

# Use of a Combined Autochthonous Starter to Ferment Peranzana Alta Daunia Table Olives †

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**Abstract:** This study aims to evaluate the use of a multiple strain cocktail (*Lactiplantibacillus plantarum*, *Candida norvegica* and *Wickerhamomyces anomalus*) as starter culture for “Peranzana Alta Daunia” olives. The effect of the veraison degree of drupes (violet or green) and brine salt concentration (6 or 8%) on the acidification kinetic was analysed by using a 2<sup>k</sup> Factorial Design. The acidification was affected by all the variables tested with the verasoin degree as the most significant one: in fact, neither salt concentration nor the presence of the starter were able to improve the acidification kinetic when unripe fruits were used.

**Keywords:** starter; table olives; Peranzana; veraison

## 1. Introduction

Table olives represent one of the most popular fermented foods in the Mediterranean area with Spain, Greece and Italy being the major producing countries [1]. According to ISTAT [2], in 2022 Italy produced about 93.000 tons of table olives, with a total area devoted to this production of about 34.000 hectares. The most important Italian varieties for table olive production are, among others, Bella di Cerignola, Nocellara Etnea, Tonda di Cagliari, Giarrappa, Termite di Bitetto, Cellina di Nardò, and Leccino, treated by either Spanish or natural styles. In Apulia (25% of national production), “Peranzana Alta Daunia” is a pure olive cultivar present in the province of Foggia, in a limited area called “Tavoliere delle Puglie”, particularly in the cities of San Severo, San Paolo di Civitate, Serracapriola and Torremaggiore. It occupies an area of about 10.000 hectares and it’s cultivated by 6.700 olive farms including about 6.8 million plants. The average annual production is 25.000 tons of olives for oil and 3.000 tons of table olives, being a dual-purpose cultivar. In fact, thanks to the excellent consistency of its pulp and its sweet and balanced taste, Peranzana is also an excellent table olive, especially if prepared by the Greek style through fermentation in brine without any preliminary treatment with NaOH, whose primary scope is to remove bitterness, due to the presence of oleuropein. Nowadays, the processing method relies on a fermentation process which involves, in different ways, mixed populations of microorganisms, mainly represented by lactic acid bacteria (LAB) and yeasts [3]. However, spontaneous fermentations are often unpredictable, thus many efforts are recently aimed to the general knowledge of this process in order to control it and obtain replicable and high-quality products: the use of a suitable starter could be an important way to standardize production and provide a correct course to the fermentation.

In this context, the aim of this study was to evaluate the use of a multiple strain cocktail as starter culture for “Peranzana Alta Daunia” olives to drive the fermentation; in particular, the effect of the ripening degree (veraison) of drupes and of the salt concentration in brines on the acidification kinetic was analysed by using a 2<sup>k</sup> Factorial Design.

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## 2. Materials and Methods

The fermentation was performed on “Peranzana Alta Daunia” olives, kindly provided by Masseria del Vicario D’Aries S.r.l. (Lucera, Foggia, Italy). Fruits were subjected to grading by removing damaged drupes and dividing the ripest fruits (violet) from the unripe ones (green). Then, fruits were washed with tap water and brined at 6% or 8% of NaCl (*w/v*); in four combinations, the starter inoculum was performed (2%). More specifically, Table 1 shows the eight combinations tested during the experiment; they were obtained by using a 2<sup>k</sup> Factorial Design where the variables tested were: veraison degree (violet or green olive), concentrations of salt (6 or 8%, *w/v*), and presence/absence of the starter cocktail.

The starter cocktail included three strains (*Lactiplantibacillus plantarum*, *Candida norvegica* and *Wickerhamomyces anomalus*) isolated and characterized in previous research [4,5]: the strains were grown at their optimal conditions and then centrifuged at 1000 × g for 10 min, washed with sterile tap water, and mixed to obtain a multiple strain starter cocktail (the initial concentration of the cocktail was ca. 7 log cfu/mL).

