for quantification. Epicatechin, catechin, phloridzin, and procyanidin B2 were quantified at 280 nm, and chlorogenic acid was quantified at 330 nm. The presence of a *p*-coumaric acid ester was revealed by alkaline hydrolysis (*14*). *p*-Coumaric acid ester was quantified at 311 nm by 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 by the Vanillin Assay. 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 into 1 mL of 0.1 M phosphate buffer, 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; the cartridge was washed with 1 mL of 0.1 M phosphate buffer, pH 7.0, and 1 mL of water prior to procyanidin elution with 1 mL of methanol (*16*). Procyanidin content was evaluated by the vanillin assay (*14*) using a calibration curve built with catechin. Results were expressed as milligrams of catechin equivalents per kilogram of dry product. Duplicate extracts were analyzed for each sample.

Total Phenolics by the Folin−Ciocalteu Assay. Total phenolics were evaluated in the acetone/water (70: 30, v/v) extracts according to the method of Singleton et al. (*17*). The reaction mixture contained 6 mL of distilled water, 76 μL of apple extract, 380 μL of Folin−Ciocalteu reagent, and 1.14 mL of 20% Na_2CO_3. Samples were incubated for 45 min at room temperature, and then the absorbance was recorded at 760 nm. A calibration curve was built using gallic acid. Total phenolics were expressed as milligrams of gallic acid equivalents per kilogram of dry product. Duplicate extracts were analyzed for each sample

DPPH Scavenging Test. This assay was performed as described previously (*18*). 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). Duplicate extracts were analyzed for each sample.

Determination of Fructose-Induced Glycation of BSA. The inhibition of fructose-induced glycation of BSA was conducted with the 70% acetone extracts according to the procedure described by McPearson et al. (*19*) with modification. The reaction mixture consisted of 50 μL of sample or standard, 900 μL of phosphate buffer (200 mM potassium phosphate buffer, pH 7.4, with 0.02% sodium azide, treated with Chelex resin), 300 μL of BSA solution (50 mg/mL of BSA in Chelex-treated phosphate buffer), and 300 μL of fructose solution (1.25 M fructose in Chelex-treated phosphate buffer). A control reaction was incubated with no addition of sample or standard. The reaction mixtures were incubated at 38 °C for 72 h. Following incubation, samples were analyzed for fluorescence on a Perkin-Elmer LS 55 luminescence spectrometer (Perkin-Elmer, U.K.) with an excitation/emission wavelength pair 370/440 nm, 5 nm slit width, against phosphate buffer blank. Each standard or sample was evaluated at five dilutions in triplicate over two separate days. Acetone/water (70:30, v/v) solutions of catechin, epicatechin, chlorogenic acid, procyanidin B2, and phlorizin were evaluated as external standards. Results were reported as percent inhibition of fructose-induced glycation of BSA, where percent inhibition = (fluorescence intensity of sample or standard − intensity of BSA)/(intensity of control reaction − intensity of BSA). A dose−response curve was constructed, and the amount of standard (nmol) or sample (mg) required to inhibit the reaction rate by 50%, I_{50}, was interpolated. The inhibition efficacy of protein glycation of apple samples was expressed as catechin equivalents, which was the ratio of I_{50} of catechin (nanomoles) to I_{50} of sample (milligrams, dry weight).

PPO Activity. Apple powder (0.10 g) was added to 1 mL of a buffer containing 0.03 M acetic acid, 0.14 M K_2HPO_4, 1 M NaCl, and 5% (w/w) polyvinylpolypyrrolidone, pH 6.5. The mixture was centrifuged at 10000*g* for 10 min at 20 °C. The supernatant was filtered through Whatman no. 1 filter paper. For PPO activity evaluation, 0.1 mL of the supernatant was added to 0.9 mL of 10 mM chlorogenic acid in the above-indicated buffer. The mixture was incubated at 25 °C, and the reaction rate was estimated from the linear increase in absorbance at 400 nm (*20*).

