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Fermented Vegetables Containing Benzoic and Ascorbic Acids As Additives: Benzene Formation during Storage and Impact of Additives on Quality Parameters

Francisco Javier Casado, Antonio Higinio Sánchez, Antonio De Castro, Luis Rejano, Víctor Manuel Beato, and Alfredo Montaño*

Instituto de la Grasa, Consejo Superior de Investigaciones Científicas (C.S.I.C.), Avenida Padre García Tejero 4, 41012 Seville, Spain

Supporting Information

ABSTRACT: Chemical and sensorial changes related to the use of benzoates and ascorbic acid as additives in packed fermented vegetables were investigated. For this, three selected vegetables (green olives, cucumbers, and caperberries) stored under different conditions (glass or plastic containers, ambient or refrigerated storage) were used. In all cases, benzoic acid remained unchanged (glass bottle) or decreased slightly (plastic pouch) at prolonged storage. Ascorbic acid was partially or totally degraded during storage, the degradation rate depending on the storage conditions and the vegetable matrix. Benzene levels higher than 10 μg/L were found in cucumbers and caperberries containing both additives, but only when packed in plastic pouches and after prolonged storage at room temperature. In these conditions, an appreciable browning of brine, related to AA degradation, was also found. The use of benzoate alone had a significant influence on vegetable color, but flavor was not significantly affected at the benzoate levels tested. On the basis of the present study, benzoates should be removed from fermented vegetable formulations containing ascorbic acid to eliminate possible benzene formation during long-term storage.

KEYWORDS: Table olives, cucumbers, caperberries, fermented vegetables, benzoic acid, ascorbic acid, preservative, additive, benzene

INTRODUCTION

Fermented vegetables are popular throughout the world and in some regions make a significant contribution to the diet of millions of individuals. Among the most typical fermented vegetables in the Mediterranean area are green olives, cucumbers, and caperberries, whose fermentation processes have been extensively studied.[1−3] These vegetable products are usually packed and preserved by their own physicochemical characteristics (relatively high values of acidity and salt), although a preservation method such as pasteurization is frequently used to guarantee the best preservation when milder levels of acidity and salt are desirable.[4]

Sorbates (sodium or potassium sorbate, sorbic acid, SA) and benzoates (sodium or potassium benzoate, benzoic acid, BA) are commonly added to fermented vegetables as adjuncts to preservation. In the European Union, the maximum permitted concentration of SA and/or BA in vegetable products is 2000 mg/kg, with the exception of olives and olive-based products (1000 mg/kg and 500 mg/kg for SA and BA, respectively; or 1000 mg/kg if both preservatives are used in combination).[5] Another additive widely used in packed vegetables, either alone or with sorbates or benzoates, is ascorbic acid (AA). Apart from its role as a nutrient, AA is an antioxidant commonly used for maintaining a "good" color in many foods.[6] Sorbate and/or AA stability studies during long-term storage of fermented vegetables, and their impact on product quality, have been reported,[7−9] but little is known about the stability of benzoate or its effect on the quality attributes in these products. Benzoates are normally added to prevent the fermentation of any fermentable residues in fermented vegetables. The minimum inhibitory concentration (MIC) for benzoic acid on a native yeast cocktail from table olives at a selected pH value (3.5−4.5) has been reported to be higher than MIC for sorbic acid.[10] It is known that benzoates may modify the flavor profile of foods.[11] Furthermore, BA in the presence of AA may decarboxylate to benzene, which is classified as carcinogenic in humans.[12,13] In a previous survey of benzene in foods, no benzene (<1 ng/g) was found in pickled vegetables containing added benzoates.[14] However, the study did not specify the type of vegetable, the packing conditions (e.g., packaging material) or the storage time. Using a more sensitive method with a detection limit of 0.04 μg/kg, benzene was found in all analyzed canned and jarred carrot samples at an average concentration of 0.2 μg/kg.[15] It is known that the formation of benzene from the reaction of benzoate with AA depends on various factors, such as temperature, concentration of benzoate and AA, occurrence of trace amounts of copper and iron salts, presence of oxygen in the food container, etc.[12,16,17]

The objectives of this work were to study (1) the stability of BA and AA, and possible benzene formation in fermented vegetables (green olives, cucumbers, and caperberries) containing both additives under different storage conditions, and (2) the impact of the addition of BA and AA on quality parameters such as brine color, vegetable surface color, and product flavor.

