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REGULAR ARTICLE

Quality evaluation of Aitana, Caiazzana and Nocellara del Belice table olives fermented with a commercial starter culture

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ABSTRACT

The present work studies the effects of a commercial starter culture (*L. plantarum* Lyoflora V3, Sacco) on microbiological and chemical parameters during fermentation of Aitana and Caiazzana black olives and Nocellara del Belice turning colour olives. Total phenol content and carpological parameters were assessed on raw olives, while pH, free acidity and different single phenols of brine samples were analysed. The two black olive cultivars were ready to eat after two months while Nocellara del Belice after five months. Different microbial populations were assessed throughout the brining period. *E. coli* was never detected in all the analysed samples, while *S. aureus* was detected (2.23 Log_{10} CFU mL^{-1}) only at beginning of fermentation in Nocellara del Belice control sample. Both hydroxytyrosol and tyrosol trends depended on starter, cultivar and time of fermentation, while oleuropein and verbascoside showed a different behaviour among cultivars. The starter addition has resulted in faster acidification, but a low pH value (3.39 min and 3.71 max values in the final products) was reached in both inoculated and control samples of the three cultivars. According to the Principal Component Analysis, hardness, bitter and acid parameters had an incisive role in the explanation of the variability among the sensory data of the three cultivars. The sensory profiles evaluated at the end of fermentation highlighted significant differences among the samples of the three cultivars for all descriptors.

Keywords: Lactic acid bacteria; Phenols; Sensory analysis; Starter culture; Table olives

INTRODUCTION

In the South of Italy, the most common processing method for table olives is the natural fermentation process, according which green, turning-colour or black olives are washed, put into containers and then filled with freshly prepared brine. Both chemically treated and untreated olives have to be fermented in order to complete the debittering process and to enhance their safety shelf-life by reducing the pH value of the brine. The fruits are maintained in brine until they lose their natural bitterness (Arroyo-López et al., 2008). Usually few elements, as olive variety and size, salt concentration of brine and fermentation temperature, regulate the time necessary to complete the process (Cardoso et al., 2010). Each producer according to personal criteria decides the end of fermentation process, since there is no chemical or microbiological method to be sure that the olives are ready for consumption (Hurtado et al., 2008).

The control of temperature during the olive fermentation often leads to process improvement especially in those regions, such as the South of Italy, where the fermentation temperature follows environmental fluctuations. However, in most companies, the temperature control of the process is not applied because it is expensive (Romeo, 2012). In addition to temperature control and pH lowering, other measures have been proposed to olive companies to prevent fermentation problems and olive alterations: extra salt addition and use of starter cultures. The addition of a high percentage of salt is not well accepted by consumers while starters are associated with the concept of health because lactic acid bacteria (LAB) are important members of the healthy human microbiota. LAB could act as probiotics (Randazzo et al., 2010) and have a number of beneficial health effects, such as antimicrobial and antitumorigenic activities (Caggia et al., 2004; Bevilacqua et al., 2010) and cholesterol reduction (Nguyen et al., 2007; Remagni et al., 2013).

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Consequently, interest in developing starter cultures for table olive fermentation is increasing. LAB have been used for a long time in fermentations because of their ability to acidify the brine and tolerate the presence of salt. Among LAB, *Lactobacillus plantarum* plays an important role in oleuropein degradation (Zago et al., 2013). Moreover, LAB addiction could standardize the fermentation process, reduce the use of pollutants as NaOH solution and extend the shelf-life of table olives by preventing microbial spoilage, and finally enhance olive flavour. Commercial starter cultures are already available on the market, but their use is still not common (Chranioti et al., 2018), whereas in the last years, table olive companies are increasingly demanding support for the correct use of starters.

'Aitana' is a synonym of 'Itrana' or 'Oliva di Gaeta' cultivar, a dual-purpose olive originated from Lazio region and well diffused in Campania region (Italy). The shape of Aitana olives is spherical and slightly asymmetric, with a flesh to pit ratio of 4.5 (Muzzalupo, 2012; Giuffrè, 2017). This olive cultivar is appreciated as black table olives in Lazio region, where it is a typical production, 'Oliva di Gaeta' PDO. 'Caiazzana' is a dual-purpose olive originated from Campania region where it is a typical production (Italy). The olive fruit is characterized by its ellipsoid shape, rounded tip, slightly flattened base and purplish-black flesh from the skin through to the stone. The Caiazzana Table Olive Presidium was established as part of the project 'Presidium of Biodiversity' organized by Slow Food Campania and Slow Food Foundation for Biodiversity in 2016 (Slow Food, 2017).

