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Degradation Kinetics of the Antioxidant Additive Ascorbic Acid in Packed Table Olives during Storage at Different Temperatures

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The kinetics of ascorbic acid (AA) loss during storage of packed table olives with two different levels of added AA was investigated. Three selected storage temperatures were assayed: 10 °C, ambient (20−24 °C), and 40 °C. The study was carried out in both pasteurized and unpasteurized product. The effect of pasteurization treatment alone on added AA was not significant. In the pasteurized product, in general AA degraded following a first-order kinetics. The activation energy calculated by using the Arrhenius model averaged 9 kcal/mol. For each storage temperature, the increase in initial AA concentration significantly decreased the AA degradation rate. In the unpasteurized product, AA was not detected after 20 days in samples stored at room temperature and AA degradation followed zero-order kinetics at 10 °C, whereas at 40 °C a second-order reaction showed the best fit. In both pasteurized and unpasteurized product, the low level of initial dehydroascorbic acid disappeared during storage. Furfural appeared to be formed during storage, mainly at 40 °C, following zero-order kinetics.

KEYWORDS: Ascorbic acid; dehydroascorbic acid; furfural; degradation; kinetics; storage; table olives

INTRODUCTION

Ascorbic acid (AA) is, apart from its role as a nutrient, an antioxidant commonly used for maintaining organoleptic quality in many food systems (*1*). Its antioxidant effect is due to its ability to scavenge oxygen and protect double bonds (*2*). The metal-sequestering activity of AA, which forms metal−ascorbate complexes that are less reactive with oxygen than are metal ions alone, also provides antioxidant capacity (*1*). Ascorbic acid is known to be thermolabile. The degradation kinetics of AA in citrus juices and various vegetables during thermal treatment (*3*−*6*) or during storage (*7*−*13*) has been studied. All of these publications are focused on natural AA—no documentation has been found about the degradation kinetics of added AA.

Green table olives constitute a basic food in the Mediterranean area and in all olive-producing countries (*14*). The addition of AA as an antioxidant in the packing of this product and other fermented vegetables is a widespread industrial practice (*15*). The thermal treatment of pasteurization is frequently used to ensure microbiological stability in table olives (*16*). It has been postulated that this treatment may stabilize the added AA (*15*).

Added AA may be oxidized to dehydroascorbic acid (DHAA), which may subsequently be degraded to other products (e.g., 2,3-diketogulonic acid, hydroxyfurfural, furfural) (*8*). It has been reported (*17*, *18*) in citrus juices that furfural is the major product of ascorbic acid degradation, both anaerobic and aerobic.

The objectives of this work were (a) to measure the amount of AA lost as a consequence of pasteurization treatment in packed table olives with two different levels of added AA, (b) to study in each case the degradation kinetics of AA during storage in both pasteurized and unpasteurized product, and (c) to investigate the changes in DHAA and furfural during storage.

MATERIALS AND METHODS

Reagents. Distilled water was purified with a Milli-Q water system (Millipore, Bedford, MA) prior to use. L-Ascorbic acid, furfural, ethylenediaminetetraacetic (EDTA) disodium salt, metaphosphoric acid, and dithiothreitol (DTT) were purchased from Sigma-Aldrich (St. Louis, MO). Methanol was purchased from Romil (Teknokroma, Barcelona, Spain). All other reagents were of analytical grade and supplied by Panreac (Barcelona, Spain).

Samples. Pitted Spanish-style green table olives, Manzanilla cultivar, were supplied by a local processor. To obtain two different levels of added AA, the olives were divided into two portions. One portion was packed in an acidified brine (containing lactic acid and sodium chloride to give equilibrium values of 0.5% titratable acidity and 5.0% salt) with ascorbic acid added to a final level of ∼200 ppm (batch I); the other portion was packed with the same acidified brine, except that AA was added to a final level of ∼400 ppm (batch II). Each batch was packed in "3 1/2 CYL" glass bottles (51 g of pitted olives plus 55 mL of brine capacity) and then divided into two lots. One lot was pasteurized at 80 °C for 6 min, and the other was left as unpasteurized control. Bottles of each lot were divided into three sublots, each stored in darkness at a different temperature, 10 °C, room temperature (20−24 °C), and 40 °C, over a period of 54 weeks. After intervals of 0, 3, 5, 13, 22, and 54 weeks, the samples were analyzed for AA, DHAA, furfural, and microbial population.

