

# Effect of Packaging on Microbial Survival and Physicochemical Characteristics of Non-Thermally Preserved Green Spanish-Style Olives <sup>†</sup>

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**Abstract:** Spanish-style green olives are one of the main trade preparations in the international market. The purpose of this work was to investigate the effect of modified atmosphere packaging of Spanish-style green olives in multi-laminated pouches on the microbiological and physicochemical characteristics of olives. Green pitted olives of cv. Conservolea and Halkidiki were packaged in high barrier multi-laminated pouches under modified atmospheres and stored at room temperature for 12 months. Microbiological [lactic acid bacteria (LAB), yeasts, and *Enterobacteriaceae*] and physicochemical changes (pH, acidity, salt content, texture and color) were monitored throughout storage together with sensory assessment. Results showed that the initial microbiota consisted of LAB (5.9–6.3 log CFU/g) and yeasts (4.8–5.3 log CFU/g). Yeasts started to decline and could not be detected after 90 days of storage. In the end, LAB dominated in populations exceeding 5.4 and 6.1 log CFU/g for cv. Halkidiki and Conservolea, respectively. No *Enterobacteriaceae* could be detected in both olive varieties. In addition, pH values increased gradually from 3.51 to 4.19, the mean acidity was *ca.* 0.2% (expressed as lactic acid) and the salt content fluctuated between 2.0–4.4%. Color parameters *L\** (luminance), *a\** (greenness/redness) and *b\** (yellowness/blueness) did not change during storage in both varieties. Texture analysis showed a decrease in olive hardness from 15–20 N to 8 N throughout storage. Sensory analysis revealed that cv. Halkidiki olives were crisper with increased fibrousness compared to cv. Conservolea.

**Keywords:** green olives; modified atmosphere packaging; multi-laminated pouches; lactic acid bacteria; yeasts

## 1. Introduction

Table olives are one of the oldest and most popular fermented foods with significant economic impact in the Mediterranean countries [1]. Nowadays, there is an increasing trend to use plastic packaging due to reduced weight, lower costs, flexibility and convenience, while modified atmosphere packaging (MAP) has been used for several years to extend the shelf-life of packaged foods [2,3]. The aim of this study was to investigate the effect of modified atmosphere packaging of Spanish-style green olives in multi-laminated pouches on the microbiological, physicochemical and sensory characteristics of cvs. Halkidiki and Conservolea olives.

## 2. Materials and Methods

### 2.1. Olive Samples and Packaging Conditions

Samples of cv. Halkidiki and Conservolea pitted green olives processed by the Spanish-method were packaged in high barrier multi-laminated pouches (113  $\mu\text{m}$  thickness;  $\text{O}_2$  permeability  $< 1.5 \text{ mL/m}^2/24 \text{ h}$ ) under modified atmospheres (30%  $\text{CO}_2/70\% \text{ N}_2$ ) and stored at room temperature for 12 months. Sampling was repeated every 30 days for 12 months.

### 2.2. Microbiological Analyses

Microbiological analyses were undertaken in olive samples every 30 days to determine the population dynamics of total viable counts (TVCs), lactic acid bacteria (LAB), yeasts and *Enterobacteriaceae* as detailed elsewhere [4].

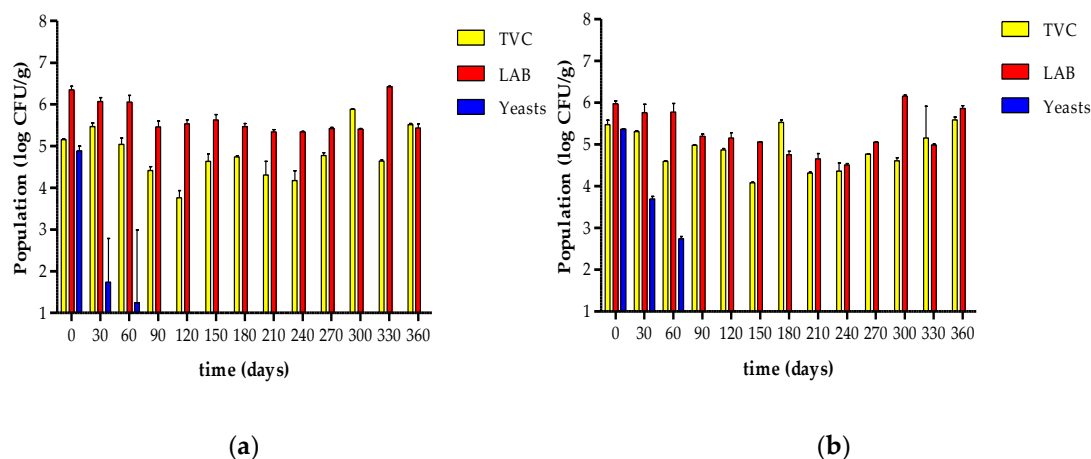
### 2.3. Physicochemical and Sensory Analyses

