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Influence of *Hanseniaspora uvarum* AS27 on Chemical and Sensorial Characteristics of Aglianico Wine

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Abstract: In this work was evaluated the effect of sequential inoculum of *Hanseniaspora uvarum* AS27 strain and a commercial *Saccharomyces cerevisiae* yeast on the physical–chemical and organoleptic features of Aglianico, a traditional red wine of Southern Italy. Four fermentation treatments on a pilot scale were performed. In fermentation treatment A, the alcoholic fermentation was spontaneously conducted by the indigenous yeasts present in grape must. In the fermentation treatments B and C were inoculated respectively *S. cerevisiae* FE and *H. uvarum* AS27 strains, as a single starter. The fermentation treatment D was initially inoculated with *H. uvarum* AS27, and *S. cerevisiae* strain was added after 72 h (sequential inoculation). Microbiological, physical–chemical parameters and sensory profiles of the wines have been defined. The results showed that the use of *H. uvarum* AS27, in sequential inoculum with *S. cerevisiae* FE, influenced the wine composition, enriching it in polyphenolic and volatile compounds. Further, the sensory evaluation showed that the use of *H. uvarum* AS27 strain, in co-culture with *S. cerevisiae*, gives the wine more pleasant characteristics. Therefore, the results have highlighted how the use of particular non-*Saccharomyces* yeasts can represent a biotechnological resource in red wine production.

Keywords: aglianico wine; *Hanseniaspora uvarum*; sequential inoculation; aroma compounds; sensory evaluation

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1. Introduction

In recent years, the use of commercial starters has allowed vignerons to enhance wine quality [1]. Consumers, who are increasingly more demanding, are looking for distinctive characteristics in wines and this encourages producers to develop biotechnological strategies to improve the aromatic complexity of wines. The metabolic activities of yeast, as well as of lactic acid bacteria, determine the production of several compounds that significantly influence wines' aroma [2]. In particular, the use of commercial yeasts ensures complete and linear fermentations and allows vignerons to obtain wines without defects free from off-flavors. However, at the same time, the exclusive use of *Saccharomyces* yeasts could reduce wine diversification [3]. Recently, the role of non-*Saccharomyces* yeasts in winemaking has been re-evaluated based on their enzymatic pool, which can be important in the valorization of wines [4]. The fermentation conducted by multiple starters, composed of *Saccharomyces* and non-*Saccharomyces* yeasts, turned out to be an advantageous biotechnological strategy that allows emulating, as far as possible, what happens in spontaneous fermentation; this technique produces wines without defects and with more complex and distinctive aromatic characteristics [5]. The limited use of non-*Saccharomyces* yeasts has often been conditioned by their low resistance to alcohol and the production of undesirable

compounds such as acetic acid, sulfur compounds, etc.; today, however, the technologies and equipment used make it possible to work in conditions that limit the production of unwanted volatile compounds [6–8].

Unlike *Saccharomyces* species, non-*Saccharomyces* yeasts possess different enzymes: glycosidase, pectinases, proteases β-glucanases, lichenases, β-glucosidases, cellulases, xylanases, amylases, lipases, esterases, etc [9]. These can play an important role both in the technological extractive phase and during the fermentation, in the release or production, of terpenoids, fatty acid esters, higher alcohols, esters, etc [10,11]. The use of these yeasts in fermentation could constitute a valid alternative to the use of enzymes produced by filamentous bacteria and fungi [12]. Numerous aromatic compounds are present in the grapes as glycosidic precursors without sensory properties [13,14]. The enzyme glucosidase hydrolyzes the β-D-glucosidic bond, favoring the formation of volatile compounds such as norisoprenoids, benzenoids, aliphatic alcohols, and terpenes that contribute to the definition of the organoleptic characteristics of the wine. The esterase of the yeast can have a significant effect on the fruity flavors of the wine. Non-*Saccharomyces* yeasts in possession of proteolytic and pectinolytic enzymes can be useful in different stages of the winemaking process [9]. Additionally, the pectic enzymes can accelerate the extraction of juice from grapes and promote the release of phenolic compounds during the maceration phase [15,16]. To maximize the metabolic activity of non-*Saccharomyces* yeasts, various methods of use have been evaluated, but sequential inoculation has proved to be the best option. This technique allows non-*Saccharomyces* yeasts to play their role without the competition of *Saccharomyces* which, inoculated in a second phase, complete the fermentation process [7]. Several researchers have shown that with this biotechnological strategy it is possible to produce wines with distinct aromatic profiles and improve the complexity of the wine [17]. In recent years, the genus *Hanseniaspora* has been the subject of numerous studies and it has been shown that several oenological characteristics belonging to this genus can positively influence the color, taste, aromas, and stability of wines [18–24]. Based on the above considerations, in this study we evaluated the oenological potential, as a starter, of the *H. uvarum* AS27 strain in Aglianico wine production. Aglianico (*Vitis vinifera* L.) is a renowned red grape cultivar widespread in Southern Italy [25] rich in polyphenolic compounds [26–28].