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Table 1. Changes in Lactic Acid Bacteria (LAB), Yeasts (Y), pH, and Titratable Acidity in Brine of Unpasteurized Packed Table Olives during Storage at Room Temperature (20–24 °C) and 10 and 40 °C

storage time (weeks)	AA level (mg/100 g)	stored at room temperature				stored at 10 °C				stored at 40 °C			
		LAB (cfu/mL)	Y (cfu/mL)	pH	acidity (%)	LAB (cfu/mL)	Y (cfu/mL)	pH	acidity (%)	LAB (cfu/mL)	Y (cfu/mL)	pH	acidity (%)
3	15	2.5×10^4	1.4×10^3	3.60	0.47	4.5×10^3	8.0×10^4	3.64	0.46	<10	5.0×10^2	3.60	0.48
	36	3.5×10^4	5.0×10^3	3.61	0.50	7.2×10^3	4.0×10^4	3.61	0.45	<10	3.2×10^2	3.64	0.46
13	15	2.6×10^4	1.3×10^3	–[a]	–	8.5×10^3	1.5×10^4	–	–	<10	1.1×10^2	–	–
	36	2.3×10^5	8.7×10^2	–	–	2.5×10^4	3.1×10^4	–	–	<10	2.7×10^2	–	–
22	15	5.0×10^5	1.8×10^3	4.04	0.51	1.5×10^4	1.1×10^4	3.61	0.48	<10	3.5×10^2	3.57	0.51
	36	5.2×10^5	1.0×10^3	4.03	0.53	5.6×10^3	1.0×10^4	3.57	0.47	<10	1.1×10^2	3.56	0.49
54	15	1.0×10^4	3.6×10^2	4.20	0.58	7.4×10^3	1.7×10^3	3.66	0.51	–	–	–	–
	36	1.7×10^4	4.1×10^2	4.20	0.58	6.6×10^3	3.6×10^3	3.59	0.50	–	–	–	–

[a] Not analyzed.

Chemical Analyses. The pH and titratable acidity of brine samples were measured using a Metrohm 670 Titroprocessor (Herisau, Switzerland). Titratable acidity was determined by titrating up to pH 8.3 with 0.2 N NaOH and expressed as percent (w/v) of lactic acid. For the determination of AA, DHAA, and furfural, three replicate bottles of each sublot were pooled and blended with the same weight of 6% metaphosphoric acid containing 1 mM EDTA. In duplicate, a portion (40 g) of slurry was taken and diluted with 3% metaphosphoric acid containing 1 mM EDTA in a 100 mL volumetric flask. After filtration through Whatman no. 41 filter paper, an aliquot of the filtrate was clarified by filtration through a 0.45 μm filter (GHP Acrodisc, Gelman, Ann Arbor, MI). AA and DHAA were analyzed using the HPLC method described by López et al. (*15*). The DHAA content was calculated by subtracting the initial AA content from the total AA after reduction with DTT solution. The HPLC system consisted of a Waters 2690 separations module connected to a Waters 996 photodiode array detector, controlled with Millenium 32 software (Waters, Milford, MA). The separation was performed on a Luna 5μ C18(2) (250 × 4.6 mm) column (Phenomenex, Torrance, CA) with a Tracer C18 guard column (Teknokroma, Barcelona, Spain). The mobile phase consisted of (A) deionized water, adjusted to pH 2.3 with orthophosphoric acid, and (B) methanol. The chromatographic conditions were as follows: 0−6 min, 0% B; 6−7 min, linear gradient of 0−20% B; 7−16 min, isocratic 20% B. The flow rate was 1.0 mL/min. To purge the column, phase B percentage was further increased to 80% within 3 min and maintained for 10 min. Peaks were measured at wavelengths of 245 nm (ascorbic acid) and 280 nm (furfural). Peak identification in samples was based on the retention time and absorption spectrum.