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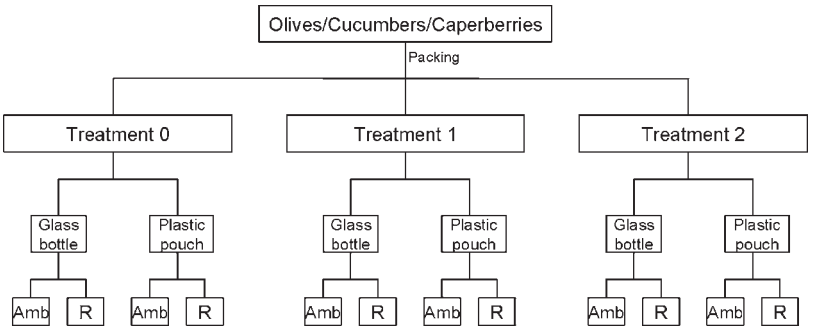

Figure 1. Experimental design for the study of chemical and sensorial changes related to the use of benzoates and ascorbic acid as additives in packed fermented vegetables. Treatment 0, packing without any additive added to the acidified cover brine; treatment 1, packing with sodium benzoate added to the acidified cover brine; treatment 2, packing with sodium benzoate plus ascorbic acid added to the acidified cover brine; Amb, ambient temperature (20−24 °C); R, refrigeration (6−9 °C).

Experiments in model brines were also carried out for comparison purposes.

MATERIALS AND METHODS

Materials and Chemicals. The selected fermented vegetables (green olives, cucumbers, and caperberries), all of them in brine, were supplied in bulk by a local processor. Physicochemical characteristics of the corresponding brines were *Green olives*: pH, 3.78; titratable acidity, 0.61% (as lactic acid); salt, 5.4% NaCl; *Cucumbers*: pH, 3.37; titratable acidity, 4.83% (as lactic acid); salt, 4.5% NaCl; *Caperberries:* pH, 3.71; titratable acidity, 0.40%; salt, 18.7% NaCl. Olives and cucumbers were directly packed without any conditioning (washing) step, but caperberries were desalted by immersion in acidified water (0.5% as lactic acid) for 3 days. For the corresponding calculations, the moisture content of green olives, cucumbers, and caperberries were assumed to be 75, 90, and 80% (w/w), respectively.

Cylindrical glass bottles (type "B250", 125 g vegetable plus 120 mL brine capacity) were obtained from Juvasa Co. (Dos Hermanas, Spain). Flexible plastic pouches (type "XSARAN/PLTN", 75 g vegetable plus 105 mL brine capacity, oxygen permeability of 7.6 mL/m^2/day; Plastienvase Co., Córdoba, Spain) were a gift of Jolca Co. (Seville, Spain).

Sodium benzoate, AA, and benzene were purchased from Sigma-Aldrich (St Louis, MO). Deionized water was obtained from a Milli-Q system (Millipore, Bedford, MA). De Man, Rogosa, Sharpe (MRS) agar and oxytetracycline-glucose-yeast extract (OGYE) agar were from Oxoid (Basingstoke, UK). All other chemicals and solvents were of analytical or chromatographic grade from various suppliers. Silicone antifoam (emulsion, 30% in water) was from Fluka (Sigma-Aldrich) and was purified in our laboratory by lyophilization followed by heating under a stream of helium at 50 °C for 24 h.