Additionally, pH, titratable acidity, salinity, color and texture measurements were undertaken at the same time intervals as for microbiological analyses. The pH of the brine was measured using a digital pH meter. Acidity and salinity were determined by titration and expressed as percentage ( $w/w$ ) of lactic acid and ( $w/w$ ) of NaCl, respectively [5]. The color changes on the surface of olive drupes were measured using a Minolta type chromatometer (Nonastru, 3 nh, NS800) based on  $L^*$  (lightness),  $a^*$  (redness/greenness), and  $b^*$  (yellowness/blueness) parameters [6]. Texture analysis was performed using a laboratory texturometer [5–7]. The sensory characteristics of packaged olives were evaluated on a monthly basis by a taste panel according to the protocol of the International Olive Council [8,9].

## 3. Results and Discussion

### 3.1. Microbiological Changes

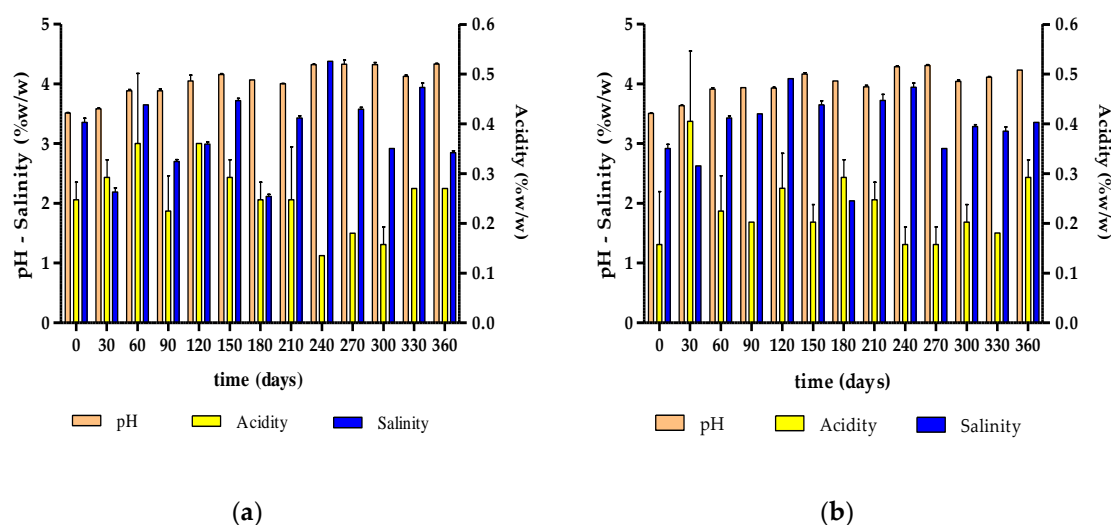
The evolution of the microbial consortia (TVC, LAB and yeasts) on olive drupes of cv. Halkidiki and Conservolea during storage in modified atmospheres is illustrated in Figure 1. *Enterobacteriaceae* could not be enumerated during storage in all samples. LAB were the dominant microbiota and ranged between 5.4–6.4 log CFU/g and 4.5–6.2 log CFU/g for cv. Halkidiki and Conservolea, respectively. Moreover, yeasts were detected within the first 60 days of storage in lower populations compared to LAB, reaching maximum counts of 4.9 log CFU/g and 5.4 log CFU/g in cv. Halkidiki and Conservolea, respectively. However, no yeasts could be detected on olives after 90 days of storage in both varieties. The evolution of the microbial consortia observed in this study is in good agreement with previous researchers who confirmed the dominance of LAB and the co-existence of yeasts at lower populations [4,10,11].