Microbiological Analyses. The microbial population during storage was determined by plating the brines on the appropriate solid media, both spreading 0.1 mL onto the surface and plating their decimal dilutions (in 0.1% peptone water) with a Spiral Plater (Don Whitley Sci. Ltd., Shipley, U.K.). De Man, Rogosa, and Sharpe (MRS) agar (Oxoid Ltd., Basingstoke, U.K.) without and with 0.02% sodium azide (Sigma, St. Louis, MO) was used for lactic acid bacteria determination, and oxytetracycline−glucose−yeast extract (OGYE) agar (Oxoid) was used for yeasts. Pasteurized samples were also tested in plate count agar (PCA, Oxoid) with the pour-plate procedure, inoculating brine portions of 1 mL. Plates were incubated at 32 °C (MRS and PCA) or 26 °C (OGYE) for up to 5 days, and colonies were enumerated using an automatic counter (Countermat, IUL Instruments, Barcelona, Spain). Analyses were carried out in duplicate and from two bottles per treatment.

Statistical Data Analysis. Regression analysis and calculation of degradation rate constants were performed with the Statistica software (*19*). The goodness of the fit was estimated using the coefficient of determination (R^2).

RESULTS AND DISCUSSION

Effect of Pasteurization Treatment on Initial AA Content. The initial concentration of AA was found to be 15.1 mg/100 g of net weight in batch I and 36.3 mg/100 g of net weight in batch II. These concentrations were not significantly ($p < 0.05$) affected by the pasteurization treatment alone (data not shown).

Degradation Kinetics of AA in Pasteurized and Unpasteurized Table Olives during Storage. Microbial population was not detected in the pasteurized product, but lactic acid bacteria (LAB) and/or yeast populations, which are normally present in fermented olives at the packing step (*14*), were detected in the unpasteurized product throughout the storage time (**Table 1**). Therefore, AA degraded in both products, but in different manners (**Figure 1**). Results of AA retention were fitted into zero-order (concentration against time), first-order (ln concentration against time), and second-order (concentration^{-1} against time) equations (**Table 2**). Examination of the R^2 shows that, at room temperature, AA was degraded in the pasteurized product following first-order reaction kinetics. It is of interest to note that the increase in initial AA concentration significantly decreased the degradation rate. This is in agreement with the results obtained by Lin and Agalloco (*20*). The relatively rapid initial loss of AA between 0 and 20 days of storage could be related to the consumption of dissolved oxygen (not measured in the present work). Such a rapid initial loss of AA has also been found in stored packed single-strength orange juice (*11*, *21*). Interestingly, in the unpasteurized product, the AA level fell dramatically at room temperature. The antioxidant was not detected after 3 weeks of storage, irrespective of the initial AA level (**Figure 1a**). The rapid disappearance of AA may be due to AA utilization by microorganisms. Evidence of this appears to exist in other fermented vegetables (*22*, *23*). Of the microorganisms present during storage (**Table 1**), LAB would probably metabolize AA in the absence of another carbon source. This topic warrants further research. Ascorbic acid utilization by species of yeasts is not likely—it has been reported (*24*) that most yeasts are unable to use AA as sole carbon source.

Under refrigerated (10 °C) storage, AA degraded following a first-order reaction in the pasteurized product but under a zero-order reaction in the unpasteurized product. The degradation kinetics of AA in the unpasteurized product would be affected by the rapid utilization of the dissolved oxygen by the microbial population (*20*). After ~1 year of storage, AA was totally degraded in the unpasteurized product, whereas in the pasteurized product, AA loss was ~60% (**Figure 1b**).

In the case of storage at 40 °C (an ambient temperature frequent in summer in many countries), AA degradation occurred more rapidly in the pasteurized product (**Figure 1c**; **Table 2**). In this product, a first-order equation was found to provide the best fit for the initial AA concentration of 15 mg/100 g (sample P-I), but for the initial content of 36 mg/100 g (sample P-II), a second-order equation was better. In the