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

RESULTS AND DISCUSSION

Initial Characterization of Antioxidant Contents and Antiglycoxidative Properties of Dehydrated Apples. The characterization of blanched dehydrated apples at the time of production is shown in **Table 1**. The values for soluble solids content, titratable acidity, pH, and antioxidant contents were consistent with those found in fresh full-ripe fruits (*21*). Recent studies have been carried out on the bioavailability of apple phenolics. With regard to procyanidins, only the low molecular weight flavan 3-ols (polymerization degree ≤3) are absorbed intact in the gastrointestinal tract. The procyanidin oligomers with polymerization degree ≥3 are supposed to exert their beneficial actions only in the gastrointestinal tract or to be cleaved into smaller units and then absorbed. For chlorogenic acid, the hypothesis has been made that it is bioavailable, but it can also be cleaved in the intestine by an esterase producing free D-(−)-quinic acid, which can then be absorbed or lost. Phloridzin is probably converted into its corresponding aglycone, as well as a glucuronide conjugate, and then absorbed (*22*). Although better information on the exact amount and form of phenolics that are available in vivo is still required, the potential health effects of these compounds are of interest.

Table 1. Titratable Acidity, pH, Soluble Solids, Antioxidant Content, DPPH-Scavenging Activity, Inhibition of Protein Glycation, and Colorimetric Parameters of Dehydrated Apples after Freeze-Drying (mean ± SD)

quality index	
titratable acidity (g of malic acid/100 g of dw)	2.48 ± 0.06
pH	3.62 ± 0.07
soluble solids (g of sucrose/100 g of dw)	72.7 ± 2.3
ascorbic acid (mg/kg of dw)	131 ± 11
chlorogenic acid (mg/kg of dw)	1060 ± 20
p-coumaric acid derivative (mg/kg of dw)	67 ± 2
epicatechin (mg/kg of dw)	319 ± 24
catechin (mg/kg of dw)	54 ± 2
phloridzin (mg/kg of dw)	87 ± 5
total procyanidins (mg/kg of dw)	2440 ± 155
procyanidn B2 (mg/kg of dw)	498 ± 54
total phenolics (mg of gallic acid equiv/kg of dw)	8100 ± 300
DPPH-scavenging activity (mmol of Trolox equiv/kg of dw)	13.7 ± 0.3
inhibition of protein glycation (mmol of catechin equiv/kg of dw)	37 ± 5
lightness index, L^*	76.3 ± 0.7
redness index, a^*	−1.26 ± 0.04
yellowness index, b^*	26.6 ± 0.5

To this aim, the inhibitory activity of apple extracts on AGE formation was studied in vitro by using a model reaction which simulates protein glycation in vivo (*19*). In fact, the amino groups of some mammalian proteins react nonenzymatically with both glucose and fructose, in vivo, to give glycosylated proteins. Subsequent reactions may result in the formation of cross-linked, fluorescent, protein derivatives, which can account for some of the complications of diabetes (*19*, *23*).

As shown in **Table 1**, protein glycation could be inhibited effectively when apple extract was added during the glycoxidative reaction. There are a few studies concerning the ability of natural compounds to counteract glycoxidative reactions; phenolics were hypothesized to act as inhibitors. The same model system as that used in this study was previously proposed to evaluate the inhibiting effect on BSA glycation of culinary herbs and spices, which are rich in phenolics (*24*). Therefore, a comparison among the anti-AGE activity of apple phenolics and those of herbs and spices can be made. To make this comparison possible, the concentration of phenolics (as mg of gallic acid equiv/mL evaluated by the Folin−Ciocalteu assay) that is required in the assay mixture to inhibit BSA glycation by 50% was calculated. For the apple extract, this value was found to be 10 mg of gallic acid equiv/L. For most culinary herbs and spices, this value is in the range of 4−12 mg of gallic acid equiv/L, but some of them are less efficient (up to 40 mg of gallic acid equiv/L is required for 50% inhibition) (*24*). Therefore, apple phenolics had a good efficiency with respect to herbs and spices in the inhibition of AGE formation. This result, together with a higher intake of

Table 2. Anti-AGE Properties (I_{50}[a]) of Apple Pulp Phenolics (mean ± SD)

compound	I_{50} (nmol)
procyanidin B2	29 ± 1
catechin	55 ± 1
epicatechin	62 ± 7
chlorogenic acid	63 ± 3
phloridzin	438 ± 50

[a] I_{50} is the amount of antioxidant (nmol) that inhibits protein glycation by 50% under the assay condition used.