2. Materials and Methods

2.1. Yeasts and Growth Conditions

In this study, a commercial yeast, *S. cerevisiae* FE (Fermol Elegance, AEB, San Polo, BS; Italy) and *H. uvarum* AS27 strain have been used. This non-*Saccharomyces* yeast was isolated from grape must [29] and belongs to the culture collection of the DiAAA (Department of Agricultural, Environmental and Food Sciences, University of Molise).

H. uvarum AS27 strain was chosen for its strong enzymatic activities (β-glucosidase, esterase and pectolytic activity) and good oenological properties, such as low production of acetic acid, good alcohol production, and good sulphite tolerance [29]. The *S. cerevisiae* strain was rehydrated before use according to the manufacturer's instructions and a pre-culture of the *H. uvarum* AS27 strain, grown in YPD medium (1% *w*/*v* yeast extract, 2% *w*/*v* peptone and 2% *w*/*v* dextrose) at 20 °C under aerobic condition for 48 h, was used. The cultures were centrifuged at 5000 rpm for 10 min at 4 °C, washed twice with sterile physiological solution (0.9% NaCl) before use, and inoculated in Aglianico grape juice to a concentration of 6.0 log CFU/mL.

2.2. Winemaking Design

For the fermentation experiments, red grapes (*Vitis vinifera* cv. Aglianico) were harvested during the 2018 vintage and transported to Mastroberardino winery (Atripalda, AV-Italy). The grapes were de-stemmed, potassium metabisulfite (70 mg/L) was added and the must was used for four different fermentation treatments (FT): FT-A, spontaneous fermentation; FT-B, inoculated with S. *cerevisiae* FE; FT-C, inoculated with *H. uvarum* AS27

strain; FT-D, initially inoculated with *H. uvarum* AS27 and after 72 h (sequential inoculum), with *S. cerevisiae* FE. For every FT, carried out in triplicate, we used stainless steel tanks (1 hL) containing 80 L of grape must with skins and the punching-down was done three times a day until the end of alcoholic fermentation. The grape must used for the experiments showed the following chemical composition: pH 3.06; sugar content 22.9° Brix, titratable acidity 9.50 g/L (as tartaric acid), malic acid 3.90 g/L, total polyphenols 940 mg/L (as gallic acid equivalent), anthocyanins 65 mg/L (as malvidin-3-glucoside), catechins 32 mg/L, YAN (yeast assimilable nitrogen) 146 mg/L. The physical–chemical analyses of the grape must were carried out in duplicate and were performed according to the corresponding European Community (EC) methods [30]. To maintain an optimal YAN level for yeasts, after 72 h of fermentation, in all the fermentation treatments 25 mg/L of ammonium phosphate was added. The alcoholic fermentation was monitored, assessing the reducing sugars and ethanol content, considering the variation during fermentation time. The temperature was set at 23–24 °C. At the end of alcoholic fermentation from every fermentation treatment, skins were pressed and the wines obtained were subjected to chemical and sensory analysis.