Packing of Vegetable Products. The experimental design for the present study is shown in Figure 1. The vegetables were each divided into three lots which were packed using acidified brine as cover liquor with the following additives: lot 1 (treatment 0), no additive was added; lot 2 (treatment 1), sodium benzoate was added to adjust the BA level to 0.05 g/100 g net weight (olives) or 0.2 g/100 g (cucumbers and caperberries); and lot 3 (treatment 3), as lot 2 plus AA adjusted to a level of 0.04 g/100 g net weight. Each lot was further divided into two sublots: one was packed in glass bottles and the other in plastic pouches. For cucumbers and caperberries, acidified cover brines consisted of acetic acid and NaCl to give the following equilibrium values: 2.5% titratable acidity (as acetic acid) and 2.5% salt in cucumbers; and 1.0% titratable acidity and 4.0% salt in caperberries. The relatively high acidity levels were used because they were the mean values previously found in a limited survey of commercial cucumbers and caperberries (data not published). For olives, acidified brine consisted of lactic acid and NaCl to give equilibrium values of 0.5% titratable acidity (as lactic acid) and 5.0% salt. When packing in glass bottles, the cover brine was added hot (≈70 °C) to achieve and maintain a vacuum inside the bottles. After packing, the containers were stored in the dark at both room temperature (20−24 °C) and under refrigeration (6−9 °C), and sampled during storage to analyze for BA, AA, benzene, as well as for the microbiological and physicochemical characteristics of brine, and vegetable color. Panel tests were also performed at the end of storage.

Packing of Model Brines. Two model brines were prepared: (1) 0.5% lactic acid +0.5% sodium lactate +5% NaCl + 0.2% sodium benzoate, pH 3.5; and (2) as (1) plus 0.04% AA. Packing and storage conditions were identical to those mentioned for the vegetables.

Chemical Analyses. AA was analyzed by HPLC as described in ref 8. BA was analyzed by spectrophotometry at 229 nm according to the method described by Montaño et al.[18]

Benzene was determined by dynamic headspace (purge and trap) gas chromatography with FID detection according to the method described by McNeal et al.[14] with modifications. To minimize benzene loss due to volatilization during sample preparation, benzene was not determined in the vegetable-brine mixture, but just in brine. Brine (30 mL) from the containers was transferred to a vial, which was immediately capped and sealed. Using a 10 μL syringe, 3 μL of purified antifoam agent was injected into the brine sample through the vial septum. An aliquot (25 mL) of sample was transferred to the sparge vessel of the purge and trap apparatus using a 25 mL gastight syringe (Hamilton, Reno, Nevada). The system consisted of a Fisons HRGC Mega 2 Series gas chromatograph (Fisons Instruments, Milan, Italy) equipped with a FID, connected to an Eclipse 4660 purge-and-trap sample concentrator (OI Analytical, Texas). A HP-5 capillary column (50 m × 0.32 mm i.d., 1.05 μm film; J&W Scientific, Folsom, CA) was used with the following oven program: isothermal for 4 min at 65 °C, temperature increased at 10 °C/min to 200 °C, and hold for 8 min. Helium was used as a carrier gas at 1 mL/min. The split ratio was 18:1 (at 70 °C) with injector and detector temperatures at 250 °C. The purge and trap analysis conditions were: trap, Tenax/silica gel/carbon molecular sieve; purge flow rate, 40 mL/min; purge time, 9 min; purge temperature, 20 °C; desorb flow rate, 30 mL/min; desorb time, 0.5 min; desorb temperature, 190 °C; bake time, 6 min; bake temperature, 210 °C. A calibration curve, constructed in the range 1−100 μg/L, produced a correlation coefficient of 0.9997. The detection limit (S/N = 2) for benzene was estimated at 1.2 μg/L. The precision of the method was found to be 9.7%, on average. Confirmation of benzene identity was carried out using static headspace sampling in combination with GC/MS according to ref 16 with slight modifications. Briefly, 10 mL of sample was placed in a 20 mL headspace vial and the pH adjusted to 10 with 30% (w/v) NaOH. The vial was sealed and heated at 50 °C for 30 min. Then, 2 mL of sample headspace was injected into the GC/MS system in splitless injection mode. The

GC/MS system used was an HP5890 series II gas chromatograph coupled to an HP5972 benchtop mass selective detector. A Zebron ZB-624 capillary column (30 m x 0.25 mm i.d., 1.40 μm film, Phenomenex) was used with helium as carrier gas. The temperature program was as follows: 25 °C for 4 min, 10 °C/min ramp to 240 °C, and held for 10 min. The temperatures for the injection port and interface transfer line were set at 220 and 280 °C, respectively. Diagnosis mass fragments (m/z 52, 77, and 78) were monitored in the selected ion-monitoring (SIM) mode. The presence of benzene was confirmed if the ratios of ions m/z 77/78 and 52/78 were within ±20% of the ion ratios from a benzene standard.