The aim of the present work was to determine the effect of a commercial starter culture compared to spontaneous fermentation on the microbiological and chemical parameters during fermentation of Aitana and Caiazzana olive cultivars and to define their sensory profiles at the end of fermentation. They were also compared to Nocellara del Belice table olives, because it is the most important Sicilian table olive cultivar (Italy), the only cultivar having two PDOs (for the same variety): 'Valle del Belice' for EVO oil and 'Nocellara del Belice' for table olives. Moreover, the work had the aim of the quality valorisation of Aitana and Caiazzana characteristics, in order to promote the use of them as table olives at industrial level.

MATERIALS AND METHODS

Plant material

Two typical Italian double-purpose cultivars, Aitana and Caiazzana, were harvested in Campania region at the end of October 2015 at full-pigmented state (black ripe), provided by Grignoli olive company (Curti, Italy). These two cultivars are mainly cultivated for oil-purpose (Olea database, 2017; Muzzalupo, 2012) but they are particularly appreciated as fermented table olives in Campania and Lazio Italian regions. Moreover, partially pigmented (at the initial turning colour stage) olive fruits of Nocellara del Belice, the most commercially important table olive cultivar from Sicily, were harvested in the same period in the orchard of olive germplasm collection of CREA-OFA located in San Giovanni Arcimusa (Lentini, Siracusa, Italy).

Experimental procedure

In order to assess the suitability to fermentation as table olives, Aitana and Caiazzana were fermented and analysed together with Nocellara del Belice olives. Carpological analyses were conducted on 50 fruits randomly sampled from the overall lots of each cultivar. Samples of table olives were washed with tap water and placed into 20 L plastic (PE) containers, then filled with 8% (w/v) NaCl brine and stored for six months at 20±1°C to promote a natural fermentation. Fruit/brine ratio were of 1.2 (about 12 kg of olives for each plastic container. After 3 days brining, the olives were inoculated with a commercial *Lactobacillus plantarum* starter (Lyoflora V3, Sacco collection, Italy). The lyophilised culture was dissolved in 2 L water supplemented with 40 g glucose and 90 g NaCl (as suggested by the producer) to allow adaptation of starter strain to the saline environment of the brine and, after 30 minutes, inoculated at a final concentration of 10^6 CFU mL^{-1} of brine (about 1 g freeze dried starter/20 L brine). Olive samples without the commercial starter were used as controls. Three replications for both inoculated and control samples were carried out for each cultivar.

Chemical reagents

Pure standards were purchased from Fluka (tyrosol and gallic acid) and Extrasynthese (verbascoside and oleuropein) while hydroxytyrosol was obtained by acid hydrolysis of oleuropein. Solvents were HPLC grade (Merck KGaA, Darmstadt, Germany). Folin-Ciocalteu's reagent was purchased from Labochimica Srl, Italy.

Total polyphenols of olives

The value of total polyphenols were obtained extracting them from 10 g homogenized olive flesh for each cultivar following the method reported by Amiot, Fleuriette and Macheix (1986) and measured spectrophotometrically at 765 nm after reaction with the Folin-Ciocalteu's reagent. The results were expressed as mg kg^{-1} of gallic acid, by using pure gallic acid standard at different concentrations to obtain the calibration curve.

Chemical analyses of brine

The pH value of the brine samples was measured at regular intervals of time during fermentation period using a Mettler DL25 pHmeter (Mettler-Toledo International Inc.). Free

acidity was determined by titring the brines with 0.1 N NaOH and expressed as g lactic acid 100 mL^{-1} of brine.