2.3. Microbiological Analysis

Samples were taken under aseptic conditions during alcoholic fermentation (0, 3, 6, 9, 12, 15, and 18 days) and subjected to yeast enumeration. Viable cell counts were evaluated by the plate-counting technique using WL agar (Oxoid, Hampshire, UK), containing 100 mg/L chloramphenicol (Sigma-Aldrich, St. Louis, USA) for bacterial growth inhibition.

The colony color and colony topography parameters were adopted to differentiate the *Saccharomyces* from non-*Saccharomyces* [31]. The various macroscopic colonies formed were counted, and representative colony forms were isolated and maintained on YPD agar slopes at 4 °C until phenotypic and genotypic characterization. The identification of typical colonies was performed using morphological, physiological, and biochemical tests according to the scheme of Barnett et al. [32]. The presence and predominance of the inoculated *S. cerevisiae* FE and *H. uvarum* AS27 starter cultures were assessed by RAPD-PCR [33]. After yeasts counting, from each different Petri plate (WL medium), 10 colonies were randomly picked and subjected to genetic characterization. Two milliliters of overnight cultures in YPD broth medium were centrifuged at 14,000 rpm for 10 min at 4 °C to pellet the cells and the pellet was subjected to DNA extraction using a yeast genomic DNA isolation kit (Norgen Biotek, Thorold, Canada). One hundred nanograms of the DNA extracted was subjected to RAPD-PCR with primer M13 (5′-GAGGGTGGCGGTTCT-3′) [34]. The amplification products were separated by electrophoresis on 1.5% (*w/v*) agarose gel (Sigma-Aldrich, Steinheim, Germany) in 0.5× TBE buffer and then subjected to ethidium bromide staining. RAPD-PCR gels were digitally captured and analyzed by GEL DOC XR System (Bio-Rad, Hercules, CA, USA) using the software Quantity One Analysis (Bio-Rad) and analyzed with the pattern analysis software package, Gel Compare II Version 2.0 (Applied Maths, Kortrijk, Belgium). The calculation of similarities in the profiles of bands was based on Pearson product-moment correlation coefficient. Dendrograms were obtained using the Unweighted Pair Group Method using Arithmetic Average (UPGMA) clustering algorithm [33].

2.4. Chemical Analytical Methods

Chemical analyses were performed according to the corresponding European Community (EC) methods [30]. The malic acid and lactic acid were determined spectrophotometrically (BioSpectrometer Eppendorf, Hamburg, Germany) using an enzymatic kit (Boehringer Mannheim, GmbH, Mannheim, Germany). Volatile compounds (µg/L) were determined by gas chromatography (GC) (Thermoquest Mod. 8000, Rodano, Milan, Italy) and flame ionization detection equipped with a fused capillary column ZB-Wax (30 m × 0.32 mm i.d., 0.50 µm film thickness, Phenomenex, Torrance, CA, USA), according to International Organization of Vine and Wine (OIV) [35]. After the addition of the internal standard (Butan-2-ol;

0.1 mg/mL in water), 1 µL of the sample was injected directly in split mode (1:50); injection port at 250 °C; the oven temperature was increased from 40 °C (5 min) to 240 °C at a rate of 7 °C/min; carrier gas helium was used with a flow rate of 60 kPa [29,36–38]. These analyses were carried out in duplicate and all the reagents were purchased from Sigma-Aldrich.