Brine color was estimated by measuring the difference in absorbance at 440 and 700 nm.[19] Total polyphenols were measured using Folin-Ciocateu reagent according to a previously described method.[20] Other physicochemical characteristics of the brine, such as pH, titratable acidity and salt content, were determined by routine methods used in our laboratory.[21]

Microbiological Analyses. The microbial population during storage was determined by plating the brines on the appropriate solid media, both by 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, England). De Man, Rogosa, Sharpe (MRS) agar without and with 0.02% sodium azide was used for lactic acid bacteria determination, and oxytetracycline-glucose-yeast extract (OGYE) agar was used for yeasts. Plates were incubated at 32 °C (MRS) or 26 °C (OGYE) for up to 5 days, and colonies enumerated using an automatic counter (Countermat, IUL Instruments, Barcelona, Spain).

Quality Parameters. The surface color of the vegetables was measured by a Color-View model 9000 spectrophotometer (BYK-Gardner, Inc., Silver Spring, MD) with a measurement area of 11 mm diameter, 45° circumferential illumination, and observation angle of 0°. All measurements were done on the CIE 1976 $L^*a^*b^*$ scale using illuminating conditions CIE type C, 10° observer. Results were expressed as the mean of 10 replicate measurements, each made on one olive/cucumber/caperberry. Hue angle ($h°$) was calculated as $\tan^{-1}(b^*/a^*)$. When a^* value was negative, $h°$ was calculated from the equation: $h° = 180 + \tan^{-1}(b^*/a^*)$.[22] Chroma ($C$) was calculated as $(a^{*2} + b^{*2})^{1/2}$. For olives, a color index (i) was also calculated from the equation: $i = (4R_{635} + R_{590} - 2R_{560})/3$, where R_{635}, R_{590}, and R_{560} are the values of reflectance at 635, 590, and 560 nm, respectively.[23]

Flavor was evaluated by a 10-member sensory panel. Evaluations were done in individual sensory booths under controlled environmental conditions (20−22 °C, incandescent lighting). Samples, coded with 3-digit numbers, were randomly provided for the judges in colorless, transparent cups covered with a watch glass. Tap water was offered between samples for cleansing the palate. Triangle panel tests were performed to determine whether the presence of BA had any significant effect on product flavor. Refrigerated samples of each product with and without additive were used in the triangle tests.

Statistical Data Analysis. Regression analysis for the calculation of degradation rate constants and their 95% confidence intervals was performed using a Microsoft Excel spreadsheet (Microsoft). Analysis of variance (ANOVA) was performed with the Statistica software (version 7, Statsoft Inc., Tulsa, OK). The Duncan test was used to compare the means. Significant differences were determined at the $p < 0.05$ level. Sensory data were compared with tabulated values, taking into account the number of judges and a confidence level of 95%.[24]

RESULTS AND DISCUSSION

Physicochemical and microbiological characteristics during storage for the three vegetable studied are given in Tables 1−3 in the Supporting Information. In general, titratable acidity and pH hardly changed during storage. No LAB or yeast populations were detected (<1.3 log cfu/mL) throughout storage for cucumbers and caperberries with added benzoate (treatments 1 and 2). Even in the absence of benzoate (control samples) these vegetables had negligible microbial populations. This may be a reflection of the harsh environmental conditions in which the vegetables were supplied, namely, the high acidity level for the cucumbers and high salt content for the caperberries, as well as the relatively high acidity levels in the packed products (3.0−3.5% as lactic acid in cucumbers, 1.4−1.6% in caperberries). On the other hand, either LAB or yeast counts, or both, were relatively high (10^3−10^5 cfu/mL) for olives during storage under different packing conditions. For each treatment (0, 1, or 2), in general, a higher LAB population but lower yeast population was found in olives packed in glass bottles than in plastic pouches.

