1 **Stability of color in Spanish-style green table olives pasteurized and stored in**
2 **plastic containers**

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12 Running title: Color stability in green table olives packed in plastic containers

ABSTRACT

BACKGROUND: There is an increasing interest in the use of pasteurizable plastic packaging by the olive industry. In order to investigate the change from traditional glass or varnished can containers to plastic packaging, the proper plastic material that is compatible with fermented olives while maintaining color quality during pasteurization treatment and storage must be selected. This work is focused on color stability in two distinct pasteurizable plastic containers with different oxygen permeability.

RESULTS: In PET + MDPE/EVOH (polyethylene terephthalate + medium-density polyethylene/ethylene vinyl alcohol) pouches, pasteurization provoked severe browning which drastically decreased their color shelf life (< 6 weeks). However, this browning did not occur in the unpasteurized product without preservatives due to the presence of microorganisms. In AlOx-coated PET + MDPE (aluminum oxide coating on polyethylene terephthalate + medium-density polyethylene) pouches, color changes were small or negligible throughout storage, especially if ascorbic acid was added to the packing solution (shelf life > 6.5 months).

CONCLUSION: The plastic material had a significant effect on the retention of color of the pasteurized product. The use of AlOx-coated PET + MDPE pouches could be an alternative to traditional packaging for the pasteurization and storage of Spanish-style green olives from a color quality standpoint.

Keywords: green table olives, plastic containers, color, storage, pasteurization, browning

INTRODUCTION

Spanish-style green olives are the most widely distributed and investigated type of table olive. This is a fermented product whose long-term preservation is usually carried out by its own physico-chemical characteristics without the need for a pasteurization treatment if an appropriate combination of pH (< 3.5), titratable acidity ($> 5 \text{ g kg}^{-1}$ as lactic acid), combined acidity ($\approx 0.025 \text{ mol L}^{-1}$), and NaCl ($> 50 \text{ g kg}^{-1}$) is used.¹ However, the increasing preference on the part of consumers for lower levels of acidity and salt has resulted in the need for modification of such conditions and the stabilization of the final product is only guaranteed by pasteurization. Also, this treatment has been progressively used to allow the commercialization of olives with incomplete fermentation.² The pasteurization step results in shelf-stable products by killing the major spoilage microorganisms, lactic acid bacteria and yeasts, as well as inactivating enzymes that may contribute to fruit softening.³

Since the development of the commercial pasteurization process, all pasteurized table olives have been packaged in glass or varnished can containers which do not react chemically with food components. The main function of food packaging is to maintain the quality and safety of products during storage.⁴ Glass and varnished can are solid materials that provide a complete barrier to gases and impart no flavors to the finished product. However, there is an increasing interest in the use of plastic packaging by the industry due to factors such as reduced weight of plastic containers, lower production costs, less apt to shatter or deform, transparent, flexible, and convenient to the consumer.⁵ In spite of all these advantages, plastic containers are likely to have at least a limited level of oxygen permeability. This could be detrimental to the quality of pickled

vegetables, which are known to be susceptible to oxidation, and reduce the shelf life of products.^{6,7}

The use of additives (ascorbic acid, sorbates and benzoates) in packed table olives is a common practice in the industry. Ascorbic acid has been demonstrated to have a positive effect on fruit color.^{8,9} Even though the use of sorbates and benzoates would not be necessary if a pasteurization treatment is applied, it is relatively frequent to find commercial samples of pasteurized packed green table olives containing one of these preservatives or even a mix of both. The addition of these preservatives may be justified if the goal is to prevent unwanted events once the package has been opened and the olives are kept at room temperature for a few days. Sorbates can prevent surface films of yeast in packed table olives.¹⁰ Benzoates are used to inhibit bacterial growth, especially lactic acid bacteria (LAB), in certain fermented and fresh-pack products.¹¹ Sodium benzoate and potassium metabisulfite have been demonstrated to be effective anti-mycotic and antibacterial agents for extending the shelf life of sauerkraut in plastic bags.⁶

The appearance of food is determined mostly by surface color and is the first sensation that the consumer perceives and uses as a tool to either accept or reject food.¹² Preliminary experiments with Spanish-style green olives pasteurized in pasteurizable plastic pouches made of PET + MDPE/EVOH (polyethylene terephthalate + medium-density polyethylene/ethylene vinyl alcohol), with oxygen permeability $\approx 3 \text{ cm}^3 \text{ m}^{-2} \text{ day}^{-1}$, showed that the visual color of both olives and brine turned unacceptable (very dark) after a few weeks of storage, but it maintained acceptable in the absence of pasteurization (unpublished data). It was hypothesized that the microbial population in the unpasteurized product consumed the oxygen entering through plastic and that the use of another plastic material with lower oxygen permeability could prevent, or at least

slow down, the browning formation. These hypotheses need confirmation. To our knowledge, no scientific study has been published on green table olives pasteurized in plastic pouches. Therefore, the objectives of this work were: (a) to study the influence of two distinct pasteurizable plastic containers made from packaging materials with different oxygen permeability on the color parameters of pasteurized packed green table olives during storage both in the absence and presence of common additives (ascorbic acid, sorbates and benzoates), and (b) to perform a similar study with the unpasteurized product in order to explain the effect of microorganisms on inhibition of browning in plastic pouches. Ascorbic acid stability and its impact on color stability were also considered.

EXPERIMENTAL

Materials and chemicals

Pitted Spanish-style green table olives (Manzanilla cultivar) were supplied in bulk by Angel Camacho SL (Seville, Spain). The physico-chemical characteristics of the corresponding brine were the following: pH, 3.47; titratable acidity, 9.1 g L⁻¹ (as lactic acid); combined acidity, 0.047 mol L⁻¹; and NaCl, 83 g L⁻¹. Two types of plastic pouches, named pouch A and B, were used. Both pouches were supplied by the SP Group (Córdoba, Spain). Pouch A was made of PET + MDPE/EVOH (polyethylene terephthalate + medium-density polyethylene/ethylene vinyl alcohol) with oxygen permeability $\approx 3 \text{ cm}^3 \text{ m}^{-2} \text{ day}^{-1}$ at 23 °C and 50% HR, and a thickness of 106 μm . Pouch B was made of AlOx-coated PET + MDPE (aluminum oxide coating on polyethylene terephthalate + medium-density polyethylene) with oxygen permeability $\approx 1 \text{ cm}^3 \text{ m}^{-2} \text{ day}^{-1}$ at 23 °C and 50% HR, and the same thickness as pouch A.

Potassium sorbate, ascorbic acid, sodium benzoate, citric acid, and sodium chloride were purchased from Sigma-Aldrich (St Louis, MO). Deionized water was obtained from a Milli-Q system (Millipore, Bedford, MA). All other chemicals and solvents were of analytical or chromatographic grade from various suppliers.