HPLC analysis of brine samples

HPLC analyses of phenol fraction of olive brines were obtained as previously described (Sorrentino et al., 2016) by directly injecting the filtered brine (Millipore filters, 0.45 μm) in the Chromatographic HPLC system. The system consisted of a liquid chromatography Waters Alliance 2695 HPLC equipped with a Waters 996 photodiode array detector (PDA) set at 280 nm and with Waters Empower software. The column was a Luna C18 (250mm × 4.6 mm i.d., 5 μm, 100 Å; Phenomenex, Torrence, CA) maintained in an oven at 30°C. Chromatographic separation was achieved by elution gradient using an initial composition of 95% of A solution (water acidified with 2% acetic acid) and 5% of B solution (methanol). The used gradient was the following: B solution increased to 30% in 15 min and to 70% in 25 min and then, after 2 min in isocratic, the mobile phase was set at the initial conditions in 8 min. A flow of 1 mL min^{-1} was used. The identification of phenolic compounds was obtained by comparing retention time with pure tyrosol, verbascoside, oleuropein, and hydroxytyrosol standards. The response factor of hydroxytyrosol was considered the same as tyrosol. All the analyses were carried out in triplicate for each sample.

Sensory analysis

The sensory evaluation of the tested olives was carried out following the guidelines of the International Olive Council (IOC, 2011a). The IOC method establishes the essential requisite and practises for the sensory evaluation of the taste, odour and texture of table olives and for their commercial classification. The sensory analysis of table olives was carry out by a group of 12 experienced tasters, chosen based on their predisposition. The guidelines for taster and panel leader training were applied according to IOC (2011b). The sensory analysis was carried out in the sensory laboratory of the CREA-OFA (Acireale, Italy) conforming to the UNI EN ISO 8589:2014 standard. For the sensory analysis, the judges used a scale of 10 cm length, for express the quantity of the intensity of the olive descriptors, where 1 represents absence descriptor and 11 the maximum intensity of the same. A standard tasting glasses, containing 3 olives with the brining liquid, was presented to each judge.

All olive samples were served at room temperature (about 20°C), coded with a 3-digit random number. The principles of Quantitative Descriptive Analysis (QDA) were used to define the characteristic sensory profile of the three varieties fermented with a commercial starter culture (Sorrentino et al. 2016).

Microbiological analyses

Decimal dilutions were aseptically prepared and plated on the following selective media and conditions. Plate Count Agar (Oxoid) for mesophilic bacteria counts incubated at 25°C for 48 h; de Man, Rogosa and Sharpe Agar (MRS, Oxoid) added with 50 mg L^{-1} Nystatin for LAB at 32°C for 48 h under anaerobic conditions; Sabouraud Chloramphenicol Agar (SAB, Bio-Rad) for yeasts and moulds at 25°C for 48 h; Chromogenic Coliform Agar Base (Bibby-Scharlau, Italy) for Coliform bacteria at 37°C for 24 h, while Mannitol Salt Agar (MSA, Oxoid) was used for *Staphylococcus* spp. at 32°C for 72 h. The analyses were done in triplicate and the results expressed as Log_{10} CFU mL^{-1}.

Statistical analyses

SPSS software (version 21.0, IBM Statistics) was used for data processing. Univariate analysis of variance was used for chemical and microbiological data to test the effects of time of fermentation, starter and olive cultivar, as fixed factors, on the measured dependent variables (phenol compounds, pH, acidity and microbial growth).

The one-way ANOVA was performed for sensory data at the end of fermentation. Furthermore, the principal component analysis (PCA) was applied to investigate relationships among samples depending on the sensory descriptors.

RESULTS AND DISCUSSION

The carpological parameters showed in Table 1 revealed that between the two black ripe cultivars, Aitana can be a good table olive and classified as 'high weight fruits' due to its weight (IOC, 2000), in fact, Aitana olives showed more than 5 g in weight with a 7.66 flesh/pit ratio. Caiazzana was considerably smaller with an average weight of about 3 g. Despite the fruit weight, Caiazzana olives showed an interesting flesh to pit ratio (about 6), to be considered for olive table production. Nocellara del Belice samples showed a good average weight (4.46 g) while the flesh to pit ratio value (4.20) was lower than those reported in bibliography in other harvesting years (Muzzalupo, 2012; Benincasa et al., 2015). The reduced dimension of the Nocellara del Belice