2.5. Sensory Profile of Wines

To evaluate the different sensorial characteristics of the wines, the samples were tasted by a panel of 20 judges (10 females and 10 males), between 22 and 61 years of age, recruited from the National Organization of Wine Tasters (ONAV, Italy) [39] according to Italian ministerial disciplines [40]. Three replicates of each FT were considered for the sensory evaluation. Wine profile evaluations took place in three sessions. In each session, the panelists evaluated four wines, obtained by the different FT, presented in a randomized order. The samples (30 mL) were presented at room temperature (18 °C) in black tulip-shaped glasses, covered with glass Petri dishes, and coded with random three-digit codes. Unsalted crackers and room temperature water were provided to rinse the mouth between samples [41]. Previous to the tasting sessions, the studied parameters were established by consensus according to ONAV methodology. The panelists were asked to rate the wines according to an unstructured scale from 0 (absent) to 9 (very intense), to rate the intensity of the following parameters: overall judgment, spiciness, herbal characters, acidity, astringency, softness, sweet cherry, red fruits, retro olfactory spiciness (ro-spiciness), retro olfactory red fruits (ro-red fruits), and color.

2.6. Statistical Analysis

The wine chemistry and the sensorial analysis data represent three biological replicates for each different FT (n = 3). The data obtained were analyzed using the software R (v 4.0.3). Analysis of variance (ANOVA) was performed. Statistical significance was attributed to values of $p \leq 0.05$.

3. Results and Discussion

3.1. Fermentation Kinetics

The different "yeast populations" and "fermentative kinetics" are shown in Figure 1. In all FT, sugars were completely fermented although with different dynamics. In FT-A the non-*Saccharomyces* yeasts were predominant in the first two days of fermentation with a cellular concentration of 5.2 log CFU/mL, and on the 6th day it was about 4.2 log CFU/mL, after which a progressive decrease was observed until reaching a concentration of about 1.0 log CFU/mL on the 12th day. *Saccharomyces* yeasts, initially present with a concentration of about 2.4 log CFU/mL, from the sixth day until the end of the alcoholic fermentation retained a concentration of about 7.3 log CFU/mL.

In FT-A, non-*Saccharomyces* yeasts were predominant after the first two days of fermentation with a cell concentration of 5.2 log CFU/mL, which gradually decreased to a concentration of about 1.0 log CFU/mL after 12 days.

In FT-B, fermentation was started and completed by the *S. cerevisiae* FE strain, inoculated at a concentration of 6.5 log CFU/mL, reached the maximum growth rate on the 3rd day of fermentation (7.7 log CFU/mL); the density of non-*Saccharomyces* yeast, initially about 3.4 log CFU/mL, quickly decreased after the addition of the starter *S. cerevisiae* FE, which instead maintained a cell concentration of 6.9 log CFU/mL at the end of alcoholic fermentation (8th day).

In FT-C, where the starter *H. uvarum* AS27 was inoculated, non-*Saccharomyces* yeasts reached a cell concentration of 7.2 log CFU mL on the 3rd day of fermentation, after which their cell density had a gradual decline. *Saccharomyces* yeasts reached their maximum cell density after six days (about 7.0 log CFU/mL) which was similar until the end of alcoholic fermentation.