Table 2. Reaction Rate Constants (k) for AA Degradation in Pasteurized (P) and Nonpasteurized (NP) Packed Table Olives Stored at Room Temperature (RT) and 10 and 40 °C

		zero order			first order			second order		
storage temp (°C)	sample[a]	$(k \pm SE^b) \times 10^3$ [mg (100 g)$^{-1}$ day^{-1}]	n^c	$R^{2\,d}$	$(k \pm SE) \times 10^3$ (day^{-1})	n	R^2	$(k \pm SE) \times 10^3$ [(100 g) mg^{-1} day^{-1}]	n	R^2
RT	P-I	27.0 ± 9.0	12	0.476	32.16 ± 3.17	10	0.928	88.05 ± 18.73	10	0.734
	P-II	67.3 ± 11.5	12	0.775	5.16 ± 0.31	12	0.965	0.55 ± 0.04	12	0.938
10	P-I	34.8 ± 5.5	12	0.798	9.20 ± 0.29	12	0.990	6.16 ± 0.69	12	0.889
	P-II	50.4 ± 5.4	12	0.895	2.26 ± 0.16	12	0.954	0.11 ± 0.01	12	0.958
	NP-I	42.52 ± 4.61	12	0.894	8.25 ± 1.15	10	0.865	1.11 ± 0.19	10	0.803
	NP-II	94.90 ± 3.43	12	0.987	2.82 ± 0.32	10	0.907	0.10 ± 0.01	10	0.879
40	P-I	80.0 ± 20.4	10	0.657	52.33 ± 1.86	8	0.992	79.72 ± 12.30	8	0.875
	P-II	152.7 ± 38.5	10	0.662	9.72 ± 1.47	10	0.845	0.75 ± 0.06	10	0.952
	NP-I	48.33 ± 8.48	10	0.803	5.20 ± 0.59	10	0.906	0.59 ± 0.04	10	0.959
	NP-II	110.52 ± 13.45	10	0.894	4.48 ± 0.35	10	0.953	0.19 ± 0.01	10	0.985

[a] I and II indicate initial AA contents of 15 and 36 mg/100 g of net weight, respectively. [b] SE = standard error. [c] n = number of experimental data. [d] R^2 = coefficient of determination.

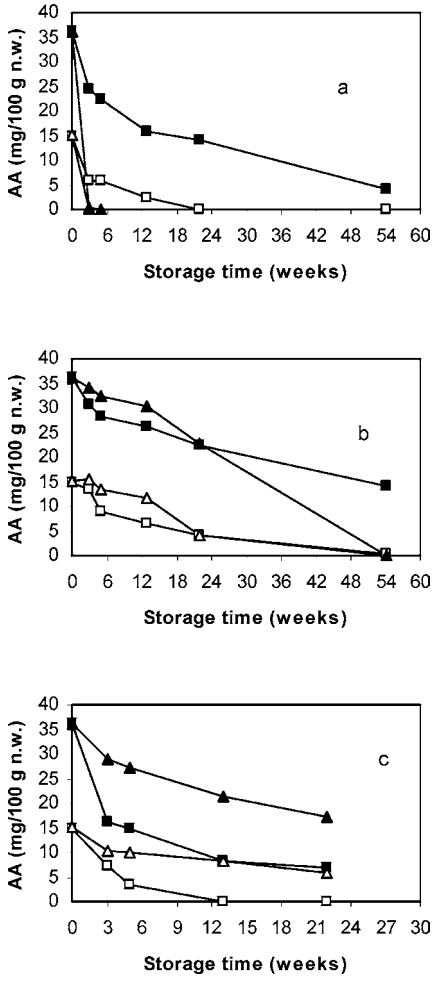

Figure 1. Changes in AA content of pasteurized and unpasteurized table olives during storage at the following temperatures: (**a**) ambient (20–24 °C); (**b**) 10 °C; (**c**) 40 °C. Samples: pasteurized (□, P-I; ■, P-II), unpasteurized (△, NP-I; ▲, NP-II); I and II indicate initial AA contents of 15 and 36 mg/100 g of net weight, respectively. Due to malfunction of the thermostated room at 40 °C, sampling at 54 weeks of storage for samples stored at this temperature was not carried out.

unpasteurized product, a second-order reaction showed the best fit irrespective of the initial AA concentration.