Table 1: Carpological characteristics and polyphenol content of olive drupes after harvesting (data are expressed as means±SD)

	Fruit length (mm)	Fruit diameter (mm)	Fruit weight (g)	Flesh/pit ratio	Total polyphenols (mg kg^{-1})
Aitana	20.69±0.90	15.15±1.16	5.09±0.71	7.66±1.98	3670±90.6
Caiazzana	16.92±1.05	11.34±0.90	2.94±0.24	5.98±1.37	9135±221
Nocellara del belice	21.10±1.26	18.96±0.86	4.46±0.51	4.20±0.63	8612±155

drupes can be depended on less water received due to the poor seasonal rainfall of 2015 in eastern Sicily.

Regarding the total polyphenol content analysed in raw olives, the cultivars showed different values: 3670 mg kg^{-1} in Aitana, 9135 mg kg^{-1} in Caiazzana and 8612 mg kg^{-1} in Nocellara del Belice. Olive polyphenol content is a parameter to be considered whenever a starter is used because high polyphenol values could negatively affect the inoculated cultures (Benincasa et al., 2015).

In Table 2, the results of chemical analyses of Aitana and Caiazzana brines during fermentation are shown. Due to the degree of olive maturation, the deamarization was fast and the olives were ready to eat after two months of fermentation. The univariate analysis of variance highlighted that both fermentation time and starter addition had an effect on pH and acidity values while the statistical differences were independent by the cultivar.

Regarding the HPLC analyses of phenols from olive brines, oleuropein, hydroxytyrosol and tyrosol were monitored as markers of debittering kinetics, while verbascoside was analysed because of the increasing interest in its biological activity (Sorrentino et al., 2016). Both hydroxytyrosol and tyrosol trends were influenced by the time of fermentation, starter and cultivar, in fact Caiazzana always showed higher value than 'Aitana' brines with an increasing trend for both cultivars. The main products of oleuropein hydrolysis consist of hydroxytyrosol and tyrosol (Charoenprasert and Mitchell, 2012), in fact, their increase is related to the oleuropein breakdown at 60 days of fermentation. Oleuropein and verbascoside, which are released into the fermenting olive brine, are usually hydrolyzed into the nonbitter components (Charoenprasert and Mitchell, 2012), but in this case, verbascoside disappeared quickly and was never detected in Caiazzana inoculated samples. No statistical differences for verbascoside were highlighted by the univariate analysis of variance.

Nocellara del Belice olives were ready to eat after five months of fermentation, and this could be related to their less degree of maturation compared to the two black ripe olives tested in the present study.

As shown in Table 3, both starter addition and time of fermentation determined means statistically different for both pH and acidity values. Also in the case of Nocellara del Belice, a safe pH value was reached in both inoculated and control samples although more slowly in the control brines than the inoculated ones. Regarding the single phenols of olive brines, all means were statistically different according to time and starter with the exception of oleuropein content, which showed high standard deviation

Table 2: Results of chemical analyses of aitana and caiazzana brines during fermentation

Analysis	Olive sample	Time (days)	
		30	60
pH value	Aitana Bio	3.51±0.017	3.44±0.020
	Aitana	3.64±0.057	3.57±0.036
	Caiazzana Bio	3.54±0.078	3.49±0.065
	Caiazzana	3.59±0.050	3.50±0.046
	Sig. Time **; Starter **; Cultivar n.s.		
Free acidity (% lactic acid)	Aitana Bio	0.570±0.026	0.707±0.056
	Aitana	0.461±0.029	0.630±0.045
	Caiazzana Bio	0.585±0.045	0.720±0.045
	Caiazzana	0.615±0.104	0.626±0.040
	Sig. Time **; Starter *; Cultivar n.s.		
Hydroxytyrosol (mg L^{-1})	Aitana Bio	227.7±25.2	252.0±17.0
	Aitana	205.9±8.26	248.1±6.96
	Caiazzana Bio	367.5±46.2	376.6±4.45
	Caiazzana	321.1±8.71	360.1±26.7
	Sig. Time **; Starter **; Cultivar **		
Tyrosol (mg L^{-1})	Aitana Bio	7.80±0.91	8.90±0.40
	Aitana	5.96±0.90	7.84±0.44
	Caiazzana Bio	13.39±1.36	12.68±0.91
	Caiazzana	11.31±0.65	13.34±1.34
	Sig. Time **; Starter **; Cultivar **		
Oleuropein (mg L^{-1})	Aitana Bio	10.78±16.7	0.00±0.00
	Aitana	22.03±19.7	0.00±0.00
	Caiazzana Bio	19.65±22.6	0.00±0.00
	Caiazzana	53.34±43.3	0.00±0.00
	Sig. Time **; Starter n.s.; Cultivar n.s.		
Verbascoside (mg L^{-1})	Aitana Bio	2.50±0.61	0.00±0.00
	Aitana	51.19±31.3	0.00±0.00
	Caiazzana Bio	0.00±0.00	0.00±0.00
	Caiazzana	20.14±12.7	0.00±0.00
	Sig. Time n.s.; Starter n.s.; Cultivar n.s.		