Experimental design

The olives were divided into two lots, one of which was packed in plastic pouch A and the other in plastic pouch B (Figure 1). Each lot was further divided into eight sub-lots which were packed using an acidified brine as cover liquor with the following additives: (1) control, no additive was added (packing solution C); (2) ascorbic acid was added to adjust the ascorbic acid level to 0.4 g L^{-1} brine at equilibrium (packing solution A); (3) ascorbic acid + potassium sorbate was added to adjust the levels of ascorbic acid and sorbic acid to 0.4 g L^{-1} brine and 1 g L^{-1} brine, respectively, at equilibrium (packing solution AS); (4) same as (3), except that ascorbic acid was excluded (packing solution S); (5) ascorbic acid + sodium benzoate was added to adjust the levels of ascorbic acid and benzoic acid to 0.4 g L^{-1} brine and 0.5 g L^{-1} brine, respectively, at equilibrium (packing solution AB); (6) same as (5), except that ascorbic acid was excluded (packing solution B); (7) ascorbic acid + potassium sorbate + sodium benzoate was added to adjust the levels of ascorbic, sorbic and benzoic acids to 0.4 g L^{-1} , 0.5 g L^{-1} and 0.5 g L^{-1} , respectively (packing solution ASB); and (8) same as (7), except that ascorbic acid was excluded (packing solution SB). For comparison purposes, olives were also packed in glass containers (type “B250”) using the packing solution ASB. In both types of pouches the drained net weight of olives was 61.5 g (17 olives) and the brine volume was 86 mL. In glass containers the drained net weight and brine volume were 96.5 g (26 olives) and 135 mL, respectively, giving the same weight-to-volume ratio (0.715) as in

the plastic pouches. Acidified cover brine consisted of citric acid and NaCl to give equilibrium values of 5 g L⁻¹ titratable acidity (expressed as lactic acid) and 47 g L⁻¹ NaCl, respectively. For the corresponding calculations, the moisture content of pitted olives was assumed to be 750 g kg⁻¹. In the case of packing in glass bottles, the cover brine was added hot (≈ 70 °C) in order to achieve and maintain a vacuum inside the bottles. After packing, for each treatment, the pouches or glass containers were divided into two lots: one lot was subjected to pasteurization (93 °C for 7 min) and then stored at 30 °C in the dark, and the other lot was directly stored at 30 °C in darkness.

In order to confirm the positive influence of microorganisms on color in the case of the product packed in pouch A without pasteurization, the following experiment was carried out where the olives were packed using acidified brine without any additive under the following conditions: (1) pasteurized control, PC; (2) non-pasteurized control, NPC; and (3) inoculated, pasteurized sample, IP; 1 mL of suspension from fermenting brine was injected into each plastic pouch after pasteurization. This suspension was obtained by centrifugation of 900 mL of brine (20,000 xg, 10 min, 15 °C) and suspending the obtained pellet in 15 mL of saline. The initial microbial populations of LAB and yeasts in the pouches due to inoculation were around 3.0 x 10⁶ cfu mL⁻¹ and 1.0 x 10⁵ cfu mL⁻¹, respectively. An injection control (IC) was also prepared by injecting 1 mL of saline per pouch into another group of pasteurized pouches.

Sampling

Three pouches per treatment were analyzed at five time points from 14 to 195 days for chemical and color parameters, and one of them was also used for the microbiological analysis.

Color parameters

The surface color of olives was measured using 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 the CIE illuminating conditions, type C, 10° observer. The results were expressed as the mean of 10 replicate measurements, each made on 1 olive. Total color difference (ΔE) was calculated as $((\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2)^{1/2}$. Differences in perceivable color can be analytically classified as very distinct ($\Delta E^* > 3$), distinct ($1.5 < \Delta E^* < 3$), and small difference ($\Delta E^* < 1.5$).¹³ In the present study, the color of olives before packing was used as a reference ($L^* = 54.05$, $a^* = 4.69$, $b^* = 35.17$).

In addition, from the reflectance curve supplied by the apparatus, a color index (*i*) was obtained, as described elsewhere¹⁴:

$$i = (4R_{635} + R_{590} - 2R_{560})/3$$

where R_{635} , R_{590} , and R_{560} are the values of reflectance at 635, 590, and 560 nm, respectively. Olive color can be analytically classified as excellent ($30.2 < i < 33.6$), good ($26.8 < i < 30.2$), acceptable ($23.7 < i < 26.8$), bad ($21.0 < i < 23.7$), and very bad ($i < 21.0$).

Brine color was estimated by measuring the difference in absorbance at 440 and 700 nm, ($A_{440} - A_{700}$), as described elsewhere.¹⁵

Analysis of additives

The ascorbic acid in brine was analyzed using an HPLC method previously used in table olives.¹⁶ The HPLC system consisted of a Waters 2690 separations module connected to a Waters 996 photodiode array detector, controlled with Millennium 32

software (Waters, Milford, MA, USA). The separation was performed on a Luna 5 μ C18(2) (250 x 4.6 mm i.d.) column (Phenomenex, Torrance, CA, USA) using deionized water (adjusted to pH 2.3 with orthophosphoric acid) as the mobile phase at a flow rate of 1.0 mL min⁻¹ at ambient temperature. Ascorbic acid was monitored at 245 nm. Its identification in samples was based on the retention time and absorption spectrum. Sorbic and benzoic acids in brine were analyzed by HPLC using the same HPLC system and column as above mentioned for ascorbic acid, except that a phosphate buffer solution (0.03 mol L⁻¹, pH 6.7) was used as the mobile phase and detection was carried out at 230 nm.¹⁷

Physicochemical and microbiological analyses

The pH and titratable acidity of brines were measured using a Metrohm 670 Titroprocessor (Herisau, Switzerland). Titratable acidity was determined by titrating to pH 8.3 with 0.2 mol L⁻¹ NaOH and expressed as acetic acid. Sodium chloride was determined by titration with silver nitrate.¹⁸ The microbial population during storage was determined by plating the brines on the appropriate solid media, spreading 0.1 ml onto the surface and plating their decimal dilutions (in 1 g L⁻¹ peptone water) with a Spiral Plater (Don Whitley Sci. Ltd., Shipley, England). De Man, Rogosa, Sharpe (MRS) agar (Biokar Diagnostics, Beauvais, France) with and without sodium azide (0.2 g L⁻¹) was used for the lactic acid bacteria determination. Since most yeast can grow on MRS agar, sodium azide was added in order to prevent their growth. Oxytetracycline-glucose-yeast extract (OGYE) agar (Oxoid, Basingstoke, UK) was used for yeast determination. Plates were incubated at 32 °C (MRS) or 26 °C (OGYE) for up to 5 days. By using this methodology, both the absence of micro-organisms in pasteurized samples (detection limit 10 cfu mL⁻¹) and the count of colonies in unpasteurized samples were

determined, being the colonies enumerated using an automatic counter (Scan 500, Interscience, St Nom, France) when growth was observed.