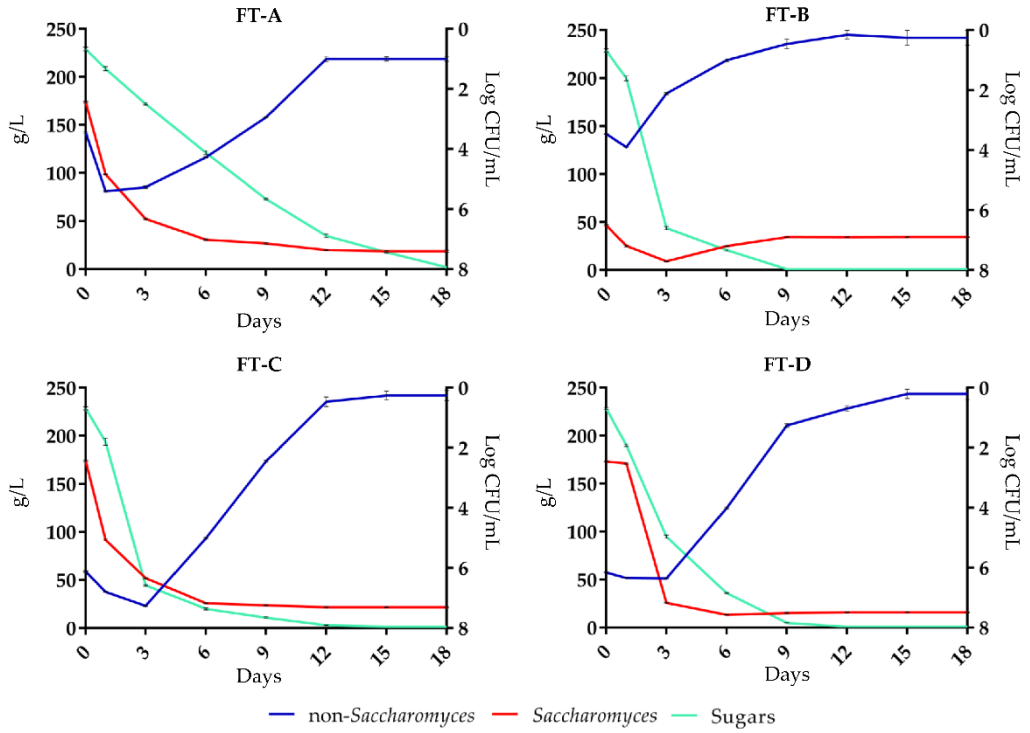

Figure 1. Evolution of *Saccharomyces* and non-*Saccharomyces* yeasts, and sugars consumption in the four fermentation treatments: FT-A (spontaneous fermentation); FT-B (*S. cerevisiae* FE); FT-C (*H. uvarum* AS27); FT-D (*H. uvarum* AS27 + *S. cerevisiae* FE).

In FT-D, in which *H. uvarum* AS27 yeast was initially inoculated, the non-*Saccharomyces* yeasts counts showed a cellular concentration of about 6.1 log CFU/mL until the 3rd day, which corresponds to the inoculation of the *S. cerevisiae* FE. From this point on, the population of the non-*Saccharomyces* yeasts had a rapid decrease, while *Saccharomyces* yeasts maintained a cellular concentration of about 7.5 log CFU/mL until the end of the alcoholic fermentation (12th day). RAPD-PCR analysis confirmed the presence and predominance of the starter of *S. cerevisiae* FE and *H. uvarum* AS27 in FT-B, FT-C, and FT-D treatments (data not shown). The data showed (Figure 1) that the best fermentation performances were detected when *S. cerevisiae* FE inoculum (FT-B) or sequential inoculum of *H. uvarum* AS27 and *S. cerevisiae* FE (FT-D) were applied. In fact, in tanks B and D, the alcohol fermentation progressed regularly and sugars were fully fermented after nine days. In FT-C, where the must was inoculated with *H. uvarum* AS27, as a single starter, the fermentation was completed, in 12 days, by indigenous *Saccharomyces*. In FT-D, the non-*Saccharomyces* were the predominant yeast until the 3rd day of fermentation when *S. cerevisiae* FE was co-inoculated.

3.2. Wine Chemistry

Table 1 shows the chemical characteristics of the wines at the end of alcoholic fermentation. The results of volatile acidity emphasize the compatibility of *H. uvarum* AS27 with the winemaking process, as also evidenced by other authors [19,29], and the use of selected apiculate yeasts in co-culture with *S. cerevisiae* did not affect the volatile acidity. In fact, in the wine obtained in FT-D, the volatile acidity value was less than 0.5 g/L. Instead, a greater increase in volatile acidity (0.97 g/L) was detected in wine obtained in FT-A (spontaneous fermentation).

Table 1. Chemical parameters of Aglianico wines obtained by different fermentation treatments: FT-A (spontaneous fermentation); FT-B (*S. cerevisiae* FE); FT-C (*H. uvarum* AS27); FT-D (*H. uvarum* AS27 + *S. cerevisiae* FE).