Data are expressed as means±SD. Bio=with starter addition.
Sig.*=significance of univariate analysis; **Significance at P<0.01; *significance at P<0.05; n.s. not significant.*

in the control sample. In the Nocellara del Belice olives, verbascoside values showed a different trend compared to the two black cultivars in which brines it disappeared quickly. In fact, in Nocellara del Belice brine samples, the verbascoside value constantly increased and remained stable between four and five months.

According to previous studies, the trend of verbascoside concentration remains consistent throughout the brine storage of ripe olives (Brenes-Balbuena et al., 1992) also when olives are subjected to oxidation (Campestre et al., 2002).

Regarding the microbiological analyses, *E. coli* was never detected in all the analysed samples, while *S. aureus* was detected only at the first sampling (30 days) in Nocellara del Belice control sample (2.23±0.75 Log_{10} CFU mL^{-1}).

The behaviour of staphylococci is in accordance to the results found by Benincasa et al. (2015); probably the LAB starter culture can affect the possibility of growth for staphylococci.

Table 3: Results of chemical analyses of nocellara del belice brines during fermentation

Analysis	Olive sample	Time (days)				
		30	60	90	120	150
pH value	NdB Bio	3.64±0.17	3.61±0.04	3.52±0.02	3.44±0.10	3.39±0.11
	NdB	4.47±0.40	4.13±0.46	3.94±0.52	3.77±0.50	3.71±0.47
	Sig. Time n.s.; Starter **					
Free acidity (% lactic acid)	NdB Bio	0.360±0.09	0.390±0.05	0.475±0.02	0.540±0.01	0.675±0.05
	NdB	0.210±0.13	0.330±0.11	0.270±0.10	0.560±0.13	0.510±0.15
	Sig. Time **; Starter *					
Hydroxytyrosol (mg L^{-1})	NdB Bio	55.70±1.39	94.50±4.5	105.8±6.4	128.5±6.5	118.7±10.1
	NdB	48.39±11.99	96.07±13.5	100.2±6.2	122.2±6.6	109.9±5.1
	Sig. Time **; Starter *					
Tyrosol (mg L^{-1})	NdB Bio	5.14±0.29	8.19±0.08	9.63±0.70	10.46±0.58	9.91±1.21
	NdB	4.19±1.47	7.58±1.56	8.39±0.93	9.43±1.05	8.28±1.01
	Sig. Time **; Starter **					
Oleuropein (mg L^{-1})	NdB Bio	32.5±19.3	6.50±5.2	0.00±0.0	0.00±0.0	0.00±0.0
	NdB	133.1±100.4	21.66±7.5	0.00±0.0	0.00±0.0	0.00±0.0
	Sig. Time n.s.; Starter n.s.					
Verbascoside (mg L^{-1})	NdB Bio	6.98±1.12	7.78±2.89	15.12±1.73	19.40±0.72	19.57±3.51
	NdB	2.46±2.26	8.21±3.66	12.80±2.35	15.22±2.48	15.97±2.79
	Sig. Time **; Starter *					

NdB=Nocellara del Belice; Bio=with starter addition. Sig.*=significance of univariate analysis; **Significance at P<0.01; *significance at P<0.05; n.s. not significant.*