Statistical data analysis

Analysis of variance (ANOVA) and regression analysis were performed with the Statistica software (version 7, Statsoft Inc., Tulsa, OK, USA). Significant differences were determined at the $p < 0.05$ level. The color changes both in olives and brine as well as the degradation of ascorbic acid during storage were fitted to different kinetic models. In the food science literature, quality changes are usually modeled by means of a zero- or first-order reaction, as pioneered by Labuza.¹⁹ A combined model or pseudo-first order kinetics has been also reported in different studies of non-enzymatic browning in foods.^{20,21} This justified that the above-mentioned kinetic models were selected in this work. The corresponding equations were the following:

$$\text{Zero-order: } C_t = C_0 - k_0 t$$

$$\text{First-order: } C_t = C_0 \exp(-k_1 t)$$

$$\text{Combined model: } C_t = K - (K - C_0) \exp(-k_1 t)$$

where C_t is the studied parameter (L^* , i , ΔE , A_{440} - A_{700} , ascorbic acid) at any given time t , C_0 is the value of the variable studied at $t=0$, k_0 is the zero-order rate constant, k_1 is the first-order rate constant, and K (i.e., k_0/k_1) expresses the maximum value that can be reached by the studied variable C for long storage times ($t \rightarrow \infty$). Data fitting was considered significant at a probability level of 95%.

Shelf life time (t_s) was estimated by determining the earliest time at which the 95% confidence limit for the mean curve intersects the proposed acceptance criterion.²²

RESULTS

The influence of factors such as pasteurization, plastic pouch, packing solution, and storage time on the pH, titratable acidity, and color parameters of packed table olives is shown in Table 1. Although all the effects were significant, pH and titratable acidity were hardly affected by the different factors. The most noticeable change in pH was due to the presence of preservatives, which resulted in slight increases (0.1-0.2 units) in comparison with packing solutions without preservatives (C and CA). Pasteurization resulted in significant decreases in pH, color parameters L^* and b^* , and color index i , which agrees with the results previously reported for Spanish-style green table olives packed in glass containers.²³ Despite this, the colors of both olives and brines of the pasteurized samples, on average, were considered acceptable ($i > 23.7$, $A_{440}-A_{700} < 0.23$) based on correlations with the visual scales previously established.^{14,15} However, factor interactions must be considered, as most of them were significant according to ANOVA (Tables S1 and S2). For the sake of clarity, in the following sections, the results with the pasteurized samples will be presented separately from those obtained with the unpasteurized samples.

Microbiological analyses did not detect (the lower limit of detection was 1.3 log cfu mL⁻¹) a microbial population in any of the pasteurized samples, but LAB and/or yeasts were detected in some samples of the unpasteurized samples throughout storage time (Table 2). Yeasts were inhibited in all unpasteurized samples containing sorbate. Both LAB and yeasts were found in some of the unpasteurized samples containing benzoate as preservative (packing solutions CAB and CB). Contrary to LAB, a yeast population was always found in the unpasteurized samples without preservatives (packing solutions C and CA). This yeast population was always higher in pouch A than

in pouch B, indicating that the higher oxygen permeability of the former favored the growth of these microorganisms.

In the samples with preservatives, regardless of the type of pouch, in general the levels of sorbates and benzoates in both pasteurized and unpasteurized samples remained stable or hardly changed throughout the 195 days of storage (data not shown). On the contrary, as will be commented in the following section, the level of ascorbic acid changed during storage with different evolutions for each type of pouch.

Influence of the plastic containers on the color parameters of olives and brine both in the absence and presence of additives (pasteurized samples)

Of the color parameters, the most useful in the present study are the index i and the brine color ($A_{440}-A_{700}$), as they are well correlated with the visual scales.^{14,15} The index i correlated well with various expressions of color, but the best correlation was obtained with the parameter L^* .²³ The influence of studied variables (type of pouch, storage time, packing solution) and their interactions on color parameters (L^* , i , $A_{440}-A_{700}$) was evaluated by factorial ANOVA. This study revealed that all three variables and their interactions were highly significant ($p<0.001$ in general) for each color parameter (Table S3). The effect of type of pouch was the most pronounced followed by the effect of storage time, as revealed by their corresponding F values.

The interaction type of pouch*storage time*packing solution for the above-mentioned color parameters is graphically shown in Figure 2. In pouch A, the lightness (L^* parameter), regardless of the packing solution used, significantly decreased with storage time whereas L^* remained unchanged or decreased slightly in pouch B. Since L^* is a measure of color on the light-dark axis, the decreasing evolution in pouch A indicates that the samples were turning darker. This evolution was fitted to three kinetic

models (zero-order, first-order, and combined model) (Table S4). For most samples, the combined model was found to provide the best fit based on the examination of the coefficient of determination (R^2). However, the kinetic parameter k_1 in this model was not significant ($p > 0.05$) for packing solutions B and SB. Therefore, the first-order kinetic model was used for comparison purposes. The corresponding rate constant k_1 hardly changed between packing solutions ranging between 0.76-1.19 day⁻¹. The presence of ascorbic acid positively affected the lightness compared to the corresponding sample without ascorbic acid, and were more pronounced in the olives packed in pouch B compared to pouch A (Figure S1a). Contrary to ascorbic acid, the presence of preservatives (sorbate, benzoate, or a mix of both, corresponding to packing solutions S, B, or SB) had no significant effect on L^* (pouches B) or produced a slight decrease in lightness (pouches A) in comparison with the control C (Figure S1a).

With regard to the color index i , a regression analysis showed that the values of i had a highly significant correlation ($R = 0.875$, $p < 0.001$) with the values of L^* , which agrees with the R value previously obtained in glass containers.²³ As occurred with L^* , the olives packed in pouch B had higher values of i than those packed in pouch A. However, differences in i values between the two types of pouches were small or insignificant in packing solutions C, S, B, and SB (Figure S1b). Again, the samples with ascorbic acid (packing solutions A, AS, AB, and ASB) in pouch B resulted in higher values of i compared to the corresponding sample without ascorbic acid (packing solutions C, S, B, and SB), but this effect was not apparent in pouch A. As occurred with the parameter L^* , the packing solutions with preservatives S, B, or SB had no significant effect or slightly decreased the i values in both types of pouches in comparison with the control C (Figure S1b).