Table 3. Rate Constants for Redness Index (a^*) Increase in Dehydrated Apples Stored at Various a_w Levels, at 20, 30, and 40 °C, As Calculated by Assuming Pseudo-Zero-Order Kinetics: $a^* = a^*_0 + kt$[a] (mean ± SE)

	k		
a_w	20 °C	30 °C	40 °C
0.1	0.014 ± 0.002	0.043 ± 0.002	0.084 ± 0.009
0.2	0.019 ± 0.002	0.050 ± 0.003	0.105 ± 0.006
0.3	0.020 ± 0.001	0.055 ± 0.005	0.111 ± 0.004
0.5	0.035 ± 0.002	0.088 ± 0.004	0.193 ± 0.009
0.7	0.056 ± 0.002	0.139 ± 0.004	0.266 ± 0.020

[a] $R^2 > 0.90$.

apples than herbs and spices, underscores the potential health benefits of this fruit. The factors involved in the inhibition of protein glycation, which explain different anti-AGE efficacy values of extracts from different food sources, could be radical scavenging activity, metal chelation ability, and steric hindrance (*24*). As shown in **Table 2**, the inhibitory efficacy of standard phenolics toward glycoxidative reactions was then evaluated. On a molar basis the dimer procyanidin B2 had a lower I_{50} value than those of the monomeric flavan 3-ols catechin and epicatechin but, when calculated on a per monomer unit, the I_{50} value was the same. The I_{50} value of chlorogenic acid was the same as that of the monomeric flavan 3-ols, whereas that of phloridzin was about 7-fold higher.

The DPPH-scavenging activity of blanched dehydrated apples was 13.7 ± 0.3 mmol of TE equiv/kg (**Table 1**), which is in the range of the values found for unblanched dehydrated apples (*14*). The DPPH-scavenging efficacy values of phenolics of apple pulp have been reported previously. The DPPH-scavenging activity of procyanidin B2 is similar to that of epicatechin (on a per monomer unit), double that of chlorogenic acid, and about 5-fold higher than that of phloridzin (*25*). As expected, the inhibitory efficacy of standard antioxidants in the fructose−BSA model system was not exactly the same as their radical scavenging activity toward the synthetic radical, although in the protein glycation process free radicals are produced (*19*).

Color Variations. The time course of color variation was studied in 30 blanched and dehydrated apple samples (three temperatures, five a_w levels, two replications for each of the above combinations). The increase in the redness index a^* was accelerated by increasing the a_w from 0.1 to 0.7 and the temperature from 20 to 40 °C. The kinetics of a^* variations followed a pseudo-zero-order model (**Table 3**). The lightness index L^* and yellowness index b^* decreased significantly in the products stored at $a_w \geq 0.3$ at 30 and 40 °C, whereas these colorimetric parameters remained unchanged in the products stored at lower a_w or at 20 °C (not shown). Because PPO activity was not detectable in the blanched/freeze-dried apples, changes in color could be attributed to nonenzymatic browning. Similarly, color variations in thermally treated apple products are ascribed to nonenzymatic browning (*9*). The rate of nonenzymatic browning is high in dehydrated foods, due to elevated concentrations of reagents, with maximum values at intermediate a_w levels between 0.5 and 0.8. Below 0.5 the reaction is limited by the low mobility of reactants, whereas above 0.8, the browning rate decreases with increasing a_w due to a mass action effect (*10*).