Changes in BA and AA, and Benzene Formation. Benzoate concentration in each vegetable did not significantly change during storage, with the exception of a slight decrease in the plastic pouches at the end of storage (Table 1). This occurred both in the absence and presence of AA (only data in the presence of AA are shown). On the other hand, AA was significantly degraded in all the three vegetables in both glass and plastic containers (Table 1). In olives, in contrast to cucumbers and caperberries, AA degradation rate was higher in glass bottle than in plastic pouch (Table 2), which is consistent with the aforementioned higher LAB population found in glass bottles. LAB are considered the microorganisms responsible for the rapid and total degradation of AA in green table olives.[7,8] As confirmation, we recently isolated various species of LAB from olive brines, which are able to metabolize AA in MRS broth (data not published).

For all the three vegetables, no benzene (<1.2 μg/L) was detected in the refrigerated samples nor in samples with benzoate alone (treatment 1) stored a room temperature. However, benzene was found in products containing benzoate and AA (treatment 2) stored at room temperature, especially when packed in plastic pouches (Table 1). In green olives, benzene was only found in small amounts (1.5 μg/L brine) in plastic pouches at the end of the storage time. In cucumbers, benzene was found after 27 weeks and its concentration reached 5.2 (glass bottle) and 44.7 μg/L (plastic pouch) after 67 weeks storage. In caperberries, the levels of benzene formed were lower in comparison with those of the cucumbers, indicating that the vegetable matrix affected the kinetics of benzene formation. In fact, the rate of benzene formation in the model brine samples was significantly higher than in each vegetable matrix. Concentrations (μg/L) of 10.4 ± 0.9 and 13.9 ± 1.2 were found in model brine packed in glass and plastic containers, respectively, after ≈14 weeks storage at room temperature, while no benzene was found in olives, cucumbers or caperberries at this time. This finding about the higher rate of benzene formation in model solutions compared to that in fermented vegetables is in accordance with previous studies reported in beverages containing benzoate and AA.[17]

Interestingly, benzene was formed even when AA had already been totally degraded, as observed in caperberries packed in plastic pouches between 27 and 67 weeks. This could indicate that products from AA degradation can promote the oxidative decarboxylation of BA to produce benzene during prolonged storage at room temperature. It must be assumed that AA degradation products can form H_2O_2 similarly to AA in the presence of metal ions and oxygen, which then generate hydroxyl radicals; in turn, hydroxyl radicals can attack BA to produce benzene.[12] AA in acid conditions may be oxidized to

Table 1. Changes in Benzoic and Ascorbic Acids (Mg/Kg Net Weight), And Benzene Formation (μg/L brine) in Fermented Vegetables with Added Benzoate and Ascorbic Acid (Treatment 2) during Storage at Room Temperature[a]

	Glass bottle			Plastic pouch		
time (weeks)	benzoic acid	ascorbic acid	benzene	benzoic acid	ascorbic acid	benzene
			Olives			
initial[b]	447 ± 81a	289 ± 2d	nd[c]	516 ± 122b	303 ± 2e	nd
2	na[d]	216 ± 4c	nd	na	280 ± 3d	nd
7	na	172 ± 17b	nd	na	224 ± 3c	nd
14	391 ± 60a	26 ± 11a	nd	338 ± 51ab	155 ± 1b	nd
27	355 ± 27a	nd	nd	396 ± 32ab	28 ± 2a	nd
67	308 ± 36a	nd	nd	340 ± 18a	nd	1.5 ± 0.3
			Cucumbers			
Initial	1733 ± 94a	342 ± 25c	nd	1828 ± 71b	360 ± 2e	nd
2	na	348 ± 10c	nd	na	343 ± 4d	nd
7	na	339 ± 5c	nd	na	313 ± 13c	nd
14	1830 ± 131a	309 ± 2bc	nd	1711 ± 25ab	228 ± 2b	nd
27	1844 ± 31a	265 ± 53b	1.3 ± 0.3a	1776 ± 72ab	46 ± 1a	6.5 ± 0.9a
67	1661 ± 12a	173 ± 6b	5.2 ± 0.7b	1659 ± 42a	nd	44.7 ± 9.4b
			Caperberries			
Initial	1763 ± 103a	379 ± 2f	nd	1760 ± 75b	366 ± 2d	nd
2	na	358 ± 13e	nd	na	326 ± 1c	nd
7	na	295 ± 2d	nd	na	250 ± 9b	nd
14	1745 ± 95a	249 ± 5c	nd	1706 ± 55b	83 ± 0a	nd
27	1740 ± 39a	197 ± 3b	nd	1737 ± 19b	nd	4.8 ± 0.1a
67	1640 ± 50a	181 ± 1a	3.6 ± 0.6	1558 ± 6a	nd	14.9 ± 2.2b