The above observations concerning the samples with ascorbic acid can be explained by the different evolution of this acid in pouches A and B (Figure 3). Thus, the ascorbic acid level fell rapidly in pasteurized pouches A (ascorbic acid was not detected after 50 days of storage in any case), whereas in pouch B the ascorbic acid degraded slowly. In these cases, with the exception of packing solution AB, where ascorbic acid degradation was minimal and data did not fit well to zero or first kinetic models, ascorbic acid was degraded at the same rate and this rate was not significantly different from the degradation rate in glass bottles (Table S5).

It can be noted that, at the end of storage, only the ascorbic acid-containing samples in pouch B showed olive colors classified as “good” based on the known correlation of index i with a visual scale¹⁴ (Figure 2b). The evolution of this parameter during storage in both types of pouches was fitted to zero-order and first-order kinetics, and the first order model was found to explain the variation of i with time better than a zero order model (Table 3). The values of k_1 were not significantly different among packing solutions in the case of pouch A ($k_1 = 1.7 \text{ day}^{-1}$, on average). However, poor fits ($R^2 = 0.362\text{-}0.546$) were obtained for packing solutions A, AB, and ASB in pouch B, due to the fact that the values of i appeared to reach equilibrium after 85 days (packing solutions AB and ASB) or 120 days (packing solution A) as shown in Figure 2b.

The estimation of shelf life time (t_s) was carried out taking into account the limit of $i = 23.7$, below which the olive color is considered unacceptable¹³, for the different packing conditions based on the first-order kinetic model (Table 3). In pouch A, shelf lives ranged from 89 to 128 days, whereas in pouch B they ranged from 142 to 288 days (excluding packing solutions A, AB, and ASB). The addition of ascorbic acid in pouch B appeared to be effective in prolonging the shelf life of the product. The positive

effect of ascorbic acid on olive color both in the absence and presence of preservatives was previously demonstrated in packed Spanish-style green table olives.^{8,9} Ascorbic acid could slow down the chemical oxidation of phenolic compounds and consequently would help to increase the shelf life of the product.

Regarding brine color, the olives packed in pouch A had darker brine colors (higher values of A_{440} - A_{700}) than those in pouch B (Figure 2c and Figure S1c). In general, this parameter remained unchanged during storage in packing B with values lower than 0.15 AU, which is below the limit of 0.23 AU above which the brine color is considered unacceptable.¹⁵ **However, it increased progressively during storage time in pouch A exceeding much of the above mentioned limit in all cases. A combined kinetic model based on a two-stage mechanism²¹ was found to provide the best fit regardless of the packing solution (Table 4). No significant differences ($p>0.05$) were obtained for the kinetic parameters k_1 and K between the different packing solutions, but differences were found for the parameter C_0 . Based on this model, the shelf life times (t_s) for olives pasteurized and stored in pouch A were estimated taking into account the acceptance limit of A_{440} - $A_{700} = 0.23$ AU (Table 4). These times ranged between 16 days (packing solution B) and 40 days (packing solution A), which were shorter than those mentioned above based on the color index i . Therefore, in the case of pouch A the primary parameter to establish the shelf life of the product should be the brine color, whereas in pouch B it should be the color index of olives.**

One of the best parameters for describing color variation is the total color difference ΔE^* , since it is a combination of the parameters L^* , a^* and b^* . In pouch A, all packing solutions showed a significant increases in ΔE^* with storage time and high values ($\Delta E^* = 15$ -18) were reached at the end of storage (Figure 4). In pouch B, samples with ascorbic acid (packing solutions A, AS, AB, and ASB) showed relatively

low values of ΔE^* ($\Delta E^* < 2$), which did not significantly change during storage; the remaining samples showed higher values from the beginning of storage ($\Delta E^* > 2$) reaching values between 5 and 7 at the end of storage. For this color parameter, a combined kinetic model provided better fit than zero-order and first-order models (Table 5). No significant differences in the parameter K among packing solutions was found (mean value of K was 19) but some significant differences were obtained for the kinetic parameters C_0 and k_1 .

Comparison between pasteurized and non-pasteurized samples

The interaction type of pouch*storage time*packing solution for the color parameters L^* , i , and $A_{440}-A_{700}$ in the non-pasteurized samples is shown in Figure 5. The most noticeable differences in comparison with the pasteurized samples (Figure 2) was that both L^* and i values of olives packed with packing solution C or A hardly changed during storage in both types of pouches (Figure 5a and 5b). Another important observation compared to the pasteurized pouches was that olives packed with packing solution AB or B in pouch A showed higher values of L^* and i throughout storage.

With regard to brine color, the most noticeable observation was that, in pouch A, the parameter $A_{440}-A_{700}$ remained unchanged during storage time in samples C, A, and B (Figure 5c), while it increased considerably in the corresponding pasteurized samples. In addition, the other samples showed appreciable browning during storage, but the evolution of this parameter and values reached at the end of storage were lower than those showed by the pasteurized samples. Thus, the brine color in sample AS or ASB appeared to reach equilibrium after 50 days of storage.

Regarding ascorbic acid in the unpasteurized samples, the most noticeable observation in pouch A was that ascorbic acid degraded more slowly, mainly in sample

A, compared to the pasteurized product. This was also observed for sample A in pouch B, but the differences between pasteurized and unpasteurized samples for the other samples were not so clear (Figure 3, Table S5).

With regards to ΔE^* in the non-pasteurized samples (Figure 4), in pouch A, the highest values of ΔE^* were found in the sorbate-containing samples, followed by samples B and AB. Samples C and A in pouch A showed the lowest values of ΔE^* and hardly changed during storage, which is consistent with the evolutions of other color parameters as mentioned above. The main observation in pouch B was that ΔE^* hardly changed in sample C during storage whereas it significantly increased in the corresponding pasteurized sample.

DISCUSSION

From the above-mentioned results, it can be deduced that pasteurization in pouch A produced color changes (browning) which drastically decreased the shelf life of the product. Oxygen entering through the plastic material could oxidize polyphenols from olives, mainly hydroxytyrosol, which is the major phenolic compound in the olives after fermentation²⁴, forming a quinone or quinone-like compound that polymerizes to form brown pigments in a similar way to the mechanism proposed in the browning of California-style black ripe olives²⁵ or in the non-enzymatic oxidation of wines.²⁶ This did not occur in pouch B due to their low oxygen permeability. Therefore, this type of pouch is recommended in the case of pasteurizing the product.