The results of microbiological analyses of Aitana and Caiazzana brines are reported in Fig 1, while those related to Nocellara del Belice ones are showed in Fig 2. An initial concentration of 6 Log_{10} CFU mL^{-1} live cells of starter culture was inoculated only in the olives of the 'Bio' trials (Fig. 1 and 2). The lactobacilli count of black olives (MRS in Fig 1) was the same for both inoculated and control samples without statistical differences. The indigenous LAB count were higher in Aitana and Caiazzana cultivars compared to Nocellara one. Remarkable differences in initial LAB cell densities were detected in Nocellara del Belice cultivar where the inoculated samples showed an initial count (7.16 Log10 CFU mL^{-}1) considerable higher than the control (3.33 Log10 CFU mL−1) and throughout the process up to the 120th day of fermentation, when it became approximately the same. The pH and acidity values of the analysed cultivars, as already discussed in Table 2 and 3, seem to confirm this different trend. The effect of starter addition seems to have been more efficacy in Nocellara del Belice olives. Probably, the high concentration of autochthonous LAB in Aitana and Caiazzana olives produced complex mechanisms of interaction and/or competition among the different LAB strains and the added starter.

The mesophilic aerobic bacteria (PCA in Fig. 1) in black ripe olives showed the same behaviour and count than LAB. In Nocellara olives (Fig. 2), instead, the total viable count together with yeast and mould population were higher in inoculated samples up to 90 days of fermentation.

Finally, the mean values of yeast and mould population (SAB in Fig 1) were similar in both inoculated and control

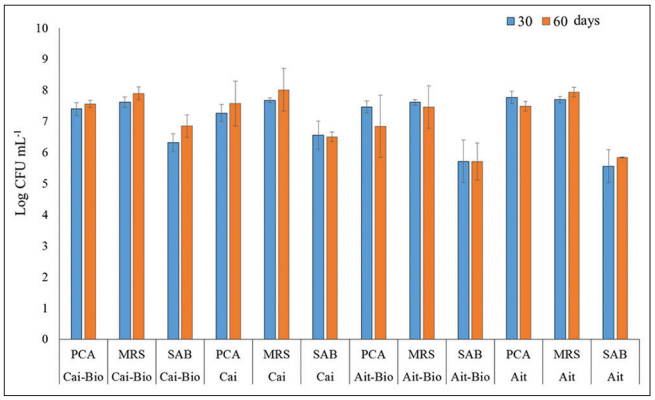

Fig 1. Results of bacterial population of Aitana and Caiazzana olives during fermentation (Data are expressed as means of Log10 CFU mL- ^{1}, error bars represent SD; Bio=with starter addition; Cai=Caiazzana; Ait=Aitana).

brines, as confirmed by other authors in previous studies (Benincasa et al., 2015; Randazzo et al., 2014).

The profile of the different inoculated olives by the spider plot (Fig 3), showed that a perception of salty and acidity characterized the Aitana olives while Caiazzana is distinguished by bitterness and finally the Nocellara of Belice for high crunchiness, as expected due to its less degree of maturation.

Moreover, the Nocellara of Belice olives, according to the judges, showed a more balanced and harmonic profile than the Aitana and Caiazzana ones.

The Anova results showed significant differences among the samples of the three cultivars for all descriptors, except

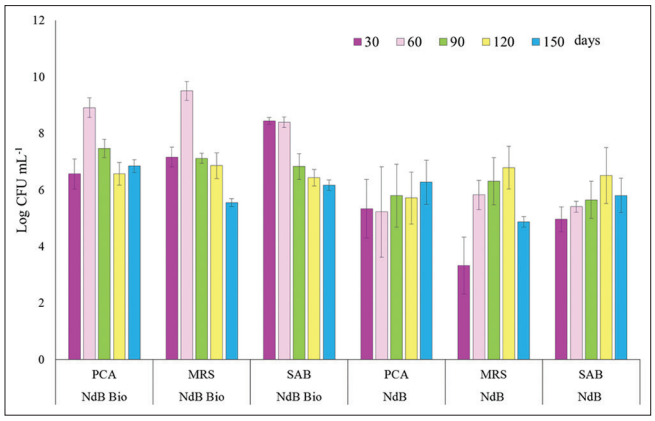

Fig 2. Results of bacterial population of Nocellara del Belice olives during fermentation (Data are expressed as means of Log10 CFU mL^{-1}, error bars represent SD; Bio=with starter addition; NdB=Nocellara del Belice).