[a] Values are means ± standard deviation of two containers, each analyzed in duplicate ($n = 4$). For each vegetable, values with the same letter within a column indicate that there are no significant differences between them. [b] Analyses were performed after 3 days storage (olives) or after 1 day storage (cucumbers and caperberries). [c] nd = not detected. [d] na = not analyzed.

Table 2. First-Order Rate Constants (k) for Ascorbic Acid Degradation in Green Olives, Cucumbers, And Caperberries under Different Storage Conditions (Treatment 2)[a]

		(k ± 95% CI[b]) x 10^3, day^{-1}		
storage temperature	packaging material	olives	cucumbers	caperberries
ambient	glass	24.12 ± 8.79d	1.52 ± 0.29b	1.51 ± 0.60b
	plastic	12.41 ± 2.60c	10.81 ± 2.91d	14.99 ± 3.88d
refrigeration	glass	1.27 ± 0.48a	0.91 ± 0.22a	0.55 ± 0.32a
	plastic	4.34 ± 0.50b	2.51 ± 0.45c	6.02 ± 0.71c

[a] Values with the same letter within a column indicate that there are no significant differences between them. [b] CI = confidence interval.

dehydroascorbic acid (DHA), which may subsequently be degraded to other products, such as 2,3-diketo-L-gulonic acid (DKG), which is relatively unstable, yielding a range of other degradation products (e.g., furfural, 2-furoic acid, 3-hydroxy-2-pyrone, etc.).[25] It has been demonstrated that preparations of DKG, similarly to AA, lead to the nonenzymatic generation of H_2O_2 in the presence of Cu^{2+}.[26]

It is known that the amount of benzene formed from the reaction of benzoate with AA depends, apart from other factors, on the concentration of BA.[16] Therefore, in case of olives, the negligible formation of benzene can at least be partially attributable to the lower concentration of added benzoate in comparison with cucumbers or caperberries. However, the possibility of partial mitigation of benzene formation due to matrix components (e.g., phenolic compounds) cannot be discarded. It is known that hydroxytyrosol and tyrosol are the major phenols in fermented green olives,[1] and these phenols exhibit peroxyl and hydroxyl radical scavenging activities.[27] A similar assumption involving polyphenols has been made for explaining the limited formation of benzene in cranberry juice compared to model solutions.[17] Determinations of total polyphenols performed at the end of storage time in a sample of each vegetable gave the following

results (expressed as mg caffeic acid/100 mL brine): green olives, 29.0 (glass bottle) and 28.6 (plastic pouch); caperberries, 17.0 and 13.4; and cucumbers, 7.6 and 6.4. Therefore, the higher polyphenol content in caperberries compared to cucumbers (with the benzoic acid level in both vegetables being similar) could explain the lower levels of benzene in caperberries. However, the use of HPLC methods that define the different phenols occurred in the brines should be more appropriate to support the relationships between phenols occurrence and benzene production. That is because the phenols occurred in the various brined vegetables are characterized by different antioxidant activity and, as a consequence, can produce a differentiate impact on the benzoate degradation. This topic merits further research.