**Figure 1.** Changes in the population of total viable counts (TVC), lactic acid bacteria (LAB), and yeasts on olive drupes of cv. Halkidiki (a) and Conservolea (b) during modified atmosphere packaging in multi-laminated pouches. Data are average values of duplicate samples  $\pm$  standard deviation.

### 3.2. Physicochemical Changes and Sensory Analyses

During storage, titratable acidity, pH and salt content of the packaged pitted olives were monitored for 12 months and the results are presented in Figure 2. The values of pH increased gradually from 3.51 to 4.19, whereas the mean titratable acidity fluctuated between 0.2–0.4% (*w/w*, lactic acid) in both cultivars. The salt content varied between 2.0–4.4% (*w/w*, NaCl) in all samples. The abovementioned changes on acidity, pH and salinity are in line with previous publications on packaged table olives of different cultivars [4,6,11,12].



**Figure 2.** Changes in pH, salt content and titratable acidity during storage of packaged pitted green olives of cv. Halkidiki (a) and Conservolea (b). Data are average values of duplicate samples  $\pm$  standard deviation.

Color parameters  $L^*$ ,  $a^*$  and  $b^*$  did not change during storage in both varieties. No statistically significant differences could be established for  $L^*$  parameter corresponding to the brightness of olives. Additionally, the values of  $a^*$  and  $b^*$  parameters indicated the prevalence of green and yellow tonalities among samples regardless of table olive variety [13]. The color of olives has a major impact on the green table olive market and consequently high brightness values and the prevalence of vivid green tonality are desirable characteristics [14].

The force needed to penetrate the olive drupe was considered as a measure of the olive's hardness. The observed values varied according to table olive variety and storage time. Initially, the mean value of hardness was 15–20 N and after 360 days of storage it decreased to 8 N. According to previously published research, packaging in modified atmospheres or in aerobic conditions resulted in reduced firmness during storage [4]. Moreover, it was reported that vacuum-packed olives presented the lowest softening rate and hence the longest shelf-life followed by modified atmosphere and aerobic packaging [4].

During sensory assessment, the taste panel evaluated negative (e.g., abnormal fermentation), gustatory (salty, bitter and acid taste) and kinesthetic sensations (hardness, fibrousness, crunchiness). No perception of abnormal fermentation (zapateria, putrid, butyric) was noticed throughout storage. Furthermore, the majority of the sensory characteristics did not vary throughout storage in both varieties. According to the outcome of the sensory assessment, olive samples from cv. Halkidiki were bitter, acid, crisper and with increased fibrousness compared to cv. Conservolea. However, after 10 months of storage all olive samples received approximately the same score with no important

differences. Overall, all samples were characterized as “Extra” according to the protocol of sensory analysis of table olives established by the International Olive Council. The composition of the modified atmosphere is important since it affects the color and texture of olives, and consequently the judgement of the panelists [4].

## 5. Conclusions

Modified atmosphere packaging of Spanish-style green pitted olives in multi-laminated pouches revealed stability with minor changes in the microbiological and physicochemical characteristics of cv. Halkidiki and Conservolea olives during 12-month storage. From the microbiological perspective, LAB dominated the olive surface from the beginning of storage whereas no yeasts could be detected after 3 months of storage. The dominance of LAB on the olives throughout storage in populations exceeding 5.0 log CFU/g combined with the probiotic potential of this microbial group creates new perspective for the development of functional table olives. In addition, the absence of *Enterobacteriaceae* throughout packaging ensures the microbiological safety of the product. The changes in the physicochemical parameters were within the acceptable limits to ensure the stability of the product during storage.

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## Abbreviations

LAB	Lactic Acid Bacteria
TVC	Total Viable Counts
MAP	Modified Atmosphere Packaging

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