Activation Energy (E_a) for AA Degradation. In the case of the pasteurized product, results in **Table 2** indicate that the rate of AA degradation increased with increasing storage temperature. The activation energy (E_a) of AA degradation was estimated on the basis of the Arrhenius relationship described by the equation

$$k = A \exp(-E_a/RT)$$

From the corresponding first-order rate constants and the above equation, values obtained for $E_a \pm$ SE were 10 ± 3 and 8 ± 1 kcal/mol for initial AA levels of 15 and 36 mg/100 g, respectively. Our value (average E_a =9 kcal/mol) is equal to that reported by Rojas and Gerschenson (*25*) in a sweet aqueous model system under anaerobic conditions in the range of 24−45 °C. However, our value is higher than that found by Lee et al. (*7*) for the anaerobic destruction of AA in tomato juice (3.3 kcal/mol) and lower than that found by Kaanane et al. (*26*) for pasteurized orange juice (13.3 kcal/mol). Substituting the E_a of 9 kcal/mol for AA degradation in the above equation, we see that a temperature rise of 10 °C caused, on average within the range of 10−40 °C, an increase in the reaction rate of 1.7 (i.e., $Q_{10} = 1.7$).

Evolution of Dehydroascorbic Acid. DHAA, the main oxidation product of AA, was found in small amounts immediately after packing. As in the case of AA, DHAA content was not significantly affected by the pasteurization treatment alone (data not shown). During storage, DHAA degraded in both the pasteurized and unpasteurized products (**Figure 2**). The lowest DHAA degradation rate occurred at 10 °C for the pasteurized product. Assuming first-order kinetics, the best fit was obtained for DHAA degradation at 10 °C in sample P-I (k = 0.010 ± 0.001 day^{-1}, R^2 = 0.888, $p < 0.001$). It is noteworthy that this degradation rate constant did not significantly differ from the corresponding degradation rate of AA. In agreement with this, Riemer and Karel (*27*) found that the degradation rate of DHAA was similar to that of AA in dehydrated tomato juice during storage at 20 and 37 °C. On the other hand, Smoot and Nagy (*8*) found that the level of DHAA in canned single-strength grapefruit juice stored at 10−50 °C remained practically unchanged during a 12-week storage period.

Furfural Production. For the analysis of furfural, in preliminary experiments we assayed the HPLC method of Yuan and Chen (*28*), using an Aminex HPX-87H column and detection at 280 nm. This method did not give satisfactory results for furfural analysis in olive extracts because the furfural peak was obscured by an unknown peak (chromatogram not shown). By using the HPLC method cited under Materials and Methods,

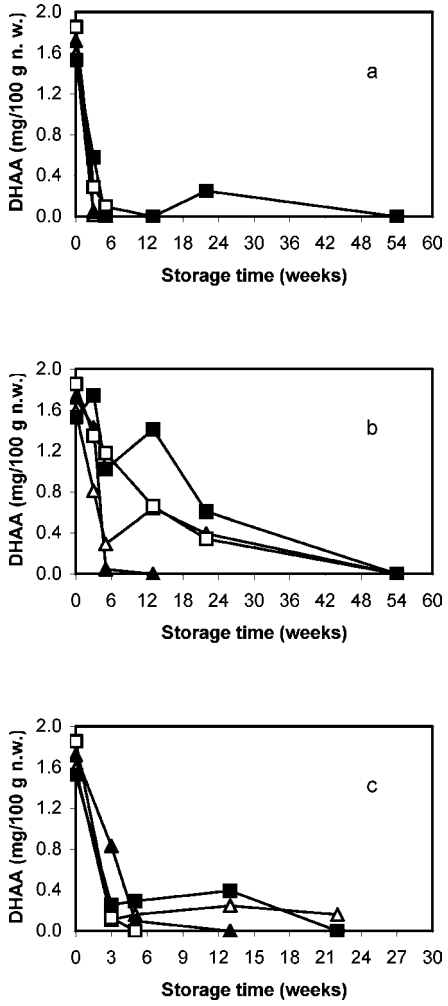

Figure 2. Changes in DHAA content of pasteurized and unpasteurized table olives during storage at the following temperatures: (**a**) ambient (20–24 °C); (**b**) 10 °C; (**c**) 40 °C. Samples: pasteurized (□, P-I; ■, P-II), unpasteurized (△, NP-I; ▲, NP-II); I and II indicate initial AA contents of 15 and 36 mg/100 g of net weight, respectively. Due to malfunction of the thermostated room at 40 °C, sampling at 54 weeks of storage for samples stored at this temperature was not carried out.