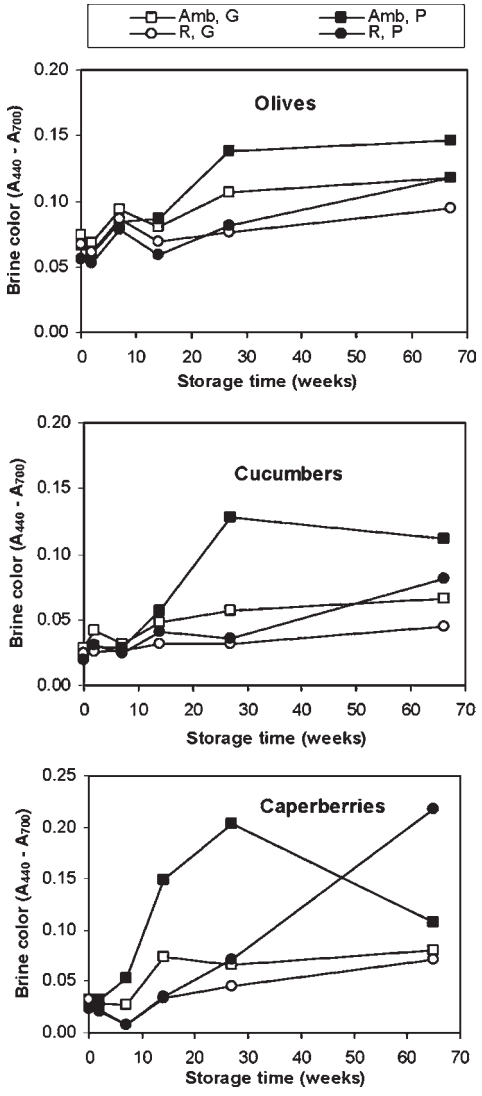

Figure 2. Changes in brine color during storage of green olives, cucumbers, and caperberries with added benzoate and ascorbic acid (treatment 2) as affected by packaging material and storage temperature. Amb = ambient temperature, R = refrigeration, G = glass bottle, P = plastic pouch. Points are means of two containers. Coefficients of variation, on average, were 7.1%, 12.8%, and 11.9% for olives, cucumbers, and caperberries, respectively.

Impact of BA and AA on Quality Parameters. In the samples treated with benzoate and AA (treatment 2), a considerable browning of brine attributable to AA degradation occurred during storage in plastic pouches at room temperature (Figure 2), reaching a maximum ($A_{440}-A_{700}$ = 0.13−0.20 AU) once AA was totally degraded. It is known that furfural, one of the main degradation products of AA,[28] may undergo polymerization to form brown melanoidins, the mechanism being apparently similar to that of the Maillard browning of pentoses.[29] Browning could be an inconvenience from a consumer standpoint, as the consumer might erroneously associate this visual color with microbial spoilage or poor preservation conditions. However, the above maximum value of the parameter $A_{440}-A_{700}$ was below the limit of 0.23 AU set for packed green olives.[19] At prolonged storage in plastic pouches, brine browning was either unchanged (olives) or decreased (particularly in caperberries). Apparently, melanoidins formed from AA degradation products in each vegetable exhibited different antioxidant activities. Maillard reaction products, especially melanoidins, have been reported to have antioxidant activity through scavenging oxygen radicals or chelating metals.[30] Browning was retarded in the refrigerated products, where AA degradation occurred more slowly than at room temperature (Table 2).

For each vegetable product, analysis of variance (ANOVA) of the surface color data (Table 3) showed significant differences between the samples containing benzoate (treatment 1) and those without additives (treatment 0). For olives, this was reflected by lower values of L^* (lightness), b^* (yellowness), C (color purity), and index i, which meant a worse color for olives, based on the known correlation of this index with a visual scale.[23] In the presence of AA (treatment 2), the color of olives improved (index i increased) in comparison with treatment 1. A similar positive effect of AA on color in sorbate-containing green olives was previously found.[8] However, when the aim is to maintain the freshness appearance of olives (i.e., the original green color of fruits), such as in case of "seasoned" Manzanilla-Aloreña table