The fact that sample C (control without additives) and sample A (only ascorbic acid added) in the unpasteurized samples in pouch A did not show any darkening during storage can be explained by the presence of microorganisms from olive fermentation,

which consumed the oxygen entering through the plastic material, thereby preventing the oxidation of phenolic compounds leading to brown pigments. This explanation is supported by the results of the microbiological analyses shown in Table 2. Thus, the presence of a high population of yeasts in samples C and A would explain the absence of browning in these unpasteurized samples and the good maintenance of both fruit color and brine color as demonstrated by the evolution of the corresponding color parameters. This is consistent with the presence of high levels of ascorbic acid in sample A throughout storage as above mentioned. On the contrary, the absence of microorganisms in the sorbate-containing samples would explain the browning along with the rapid oxidation of ascorbic acid in pouch A. Apparently, in the benzoate-containing samples the yeast population, which was lower than that in the samples without preservatives, was not sufficient to prevent color deteriorations or the total degradation of ascorbic acid due to oxygen; but some beneficial effect on color was produced in comparison with the sorbate-containing samples or with the corresponding pasteurized samples as mentioned above. However, some influence of the LAB population should not be ruled out.

In order to confirm the above-mentioned effect of microorganisms on the color stability in plastic pouches an inoculation experiment was carried out, as indicated in the Materials and Methods section. For this experiment, pouch A was used and the olives were packed without any additive. Again, no microbial population was found in the pasteurized samples PC (pasteurized control) and IC (injection control). The unpasteurized control (NPC) showed a stable population of yeasts, but not of LAB, whereas the sample pasteurized and then inoculated (IP) showed yeasts together with a decreasing population of LAB (data not shown). The evolution of color parameters

during storage clearly showed that the presence of microorganisms favorably affected the color stability of Spanish-style green olives in plastic pouches A (Figure S2).

CONCLUSIONS

The type of plastic material had a great impact on the color stability of Spanish-style green table olives which were pasteurized and stored in plastic pouches. The present study demonstrated that in pasteurizable plastic pouches the color parameter for fixing shelf life is dependent on the type of plastic material used. In pouches made of PET + MDPE/EVOH, with oxygen permeability $\approx 3 \text{ cm}^3 \text{ m}^{-2} \text{ day}^{-1}$, this parameter should be the brine color ($A_{440}-A_{700}$) whereas in AlOx-coated PET + MDPE pouches, with lower oxygen permeability, the color index i of olives should be used (as in glass containers). It can be concluded that PET + MDPE/EVOH is not a good material for the pasteurization and storage of Spanish-style green table olives due to the fact that the product's shelf life is too short (< 6 weeks at 30°C), but it would be appropriate for unpasteurized product in the absence of preservatives because oxidation reactions are prevented by microorganisms (at least for 6.5 months at 30°C). On the other hand, the obtained results suggest that, from the standpoint of color quality, the use of AlOx-coated PET + MDPE pouches could be an alternative to glass packaging for the pasteurization and storage of Spanish-style green olives, especially if ascorbic acid is added to the packing solution (shelf life > 6.5 months at 30°C). However, further studies are needed for a more accurate evaluation of the shelf life of product in this type of plastic container.

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540

FIGURE CAPTIONS

Figure 1. Experimental design for the study of color changes in Spanish-style green table olives pasteurized and stored in two distinct plastic pouches using different packing solutions. C, control without additives; A, ascorbic acid; AS, ascorbic acid + potassium sorbate; S, potassium sorbate; AB, ascorbic acid + sodium benzoate; B, sodium benzoate; ASB, ascorbic acid + potassium sorbate + sodium benzoate; SB, potassium sorbate + sodium benzoate.

Figure 2. Changes in (a) parameter L^* , (b) color index i , and (c) brine color during storage of Spanish-style green table olives pasteurized in two distinct plastic pouches using different packing solutions.

Figure 3. Changes in concentration of ascorbic acid added as an antioxidant during storage of Spanish-style green table olives pasteurized in two distinct plastic pouches in comparison with the corresponding unpasteurized product.

Figure 4. Evolution of total color difference (ΔE^*) during storage of Spanish-style green table olives pasteurized in two distinct plastic pouches compared with the corresponding unpasteurized product.

Figure 5. Changes in (a) parameter L^* , (b) color index i , and (c) brine color during storage of Spanish-style green table olives packed in two distinct plastic pouches, without pasteurization, using different packing solutions.

Supporting Information

Table S1: Factorial ANOVA for different color parameters of Spanish-style green table olives packed in plastic pouches.

Table S2: Factorial ANOVA for pH and titratable acidity (TA) of Spanish-style green table olives packed in plastic pouches.

Table S3: Factorial ANOVA for different color parameters of pasteurized packed green table olives.

Table S4: Results of zero-order, first order, and combined kinetic modelling of lightness (L^*) for olives packed in pouches A (pasteurized samples).

Table S5: Results of zero-order and first order kinetic modelling of ascorbic acid degradation in pasteurized and unpasteurized packed green table olives.

Figure S1: Effect of the interaction type of pouch*packing solution on color parameters of Spanish-style green table olives pasteurized and stored in plastic pouches.

Figure S2: Effect of inoculation with microorganisms from fermenting olives on color parameters in pasteurized pouches A.

Table 1. Main effects of pasteurization, type of plastic pouch, packing solution, and storage time on physicochemical characteristics and color parameters of packed Spanish-style green table olives^a

Main effects		pH	TA (g L ⁻¹) ^b	L*	a*	b*	Color index (i) ^c	Color brine (A ₄₄₀ -A ₇₀₀)
Pasteurization treatment								
	yes	3.45a	5.2b	49.9a	5.4b	30.6a	25.9a	0.188b
	no	3.48b	5.1a	52.2b	4.8a	33.3b	27.1b	0.148a
Type of pouch								
	A	3.47a	5.2b	49.0a	5.6b	29.4a	25.6a	0.217b
	B	3.46a	5.1a	53.0b	4.6a	34.4b	27.4b	0.119a
Packing solution								
	C	3.31a	5.2b	51.5c	5.0ab	32.5b	26.9bc	0.142a
	A	3.32a	5.3c	53.1d	5.0ab	34.9c	28.6d	0.133a
	AS	3.56d	5.1b	51.7c	5.0ab	32.9b	27.1bc	0.179bc
	S	3.56d	5.1b	49.1a	5.4c	29.4a	25.1a	0.189c
	AB	3.41b	5.3c	52.0c	5.0ab	33.3b	27.4c	0.170bc
	B	3.46c	5.1b	50.1b	4.9a	30.3a	25.3a	0.166b
	ASB	3.54d	5.2b	51.2c	5.0ab	32.3b	26.5b	0.185c
	SB	3.55d	4.9a	49.5ab	5.3bc	29.6a	25.3a	0.179bc
Storage time (days)								
	14	3.49b	5.1a	53.1e	5.1a	34.0d	29.3e	0.130a
	50	3.46b	5.2b	51.7d	5.2a	32.5c	27.5d	0.159b
	85	3.47b	5.1a	51.0c	5.0a	32.0c	26.3c	0.166b
	120	3.48b	5.2b	50.2b	5.1a	31.1b	25.4b	0.188c
	195	3.42a	5.2b	49.1a	5.0a	30.0a	24.0a	0.197c

^a Means with different letters within a column for each effect are significantly different ($p < 0.05$). ^b TA, titratable acidity expressed as lactic acid. ^c $i = (4R_{635} + R_{590} - 2R_{560})/3$, where R_{635} , R_{590} , and R_{560} are the values of reflectance at 635, 590, and 560 nm, respectively.