**Table 1.** Combinations tested.

Combinations	Veraison Degree	Salt Concentration ( <i>w/v</i> )	Starter
1	Violet	8%	yes
2	Violet	8%	no
3	Violet	4%	yes
4	Violet	4%	no
5	Green	8%	yes
6	Green	8%	no
7	Green	4%	yes
8	Green	4%	no

The fermentation was performed in 1 L containers, with 0.5 kg of olives and 0.5 L of brine; these vessels were stored at room temperature for the entire fermentation process and periodically mixed. During 90 days, microbiological analyses were performed both on olives and brines: 20–25 g of olives were diluted with sterile saline solution (0.9% NaCl) and homogenized through a Sterilmixer (Pbi Intl., Milan, Italy) at 16,000 rpm for 3 min. Serial dilutions of olive homogenates and brines were inoculated onto selective media to enumerate mesophilic bacteria, lactic acid bacteria, spore-former bacteria, Pseudomonadaceae, Staphylococci, Enterobacteriaceae and yeasts, according to Perricone et al. [6]. pH measurements were obtained through a pH-meter Crison 2001 (Crison Instruments, Barcelona, Spain).

The experiments were performed in duplicate. For each combination studied, the pH data were used as dependent variable for a multiple regression analysis through the software Statistica for Windows version 7.0 (Statsoft, Tulsa, Okhla).

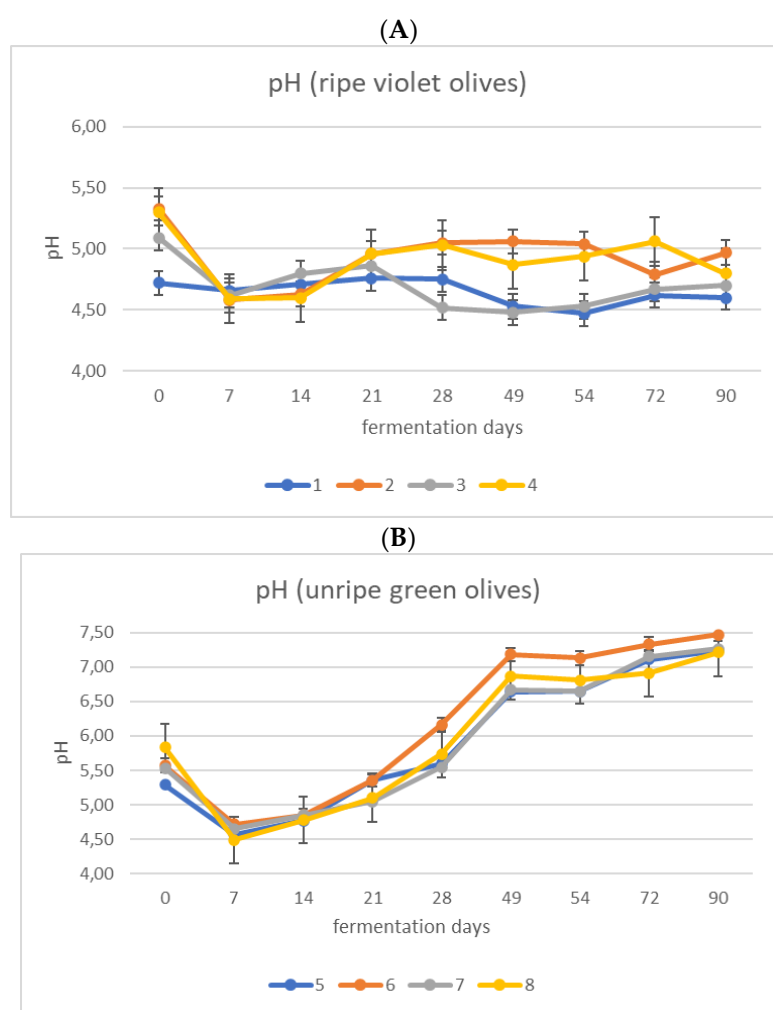
## 3. Results and Discussion

pH is a key variable for olive safety and quality. In the combinations 1–4 (ripe violet olives), the pHs of the brines showed similar values, ranging from 4.70–5.30 units recovered at the beginning of fermentation to about 4.60–4.90 after 90 days, regardless the salt concentration. A good acidification was observed in both combinations with starters (1, 3) (Figure 1A), even if pH was still too high to be considered safe. In fact, Codex Alimentarius standard [7] sets the breakpoint for olive safety at 4.3, but a pH of 4.5 is generally accepted by producers as safe, at least during storage. However, a possible solution to rapidly obtain a pH decrease of the brine to the value of 4.3 should be the inoculation of starter