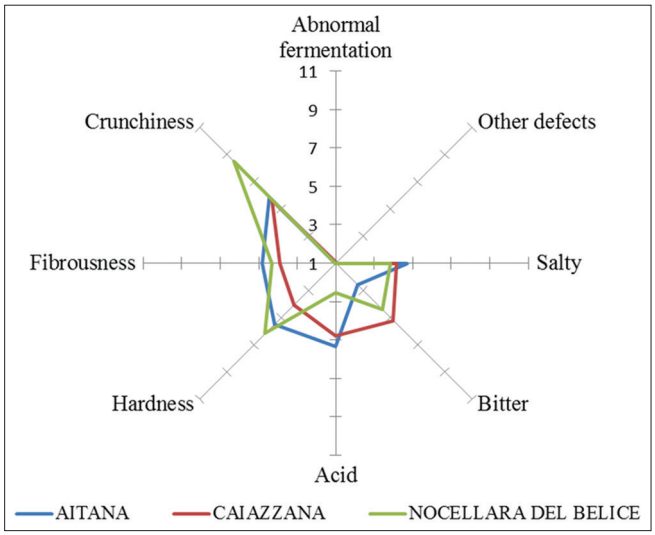

Fig 3. Evaluation profile of sensory attributes of inoculated table olive samples.

for abnormal fermentation and other defects (data not shown).

The sensory profile confirmed the evolution of results obtained by the principal components analysis. The Biplot (Fig 4) also highlighted that the distribution of samples was homogeneous within each variety. Moreover, hardness, bitter and acid parameters had an incisive role in the explanation of the variability among the sensory data of the three cultivars. The whole pattern of data separated only Nocellara del Belice and Caiazzana olives whereas Aitana and Nocellara del Belice had only a little overlapping.

CONCLUSIONS

The experimental trials carried out showed that the commercial starter culture used in the present work gave better results in Nocellara del Belice turning colour olives

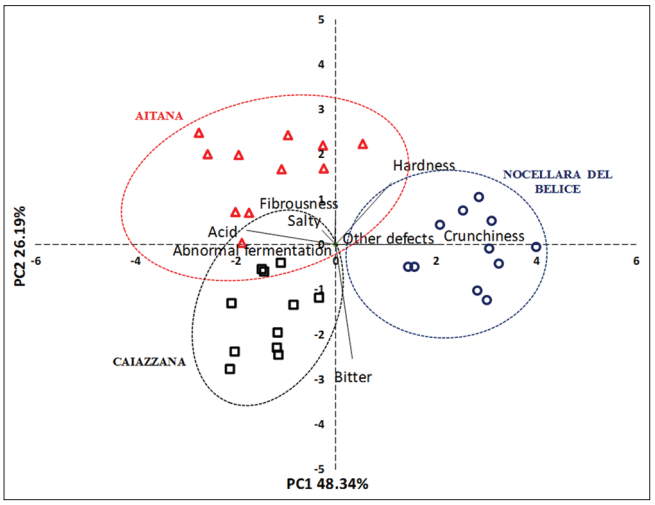

Fig 4. Principal Component Analysis (Biplot) of sensory data of the three analysed cultivars. Ellipses indicate 95% of confidence.

than in black Aitana and Caiazzana ones. However, there was a better kinetics of oleuropein degradation and a faster lowering of the pH in brines of inoculated Aiatana and Caiazzana than in the control ones. This effect on chemical results was due to the starter inoculation as statistically highlighted.

In general, the three cultivars showed good sensory characteristics, but Nocellara del Belice olives had the best profile, due to its flesh consistency and crunchiness. The sensory profile confirmed the evolution of results obtained by the principal components analysis with hardness, bitter and acid parameters showing the most discriminant role in the explanation of the variability among the sensory data of the three cultivars.

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