Table 2. Populations (log cfu mL⁻¹) of lactic acid bacteria (LAB) and yeast (Y) in brine of unpasteurized Spanish-style green table olives packed in pouches A and B during storage

Storage time (days)	Packing solution ^a	Pouches A		Pouches B	
		LAB	Y	LAB	Y
14	C	- ^b	4.52	-	4.37
	A	-	4.63	-	4.22
	AS	-	-	-	-
	S	-	-	-	-
	AB	-	3.49	-	2.67
	B	-	4.05	-	2.24
	ASB	-	-	-	-
	SB	-	-	-	-
50	C	-	4.84	-	3.87
	A	-	4.75	-	3.85
	AS	-	-	-	-
	S	-	-	-	-
	AB	2.20	3.60	-	1.48
	B	-	4.17	-	2.20
	ASB	-	-	-	-
	SB	-	-	-	-
85	C	-	4.93	-	3.86
	A	-	4.93	-	3.67
	AS	-	-	5.95	-
	S	-	-	-	-
	AB	3.69	3.65	-	2.08
	B	5.00	3.18	-	-
	ASB	-	-	-	-
	SB	-	-	-	-
120	C	-	5.00	-	3.92
	A	-	5.08	-	3.88
	AS	-	-	-	-
	S	-	-	-	-
	AB	-	3.27	-	-
	B	4.30	2.15	-	1.60
	ASB	-	-	-	-
	SB	-	-	-	-
195	C	-	5.02	-	4.38
	A	-	4.96	-	3.97
	AS	-	-	4.49	-
	S	-	-	-	-
	AB	-	4.56	-	-
	B	5.28	4.32	-	-
	ASB	-	-	-	-
	SB	-	-	2.60	-

^a See text for meaning of acronyms. ^b -, not detected (log cfu mL⁻¹ < 1.3).

Table 3. Results of zero-order and first order kinetic modelling of color index *i* for olives pasteurized and stored in pouches A and B

POUCHES A							
Packing solution	Zero order ^a			First order ^b			
	C ₀ ±SE	(k ₀ ±SE) x 10 ³ (day ⁻¹)	R ²	C ₀ ±SE	(k ₁ ±SE) x 10 ³ (day ⁻¹)	R ²	t _s ^c (days)
C	29.2 ± 0.4	40.6 ± 3.3	0.925	29.5± 0.4b	1.6± 0.1a	0.930	128
A	29.7 ± 0.6	47.8± 5.3	0.874	30.2± 0.6b	2.0± 0.2a	0.896	116
AS	29.4 ± 0.9	45.9± 7.7	0.747	29.9± 0.9b	2.0± 0.3a	0.778	113
S	27.8 ± 0.5	35.6± 4.5	0.838	28.1± 0.5ab	1.5± 0.2a	0.855	109
AB	28.6 ± 1.0	43.0± 8.7	0.671	29.1± 1.0ab	1.9± 0.4a	0.706	105
B	26.9 ± 0.5	31.6± 4.5	0.806	27.1± 0.5a	1.3± 0.2a	0.804	101
ASB	27.4 ± 0.9	41.5± 7.6	0.714	27.9± 0.9ab	1.9± 0.3a	0.755	89
SB	28.1 ± 0.4	36.2± 3.9	0.880	28.3± 0.5ab	1.5± 0.2a	0.876	115
POUCHES B							
Packing solution	Zero order ^a			First order ^b			
	C ₀ ±SE	(k ₀ ±SE) x 10 ³ (day ⁻¹)	R ²	C ₀ ±SE	(k ₁ ±SE) x 10 ³ (day ⁻¹)	R ²	t _s ^c (days)
C	29.5±0.6	32.4±4.9	0.781	29.7±0.6ab	1.3±0.2ab	0.795	159
A	30.9±0.6	17.7±5.1	0.501	30.9±0.6b	0.6±0.2a	0.502	ne
AS	30.9±0.5	21.0±4.0	0.697	30.9±0.5b	0.7±0.1a	0.700	288
S	28.8±0.6	32.5±5.4	0.751	28.9±0.7ab	1.3±0.2ab	0.745	142
AB	29.9±0.6	14.5±5.7	0.353	29.9±0.7ab	0.5±0.2a	0.362	ne
B	27.9±0.3	26.8±2.5	0.906	28.0±0.3a	1.1±0.1ab	0.914	147
ASB	29.6±0.5	17.9±4.8	0.537	29.7±0.6ab	0.7±0.2a	0.546	ne
SB	28.7±0.4	32.9±3.6	0.866	28.9±0.4a	1.3±0.1b	0.874	142
ASB (glass)	31.2±0.8	38.1±6.7	0.731	31.5±0.8	1.4±0.2	0.738	172

^a Modeled using $C_t = C_0 - k_0 t$. ^b Modeled using $C_t = C_0 \exp(-k_1 t)$. Means with different letters within a column (kinetic parameter) are significantly different ($p < 0.05$). ^c Shelf life time (t_s) was estimated by determining the earliest time at which the 95% confidence limit for the mean curve intersects the acceptance limit of color index *i* (23.7); ne, not estimated because of their low R².

Table 4. Results of zero-order, first order, and combined kinetic modelling of brine browning (A_{440} - A_{700}) for olives pasteurized and stored in pouches A