Table 3. Influence of Treatment on Color Parameters of Packed Olives, Cucumbers, And Caperberries[a]

	olives			cucumbers			caperberries		
color parameter	0	1	2	0	1	2	0	1	2
L^*	51.94b	50.74a	52.41b	41.35a	42.74a	42.29a	39.95a	39.23a	40.20a
a^*	3.68a	3.63a	3.90b	0.62b	−0.11a	1.07b	1.76b	1.30a	2.28c
b^*	35.06b	33.13a	35.77b	30.56a	29.39a	30.81a	25.30b	23.98a	26.33b
$h°$	84.00a	83.72a	83.73a	88.89a	90.59b	87.99a	86.07b	86.99c	85.06a
C	35.25b	33.33a	35.98b	30.57a	29.44a	30.83a	25.37b	24.02a	26.43b
Index i	26.11b	25.06a	26.87b	—[b]	—	—	—	—	—

[a] Means (n = 20; 2 packaging materials × 2 storage temperatures × 5 samplings) within rows, for each vegetable, followed by the same letter are not significantly different at the 5% level, according to Duncan's multiple range test. [b] —, not applicable.

olives, AA is not an effective additive.[31] For cucumbers and caperberries, the samples containing benzoate alone were the greenest (highest $h°$, lowest a^*) but the parameter L^* was not significantly affected by treatment. Hue angle and L* values have been previously used to describe color changes in fermented and nonfermented cucumbers.[32] On the other hand, treatment 1 samples had lower b^* and C values than treatments 0 and 2 samples, although the differences were statistically significant only in the case of caperberries. ANOVA for each vegetable also showed that the influence of packaging material, storage temperature, and storage time on color parameters was negligible in most cases (data not shown).

Panel tests performed for each vegetable at the end of the storage showed that the flavor differences between samples with benzoate (treatment 1) and their corresponding controls without benzoate (treatment 0) were not significant (data not shown). Although the levels of BA in cucumbers and caperberries were about 5 times higher than in olives, and were therefore more likely to be detected by the judges, it must be remembered that the former vegetables had higher acidity values (3.0% and 1.4% in cucumbers and caperberries, respectively, compared with 0.4% in olives; Tables 1−3 in the Supporting Information). This could mask possible flavor differences due to the BA.

To summarize, this work studied the main chemical and sensorial changes related to the use of benzoates in fermented vegetables, in the absence and presence of AA. In all the three fermented vegetables studied, BA remained unchanged (glass bottle) or decreased slightly (plastic pouch) at prolonged storage. AA was partially or totally degraded in all cases, the degradation rate depending on the packing conditions and on the vegetable matrix. Benzene was only formed when BA was together with AA. The amounts of benzene found were compatible with previous findings of benzene in pickled vegetables containing added benzoates,[14] but only for refrigerated storage or short storage times (<14 weeks) at room temperature. However, for prolonged storage (>1 year), benzene levels above the WHO drinking water limit of 10 μg/L[33] can be formed, depending on the vegetable matrix and packaging material. Since shelf lives of fermented vegetables are normally greater than one year and any exposure to benzene and benzene-containing products should be avoided, we believe that benzoates should be removed from fermented vegetable formulations containing AA to eliminate possible benzene formation during room temperature storage. If benzoates are added in combination with AA, fermented vegetables should be kept refrigerated. In addition, the use of oxygen-impermeable containers is also highly recommended, to minimize benzene formation. It must be stressed that the fermented vegetables analyzed in the present study are normally consumed as snacks so that consumption per person per day should not be high. Therefore, intake of these vegetables, even in excess, is not likely to exceed the U.S. Environmental Protection Agency oral reference dose (RfD) of 4 μg/kg bodyweight/day for benzene.[34] An appreciable browning of brine during storage, which can be attributed to AA degradation, was found in all products packed in plastic containers (i.e., in the presence of dissolved oxygen) with added benzoates and AA. In general however the browning intensity was not excessive and should not be an inconvenience during product marketing. The use of benzoate alone had a significant influence on vegetable color, but the flavor was not significantly affected at the benzoate levels tested. Packers or processors should be aware of the above-mentioned results if they use benzoates and AA in packed fermented vegetables.

■ ASSOCIATED CONTENT

Supporting Information. Tables of microbial and physicochemical characteristics of brine for each vegetable. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +34 95 4691054, fax: +34 95 4691262, e-mail: amontano@cica.es.

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