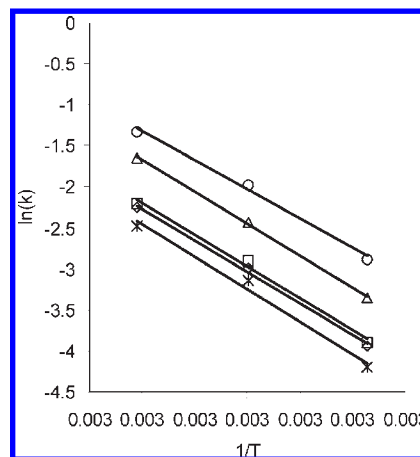

Figure 1. Arrhenius plot for the rate of increase of the colorimetric parameter a^*.

Different primary kinetic models were proposed to describe nonenzymatic browning of apples. This phenomenon involves different reaction patterns and is considered to consist of two stages: a first stage during which colored polymeric compounds are formed and a second stage in which colored polymeric compounds continued to be formed but are also destroyed (*26*). Under severe heating conditions, for instance, in apple products (11.65 −70 °Brix) heated in the temperature range between 60 and 90 °C for 50 −500 h, the logistic model is the most appropriate for describing the browning kinetics (*10*). At lower heating temperatures, for instance, in apple products (65−75 °Brix) stored in the temperature range between 5 and 37 °C for 4 months, browning follows pseudo-zero-order kinetics (*9*), as observed in this study.

The rates of increase of the redness index a^* were then used to evaluate the activation energy for color variation, by applying the Arrhenius equation. A statistically invariant E_a among a_w levels was derived, with an average value of 64000 ± 3000 J·mol^{-1} (**Figure 1**). As apple color is critical for consumer preference, from this value it could be calculated that a 10 °C increase in temperature caused a decrease in the storage time of about 2.3-fold. This value for E_a is in general agreement with those reported for apple juice concentrates and purees. In fact, Torbido and Lozano (*11*) found E_a values in the range between 69000 and 83700 J·mol^{-1} for nonenzymatic browning of apple juice concentrates during storage at 5−37 °C. E_a of 67000 J·mol^{-1} was reported by Vaikousi et al. (*10*) for the browning of apple juice concentrates in the temperature range of 60−90 °C, which was not affected by the a_w levels between 0.74 and 0.99. Burdurlu et al. (*9*) found E_a of 87900−141000 J·mol^{-1} for apple juice concentrates stored at 5−37 °C. These above-referenced studies are crucial, because the magnitude of the parameter E_a, once known for the browning process, allows prediction of color changes of the product as a function of temperature and time of storage (*27*). For instance, once a time/temperature combination is defined, “equivalent” time/temperature combinations in terms of browning effect can be calculated. These models can predict color changes; however, they may not be applied generally to predict other degradation reactions, unless the latter are closely related to browning and have the same magnitude for E_a (*28*). The oxidation of ascorbic acid and phenolics was demonstrated to occur concomitantly with the Maillard reaction (*8*). Therefore in this study, the observed E_a value was used to solve the eq 3 to find

Table 4. Antioxidant Retention Percentages in Dehydrated Apples Stored for 120 Days at 20 °C, for 50 Days at 30 °C, and for 22 Days at 40 °C[a]