Packing solution	Zero order ^a			First order ^b			Combined model ^c				
	$C_0 \pm SE$	$(k_0 \pm SE) \times 10^3 \text{ (day}^{-1}\text{)}$	R^2	$C_0 \pm SE$	$(k_1 \pm SE) \times 10^3 \text{ (day}^{-1}\text{)}$	R^2	$C_0 \pm SE$	$(k_1 \pm SE) \times 10^3 \text{ (day}^{-1}\text{)}$	$K \pm SE$	R^2	$t_s^d \text{ (days)}$
C	0.15±0.01	-1.0±0.09	0.900	0.17±0.01	-3.5±0.4	0.843	0.11±0.01a	10.8±1.8a	0.35±0.02a	0.976	34
A	0.14±0.01	-0.9±0.08	0.900	0.15±0.01	-3.6±0.4	0.843	0.10±0.01a	9.8±2.0a	0.33±0.02a	0.968	40
AS	0.16±0.01	-0.9±0.10	0.864	0.18±0.01	-3.4±0.5	0.809	0.12±0.02ab	11.0±2.9a	0.36±0.02a	0.942	21
S	0.20±0.01	-0.9±0.08	0.911	0.21±0.01	-3.0±0.3	0.862	0.16±0.01b	9.1±1.7a	0.40±0.02a	0.978	17
AB	0.18±0.01	-0.8±0.08	0.900	0.19±0.01	-2.9±0.3	0.851	0.14±0.01ab	10.2±1.8a	0.35±0.02a	0.977	26
B	0.19±0.01	-0.8±0.08	0.898	0.21±0.01	-2.8±0.3	0.851	0.16±0.01b	9.0±2.1a	0.38±0.02a	0.966	16
ASB	0.19±0.01	-0.9±0.08	0.904	0.21±0.01	-3.0±0.3	0.856	0.15±0.01b	9.3±2.0a	0.39±0.02a	0.969	17
SB	0.16±0.01	-1.0±0.11	0.881	0.18±0.01	-3.6±0.5	0.826	0.11±0.01a	11.2±2.4a	0.37±0.02a	0.957	22

^a Modeled using $C_t = C_0 - k_0 t$. ^b Modeled using $C_t = C_0 \exp(-k_1 t)$. ^c Modeled using $C_t = K - (K - C_0) \exp(-k_1 t)$. Means with different letters within a column (kinetic parameter) are significantly different ($p < 0.05$). ^d Shelf life time (t_s) was estimated by determining the earliest time at which the 95% confidence limit for the mean curve intersects the acceptance limit of A_{440} - A_{700} (0.23 AU).

Table 5. Results of zero-order and first-order kinetic models of color difference (ΔE^*) for olives pasteurized and stored in pouches A

Packing solution	Zero order ^a			First order ^b			Combined model ^c			
	$C_0 \pm SE$	$(k_0 \pm SE) \times 10^3 \text{ (day}^{-1}\text{)}$	R^2	$C_0 \pm SE$	$(k_1 \pm SE) \times 10^3 \text{ (day}^{-1}\text{)}$	R^2	$C_0 \pm SE$	$(k_1 \pm SE) \times 10^3 \text{ (day}^{-1}\text{)}$	$K \pm SE$	R^2
C	4.5±0.6	-66.9±5.1	0.934	6.2±0.6	-5.4±0.6	0.869	2.9±0.8b	6.3±2.2a	22.6±3.8a	0.962
A	1.7±0.7	-74.7±6.4	0.919	4.1±0.7	-6.9±1.1	0.808	-0.8±0.9 a (ns)	8.1±2.1a	19.4±2.6a	0.967
AS	2.4±1.0	-74.5±9.0	0.850	4.9±1.0	-6.2±1.2	0.724	-2.5±0.9a	14.8±2.2ab	16.2±0.9a	0.976
S	7.2±0.7	-55.6±6.0	0.874	8.3±0.7	-3.9±0.5	0.806	4.2±0.7bc	12.3±2.3ab	18.2±1.0a	0.968
AB	2.8±1.3	-75.3±11.2	0.788	5.3±1.2	-5.9±1.4	0.657	-4.0±1.2a	19.3±3.0b	15.7±0.8a	0.969
B	7.7±0.5	-53.2±4.1	0.932	8.7±0.5	-3.8±0.4	0.887	6.5±0.6c	6.3±2.2a	22.3±3.1a	0.962
ASB	3.6±1.2	-73.9±10.4	0.807	5.6±1.1	-5.6±1.2	0.684	-2.3±1.0a	17.5±2.6b	16.7±0.8a	0.973
SB	6.7±0.6	-59.5±5.2	0.915	7.9±0.6	-4.3±0.5	0.860	4.7±0.8bc	8.3±2.4ab	20.5±2.2a	0.958

^a Modeled using $C_t = C_0 - k_0 t$. ^b Modeled using $C_t = C_0 \exp(-k_1 t)$. ^c Modeled using $C_t = K - (K - C_0) \exp(-k_1 t)$. Means with different letters within a column (kinetic parameter) are significantly different ($p < 0.05$). ns, no significant at 95% probability level.

(a)

(b)

(c)

Table S1. Factorial ANOVA for different color parameters of Spanish-style green table olives packed in plastic pouches

Effect	L*		a*		b*		Color index (<i>i</i>)		Brine color (A ₄₄₀ -A ₇₀₀)	
	F	p	F	p	F	p	F	p	F	p
Storage time (1)	350	0.000000	7.7	0.000006	220.0	0.000000	313.0	0.000000	702.6	0.000000
Type of pouch (2)	2850	0.000000	2111.4	0.000000	2858.8	0.000000	310.5	0.000000	12143.3	0.000000
Treatment (3)	161	0.000000	27.6	0.000000	213.7	0.000000	64.9	0.000000	265.2	0.000000
Pasteurization (4)	919	0.000000	621.8	0.000000	819.8	0.000000	113.6	0.000000	2060.0	0.000000
(1)*(2)	175	0.000000	143.4	0.000000	189.4	0.000000	17.4	0.000000	454.7	0.000000
(1)*(3)	4	0.000000	4.5	0.000000	2.8	0.000008	2.0	0.002370	8.8	0.000000
(2)*(3)	25	0.000000	101.4	0.000000	22.7	0.000000	18.5	0.000000	117.2	0.000000
(1)*(4)	23	0.000000	3.9	0.003965	19.5	0.000000	6.2	0.000083	87.8	0.000000
(2)*(4)	355	0.000000	199.8	0.000000	372.9	0.000000	43.8	0.000000	1735.7	0.000000
(3)*(4)	40	0.000000	41.4	0.000000	37.9	0.000000	10.1	0.000000	118.0	0.000000
(1)*(2)*(3)	4	0.000000	3.5	0.000000	4.5	0.000000	2.8	0.000008	6.8	0.000000
(1)*(2)*(4)	21	0.000000	18.3	0.000000	22.4	0.000000	2.6	0.037251	100.1	0.000000
(1)*(3)*(4)	5	0.000000	2.7	0.000017	4.0	0.000000	2.8	0.000007	6.8	0.000000
(2)*(3)*(4)	13	0.000000	10.1	0.000000	16.8	0.000000	4.7	0.000047	39.0	0.000000

(1)*(2)*(3)*(4)	3	0.000000	1.3	0.150147	3.1	0.000001	1.7	0.020613	4.0	0.000000
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Table S2. Factorial ANOVA for pH and titratable acidity (TA) of Spanish-style green table olives packed in plastic pouches