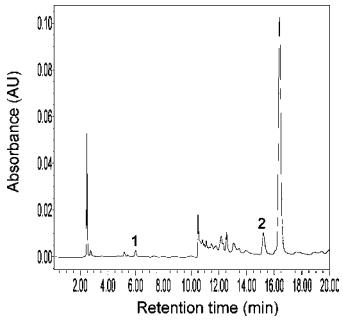

Figure 3. HPLC chromatogram of pasteurized table olives with added AA (36 mg/100 g of net weight) after 22 weeks of storage at 40 °C. Detection: 280 nm. Peaks: 1, ascorbic acid; 2, furfural. See Materials and Methods for sample preparation and chromatographic conditions.

a peak at $t_R \approx 15$ min (**Figure 3**) was tentatively identified as furfural on the basis of the retention time and the absorption spectrum maximum (276.9 nm). At room temperature, small amounts (<0.08 mg/100 g of net weight) were found only in the pasteurized product, whereas under refrigerated storage, furfural production was found to be insignificant in both the pasteurized and unpasteurized products (data not shown).

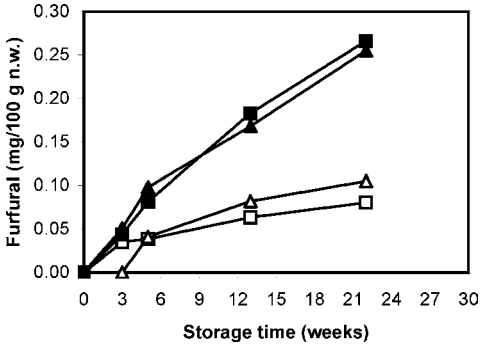

Figure 4. Furfural concentration in pasteurized and unpasteurized table olives during storage at 40 °C. Samples: pasteurized (□, P-I; ■, P-II), unpasteurized (△, NP-I; ▲, NP-II); I and II indicate initial AA contents of 15 and 36 mg/100 g of net weight, respectively.

Furfural was found in larger amounts at 40 °C (**Figure 4**). These results are similar to those of Kanner et al. (*29*) in orange juice concentrate stored at various temperatures. Furfural formation during storage at 40 °C is best described by a zero-order reaction (data not shown), which agrees with results found by other authors in citrus juices (*9*, *26*). After 6 weeks of storage at 40 °C, furfural content was ∼1 mg/kg of net weight. This concentration is similar to that found in orange juice during storage at 35 °C after 6 weeks (*26*, *30*) but lower than that found in lemon juice, for which, after 6 weeks of storage at 36 °C, furfural was ∼5 mg/L (*9*). At 40 °C, a highly significant ($p < 0.01$) correlation for the relationship between AA degradation and furfural formation was found in sample NP-II ($R = 0.978$), whereas lower correlation coefficients were obtained for samples NP-I ($R = 0.871$, $p < 0.1$), P-I ($R = 0.950$, $p < 0.05$), and P-II ($R = 0.824$, $p < 0.1$).

In conclusion, the present work is the first publication on AA degradation during storage in fermented vegetables. It is demonstrated that added AA in table olives was degraded during storage, the reaction kinetics being dependent on the presence or absence of pasteurization treatment as well as on the storage temperature. The added AA was stable at room temperature following pasteurization treatment (80 °C, 6 min), but disappeared rapidly when this treatment was not applied. This finding supports a previous hypothesis (*15*). The effect of pasteurization treatment alone on added AA was not significant. In pasteurized table olives, AA degradation followed generally first-order kinetics, and it is shown that the increase in initial AA concentration significantly decreased the degradation rate. From a practical standpoint, this means that AA degradation can be substantially retarded by increasing its concentration. In unpasteurized table olives, AA degradation followed zero- (10 °C) or second-order (40 °C) kinetics. In both pasteurized and unpasteurized products, the low level of initial DHAA disappeared during storage. Similarly to what has been reported in citrus juices, furfural was formed during storage, mainly at 40 °C, following zero-order kinetics.

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