		storage temperature and time			
antioxidant	a_w	0 days	20 °C/ 120 days	30 °C/ 50 days	40 °C/ 30 days
ascorbic acid	0.1	100 c	93 b	73 a	69 a
	0.2	100 c	69 b	67 b	39 a
	0.3	100 c	54 b	65 b	27 a
	0.5	100 b	0 a	0 a	0 a
	0.7	100 b	0 a	0 a	0 a
epicatechin	0.1	100 b	100 b	61 a	72 a
	0.2	100 c	100 c	58 b	41 a
	0.3	100 c	100 c	50 b	32 a
	0.5	100 c	100 c	34 b	16 a
	0.7	100 c	100 c	18 b	0 a
procyanidin B2	0.1	100 c	86 b	93 bc	60 a
	0.2	100 c	76 b	76 b	32 a
	0.3	100 c	65 b	49 b	14 a
	0.5	100 d	33 c	17 b	0 a
	0.7	100 c	13 b	8 b	0 a
total procyanidins	0.1	100	100	100	100
	0.2	100 b	100 b	100 b	80 a
	0.3	100 b	95 b	71 a	67 a
	0.5	100 c	84 b	60 a	51 a
	0.7	100 b	58 a	56 a	44 a
phloridzin	0.1	100 b	86 ab	81 a	92 ab
	0.2	100 b	91 ab	81 a	90 ab
	0.3	100 b	90 ab	92 ab	81 a
	0.5	100 b	97 b	86 a	78 a
	0.7	100 d	89 c	74 b	53 a
chlorogenic acid	0.1	100	100	100	100
	0.2	100	100	100	100
	0.3	100	100	100	100
	0.5	100 b	100 b	91 a	92 a
	0.7	100 c	91 b	78 a	75 a
p-coumaric acid derivative	0.1	100	100	100	100
	0.2	100	100	100	100
	0.3	100 b	99 ab	94 a	93 a
	0.5	100 b	100 b	90 a	86 a
	0.7	100 c	94 b	78 a	76 a

[a] Time—temperature storage conditions causing the same browning effect were chosen. Different letters within the same row indicate significant differences among storage conditions (LSD, $p < 0.05$).

time and temperature conditions that cause the same browning effect. Storage for 120 days at 20 °C was taken as reference, and equivalent conditions, in terms of browning effect, were 50 days at 30 °C and 22 days at 40 °C.

In a second step of the research, dehydrated apples were stored under these selected conditions and the expected color changes were observed (not shown). Dehydrated apples were then analyzed to verify whether the products with the same browning intensity had similar antioxidant components and potential antiglycation properties.

Variations in Antioxidant Contents, Inhibition of Protein Glycation, and Antioxidant Activity. After storage, catechin was not detectable in any of the products, whatever the a_w and time/temperature conditions (not shown). Catechin is also unstable in dehydrated grape seed (*29*). As shown in **Table 4**, the retention percentages for ascorbic acid, epicatechin, procyanidin B2, and total procyanidins were in general higher following storage under low-temperature/long-time than high-temperature/low-time conditions at the same a_w level and decreased with increasing a_w level. Phloridzin, chlorogenic acid, and the *p*-coumaric acid derivative had higher retention percentages compared to ascorbic acid and flavan 3-ols, with similar dependence on time/temperature and a_w conditions. In fact, after storage for 120 days/20 °C, changes in the antioxidant compounds were minimal: antioxidant retention was >86%, except for catechin, ascorbic acid, and procyanidins at the a_w level of 0.7. After storage for 22 days/40 °C, the same browning effect occurred as after 120 days/20 °C; however, antioxidant loss was higher, especially at a_w levels >0.3.

Table 5. Retention Percentages of Inhibitory Activity toward Protein Glycation and DPPH-Scavenging Activity in Dehydrated Apples Stored for 120 Days at 20 °C, for 50 Days at 30 °C, and for 22 Days at 40 °C[a]

		storage temperature and time			
antioxidant/ antiglycation activity	a_w	0 days	20 °C/ 120 days	30 °C/ 50 days	40 °C/ 22 days
DPPH-scavenging activity	0.1	100b	87 a	81 a	83 a
	0.2	100 b	78 a	76 a	74 a
	0.3	100 b	87 b	79 ab	74 a
	0.5	100 c	83 b	73 a	65 a
	0.7	100 c	86 b	78 b	52 a
anti-AGE activity	0.1	100 c	65a	75a	83 ab
	0.2	100 b	67a	74a	79 a
	0.3	100 b	65a	74a	76 a
	0.5	100 c	66b	71b	33 a
	0.7	100 c	76b	64b	23 a

[a] Time—temperature storage conditions causing the same browning effect were chosen. Different letters within the same row indicate significant differences among storage conditions (LSD, $p < 0.05$).