Effect	pH		TA	
	F	p	F	p
Storage time (1)	350	0.000000	7.7	0.000006
Type of pouch (2)	2850	0.000000	2111.4	0.000000
Treatment (3)	161	0.000000	27.6	0.000000
Pasteurization (4)	919	0.000000	621.8	0.000000
(1)*(2)	175	0.000000	143.4	0.000000
(1)*(3)	4	0.000000	4.5	0.000000
(2)*(3)	25	0.000000	101.4	0.000000
(1)*(4)	23	0.000000	3.9	0.003965
(2)*(4)	355	0.000000	199.8	0.000000
(3)*(4)	40	0.000000	41.4	0.000000
(1)*(2)*(3)	4	0.000000	3.5	0.000000
(1)*(2)*(4)	21	0.000000	18.3	0.000000
(1)*(3)*(4)	5	0.000000	2.7	0.000017
(2)*(3)*(4)	13	0.000000	10.1	0.000000
(1)*(2)*(3)*(4)	3	0.000000	1.3	0.150147

Table S3. Factorial ANOVA for different color parameters of pasteurized packed green table olives

Effect	L*		Color index (i)		Brine color (A ₄₄₀ -A ₇₀₀)	
	F	p	F	p	F	p
Storage time (1)	306.0	0.000000	256.1	0.000000	476.74	0.000000
Type of pouch (2)	2905.1	0.000000	372.4	0.000000	8676.19	0.000000
Treatment (3)	64.6	0.000000	23.5	0.000000	30.35	0.000000
(1)*(2)	174.9	0.000000	16.8	0.000000	363.54	0.000000
(1)*(3)	3.0	0.000011	2.4	0.000387	2.16	0.001527
(2)*(3)	6.2	0.000002	16.6	0.000000	13.31	0.000000
(1)*(2)*(3)	3.1	0.000007	2.4	0.000406	1.98	0.004581

Table S4. Results of zero-order, first order, and combined kinetic modelling of lightness (L^*) for Spanish-style green table olives packed in pouches A (pasteurized samples)

Packing solution	Zero order ^a			First order ^b			Combined model ^c			
	$C_0 \pm SE$	$(k_0 \pm SE) \times 10^3 \text{ (day}^{-1}\text{)}$	R^2	$C_0 \pm SE$	$(k_1 \pm SE) \times 10^3 \text{ (day}^{-1}\text{)}$	R^2	$C_0 \pm SE$	$(k_1 \pm SE) \times 10^3 \text{ (day}^{-1}\text{)}$	$K \pm SE$	R^2
C	51.30±0.38	44.1±3.3	0.937	51.47±0.37b	0.95±0.07ab	0.944	52.35±0.51	6.1±2.2	39.1±2.7	0.963
A	53.81±0.66	55.7±5.8	0.884	54.14±0.65c	1.19±0.12b	0.900	56.40±0.76	11.0±2.5	42.3±1.3	0.962
AS	53.12±0.80	53.2±7.0	0.827	53.45±0.79c	1.15±0.14ab	0.847	56.92±0.76	15.9±2.8	43.5±0.7	0.966
S	49.63±0.45	37.1±4.0	0.877	49.79±0.44a	0.82±0.08ab	0.889	51.49±0.49	11.6±2.4	42.1±0.7	0.966
AB	52.51±0.92	49.7±8.2	0.756	52.83±0.93bc	1.08±0.17ab	0.777	57.60±1.05	21.6±3.9	44.2±0.5	0.957
B	49.27±0.37	34.6±3.3	0.899	49.38±0.39a	0.76±0.07a	0.903	49.69±0.61	ns	34.9±11.3	0.908
ASB	51.77±0.77	50.7±6.8	0.824	52.09±0.76bc	1.12±0.14ab	0.844	55.60±0.65	16.6±2.3	42.7±0.5	0.977
SB	50.14±0.30	41.2±2.7	0.952	50.28±0.30ab	0.90±0.06ab	0.955	50.60±0.47	ns	32.1±10.3	0.959

^a Modeled using $C_t = C_0 - k_0 t$. ^b Modeled using $C_t = C_0 \exp(-k_1 t)$. ^c Modeled using $C_t = K - (K - C_0) \exp(-k_1 t)$. ns = no significant at 95% probability level

Table S5. Results of zero-order and first order kinetic modelling of ascorbic acid degradation in pasteurized and unpasteurized packed Spanish-style green table olives

PASTEURIZED SAMPLES ^a						
Packing solution	Zero order			First order		
	$C_0 \pm SE$	$(k_0 \pm SE)$ (mg L ⁻¹ day ⁻¹)	R^2	$C_0 \pm SE$	$(k_1 \pm SE) \times 10^3$ (day ⁻¹)	R^2
A (pouches B)	298±18	0.8±0.2	0.649	313±21	3.9±0.8	0.684
AS (pouches B)	303±9	0.7±0.1	0.859	312±10	3.1±0.4	0.868
AB (pouches B)	250±15	0.4±0.1	0.368	252±17	1.7±0.7	0.365
ASB (pouches B)	307±12	0.9±0.1	0.831	320±14	4.0±0.5	0.838
ASB (glass)	282±16	0.6±0.1	0.557	293±18	2.8±0.7	0.600

UNPASTEURIZED SAMPLES ^b						
Packing solution	Zero order			First order		
	$C_0 \pm SE$	$(k_0 \pm SE)$ (mg L ⁻¹ day ⁻¹)	R^2	$C_0 \pm SE$	$(k_1 \pm SE) \times 10^3$ (day ⁻¹)	R^2
A (pouches A)	340±8	0.6±0.1	0.855	347±8	2.2±0.2	0.885
A (pouches B)	351±4	0.4±0.0	0.924	354±4	1.4±0.1	0.937
AS (pouches B)	261±11	0.6±0.1	0.785	265±14	2.9±0.6	0.754
AB (pouches A)	192±29	1.3±0.3	0.648	390±21	30±2	0.979
AB (pouches B)	290±8	0.7±0.1	0.906	297±10	3.3±0.4	0.890
ASB (pouches B)	244±20	0.6±0.2	0.535	257±23	3.9±1.1	0.576
ASB (glass)	250±40	1.5±0.4	0.562	355±74	17±5	0.633

^aDegradations not modelled in pouches A due to rapid disappearance of ascorbic acid. ^bDegradations not modelled in samples AS (pouches A) and ASB (pouches A) due to rapid disappearance of ascorbic acid.

Figure S1. ANOVA study: Effect of the interaction type of pouch*packing solution on color parameters of Spanish-style green table olives pasteurized and stored in plastic pouches

Figure S2. Effect of inoculation with microorganisms from fermenting olives on color parameters in pasteurized pouches A. PC, pasteurized control; NPC, non-pasteurized control; IP, sample pasteurized and then inoculated; IC, injection control. Values are means $\pm$ SE