cultures combined with the addition of small quantities of glucose, as suggested by some authors [6,8].

In the combinations 5–8 (unripe green olives), after a slight initial acidification, an increase in pH values was recorded by the 14th day: the pH reached values of about 7.3–7.5 in all samples which appeared visibly altered due to the onset of putrid fermentative phenomena (Figure 1B).

At the beginning of fermentation, the concentration of lactic acid bacteria was about 6.5 log cfu/mL and 8.30 log cfu/mL in the combinations without (2, 4) and with the starter (1, 3), respectively; however, starting from the 14th day, the cell load remained constant and equal to 8 log cfu/mL in all batches (data not shown). The initial concentration of yeasts ranged between 5.3 and 6.7 log cfu/mL at the beginning of the experiment, reaching approximately 7 log cfu/mL after 14 days, and then remaining unchanged (data not shown).



**Figure 1.** pH values in the eight combinations tested. 1–4, ripe violet olives; 5–8, unripe green olives. Data are presented as mean  $\pm$  standard deviations.

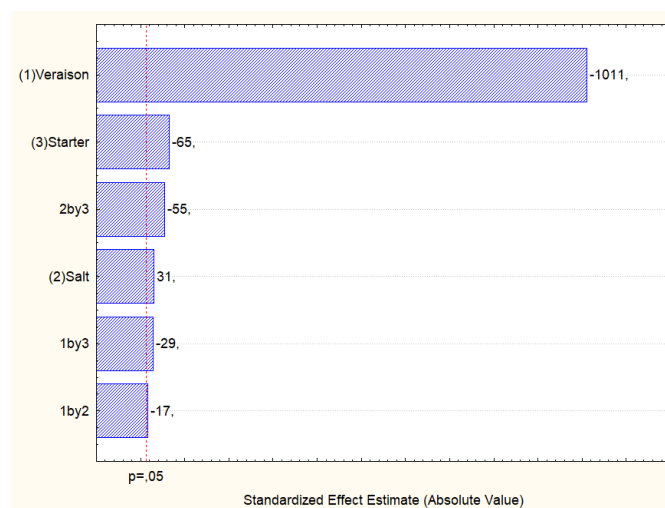
To evaluate the effects of the veraison degree and of the brine salt concentration on the acidification kinetics, for each batch the pH data were used as dependent variable for a multiple regression analysis: the results obtained at the end of fermentation (90 days) are shown in Table 2.

The acidification was affected by all the variables as individual terms with the veraison degree as the most significant one; namely, the pH values decreased with the increase of the fruit ripening. As generally recognized, the veraison or olive maturity index

indicates how ripe the fruit is by assessing the changes in the colour of the olive drupe. The most appropriate veraison index for starting harvest is that which will ensure the best balance for amounts and organoleptic characteristics of olives. The veraison index varies according to the geographical area and olive cultivar: in some cases, the fruit should be harvested when the drupe skin is green to yellow, in other cases, when it is reddish or pale purple, in yet others, when it is black. This is the reason why the effect of this variable on the acidification was tested; the results underlined that the fruit maturity may exert a strong effect on the fermentation course. A significant effect for the variables in interaction was also recorded (Table 2). Figure 2 shows the Pareto chart reporting on the vertical axis the individual and interactive terms of the studied variables and on the horizontal axis the values of standardized coefficient, evaluated as the ratio between the regression coefficient of each term and the relative standard error. The vertical line is the significance breakdown ( $p = 0.05$ ). This figure re-emphasizes that the acidification of brines was significantly influenced by all the investigated factors and, in particular, by the veraison degree (the standardized effect was in fact the highest,  $-1011.00$ ).