The variation in inhibition efficacy on AGE formation was then studied. As shown in **Table 5**, products stored for 120 days/20 °C and for 50 days/30 °C retained on average >64% of the inhibition effectiveness toward protein glycation. The products stored at 40 °C retained the same efficacy after storage at $a_w \leq 0.3$ as those stored at lower temperatures, but had only 33 and 23% of the initial efficacy after storage at a_w levels of 0.5 and 0.7, respectively.

The trend of decrease of DPPH-scavenging activity was similar to that observed for the antiglycation activity.

Although flavan 3-ols play a major role in the inhibitory activity toward protein glycation (**Table 2**) and DPPH-scavenging activity (*25*), the fact that they decreased during storage of dehydrated apples at 20 and 30 °C was not correlated with a decrease of the antioxidant and antiglycation properties ($p > 0.05$ for each component identified and for their sum). It may be hypothesized that at 20 and 30 °C antioxidant degradation led to formation of derivatives which retained some of the original properties. On the contrary, in the products stored at 40 °C the decrease of procyanidins was correlated to both the decrease in the inhibitory activity toward protein glycation ($R = 0.91$) and the decrease in DPPH-scavenging activity ($R = 0.97$) (**Figure 2**).

Overall Effect of Storage on Dehydrated Apples. It is generally agreed that most of the steps of the Maillard reaction are based on ionic mechanisms, but a free radical mechanism has also been demonstrated to occur in the very early stages of the sugar—amino acid interaction (*30*). Phenolics and ascorbic acid take part in this pathway, thus being degraded (*8*).

Increasing storage temperature from 20 to 40 °C not only changed the time scale for equivalent color variation but also promoted changes in the patterns of antioxidant degradation. In general, the extent of degradation was minimum in dehydrated

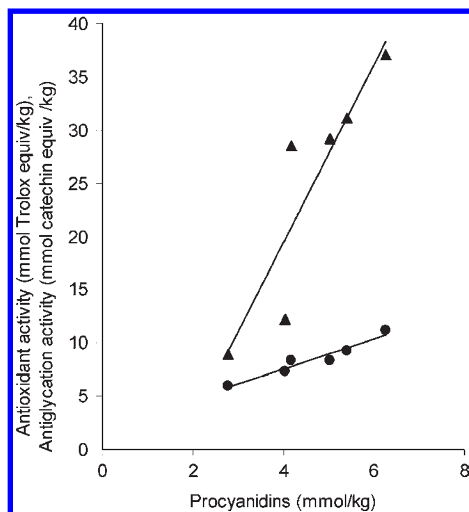

Figure 2. Correlation between the procyanidin content (measured by the vanillin assay) and both the total antioxidant activity measured by the DPPH scavenging test (●; $R = 0.97$) and the antiglycation activity (▲; $R = 0.91$) in dehydrated apples stored at 40 °C.

apples stored for 120 days/20 °C at a_w levels up to 0.3. Conversely, the extent of degradation was maximum in the product stored at the a_w level of 0.7 for 22 days/40 °C.

This sharp increase in oxidation rates relative to browning rate at temperatures ≥30 °C could be due to increased solubility of both antioxidants and metal catalysts and/or changes in the solid matrix structure, enhancing exposition of new surfaces for oxidation. The same phenomena could also explain the increase in oxidation rates with increasing a_w above the level of 0.3 (*12*). Therefore, low-temperature/long-time storage conditions were found to be optimal for dehydrated apples.

Apples have previously been proposed as dietary components for diabetic people, due to their contents of highly methoxylated pectins, which reduce blood glucose and plasma insulin in rats, and phloridzin, which suppresses glucose absorption in the small intestine of diabetic mice. Because diabetes is a complex process, the concomitant assumption of apple phenolics with complementary health-relevant activities may lead to enhanced preventive effects.

ABBREVIATIONS USED

AGE, advanced glycation endproducts; a_w, water activity; BSA, bovine serum albumin; CU, colorimetric unit; DPPH, 2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl; E_a, activation energy; PPO, polyphenol oxidase.

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