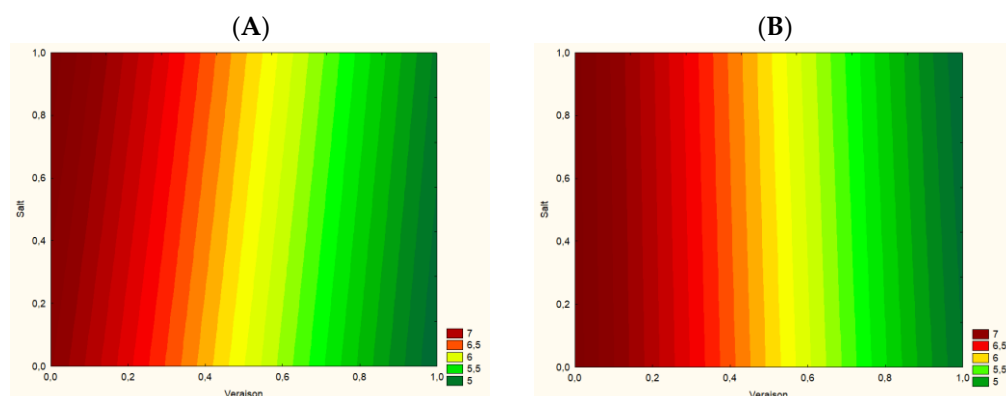
**Table 2.** Effects of the veraison degree, salt concentration and presence/absence of the starter culture on the acidification recorded in the brines after 90 days.

R-sqr = 1; Adj:0.9999 (regression coefficients)	Effect Estimates	Std.Err.
[Veraison degree]	-2.5275	0.0025
[Salt concentration]	0.0775	0.0025
[Starter]	-0.1625	0.0025
[Veraison degree] * [Salt concentration]	-0.0425	0.0025
[Veraison degree] * [Starter]	-0.0725	0.0025
[Salt concentration] * [Starter]	-0.1375	0.0025



**Figure 2.** Pareto chart.

For a quantitative estimation of the effects of the three parameters, contour plots were also built (Figure 3). As evidenced by the interactions [Veraison degree] \* [Salt concentration] with (A) and without (B) the starter cocktail, the lower pHs were obtained at the highest fruit ripening, regardless the salt concentration in the brines and, above all, the presence of the starter cultures.



**Figure 3.** Contour plots of the interaction [Veraison degree] \* [Salt concentration] with (A) and without (B) the starter cocktail.

The use of starter cultures for table olives fermentation is highly recommended, since it may reduce spoilage, inhibit the growth of pathogens, and help to achieve a controlled process. Despite these benefits, the application of starter cultures for olive fermentation is still limited and most of the olive varieties are actually produced without their use [9]. In addition, very few studies report the application of autochthonous starter cultures, even if the application of these cultures could be useful for achieving IGP and PDO (Protected Designation of Origin) product specifications, linking the fermented final product to the region where it comes from [10]. Currently, four Italian PDO are recognized: “Nocellara del Belice” (Reg. EC 134/1998), “La Bella della Daunia” (Reg. EC 1904/2000), “Oliva Ascolana del Piceno” (Reg. EU 1855/2005) and “Oliva di Gaeta” (Reg. EU 2016/2252), but recently numerous efforts have been made to promote “Peranzana Alta Daunia”, by obtaining the recognition of TAP “traditional agrifood product” with Mipaaf Decree n. 8663 of 06/05/2009 (IX revision of the list National TAP (Official Gazette No. 149 of 06/30/09) and moving towards the recognition of the PDO table olive “Alta Daunia” (cv. Peranzana). In this context, the present study is a precious step, since it offers a first analysis on the possibility to use autochthonous starters to ferment this variety that is the most rationale approach to obtain safe products and a high standardized quality.

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