Parameters	FT-A	FT-B	FT-C	FT-D
pH	3.18 ± 0.07	3.23 ± 0.15	3.21 ± 0.10	3.21 ± 0.10
Titratable acidity * (g/L)	9.32 ± 0.16	9.10 ± 0.30	9.35 ± 0.15	9.26 ± 0.15
Reducing sugars (g/L)	1.90 ± 0.10	1.20 ± 0.20	1.47 ± 0.60	1.18 ± 0.60
Alcohol (% *v*/*v*)	13.0 ± 0.1	12.9 ± 0.1	12.9 ± 0.2	13.0 ± 0.1
Glycerol (g/L)	7.00 ± 0.18 ^{a}	8.76 ± 0.35 ^{b}	7.23 ± 0.25 ^{a}	8.36 ± 0.25 ^{b}
Volatile acidity ** (g/L)	0.97 ± 0.06 ^{d}	0.29 ± 0.02 ^{a}	0.62 ± 0.03 ^{c}	0.46 ± 0.02 ^{b}
Malic acid (g/L)	3.21 ± 0.20	3.13 ± 0.02	3.25 ± 0.10	3.11 ± 0.12
Lactic acid (g/L)	0.02 ± 0.00 ^{a}	0.04 ± 0.01 ^{a}	0.02 ± 0.00 ^{a}	0.03 ± 0.00 ^{b}
Catechins (mg/L)	384 ± 4 ^{a}	512.03 ± 7 ^{c}	460 ± 6 ^{b}	543 ± 5 ^{d}
Total polyphenols *** (mg/L)	2267 ± 2 ^{a}	2451 ± 3 ^{c}	2371 ± 6 ^{b}	2550 ± 7 ^{d}
Anthocyanins **** (mg/L)	463 ± 1 ^{a}	522 ± 1 ^{b}	450 ± 9 ^{a}	552 ± 9 ^{c}
Color intensity (IC)	13.7 ± 0.7 ^{a}	15.6 ± 0.3 ^{b}	13.8 ± 0.4 ^{a}	16.6 ± 0.3 ^{b}
Color tonality (CT)	0.49 ± 0.04	0.47 ± 0.04	0.42 ± 0.04	0.46 ± 0.03

Data are expressed as mean values ± standard deviations (n = 3); a–d: within a row, different letters indicate significant differences ($p \leq 0.05$); * as tartaric acid; ** as acetic acid; *** as gallic acid equivalent; **** as malvidin-3-glucoside.

The concentrations of glycerol in the wines, obtained in FT-B and FT-D, 8.36 g/L and 8.76 g/L respectively, were not significantly different from each other. As already reported in literature [42], certain *S. cerevisiae* strains could affect glycerol content and the presence of *H. uvarum* AS27 in the early stages of the alcoholic fermentation did not affect the total production of this compound. In contrast, in FT-A and FT-C the glycerol amounts, 7.0 g/L, and 7.23 g/L respectively, were significantly different from amounts detected in the wines produced in FT-B and FT-D.

Further, significant differences were found in the amounts of polyphenolic compounds in the wines. In FT-D the highest amounts of total polyphenols, anthocyanins, and catechins were detected. This result could be attributable to the more intense enzymatic activity of the *H. uvarum* AS27 during the grape maceration phase; this would also explain the highest color intensity (IC) in wine produced in FT-D. The results of the analysis of the volatile compounds are reported in Table 2.

The use of *H. uvarum* AS27 starter, in FT-C and FT-D, regardless of combination with *S. cerevisiae*, contributed to obtaining wines with lower amounts of acetaldehyde and ethyl acetate than wine obtained by spontaneous fermentation (FT-A). Several studies reported the negative effects of these compounds on wine quality. In detail, high values in acetaldehyde could result in the appearance of oxidation off-flavor [43,44].

Some species of yeasts have a propensity to produce more ethyl acetate than others.

Ethyl acetate is the major ester produced by yeast and at low levels it imparts a fruity character to the wine. The aroma threshold in wine is around 18 mg/L and levels up to around 60 mg/L are considered to have a positive effect on the wine [45].

In the wines obtained in the four fermentation treatments, significantly different amounts of the higher alcohols were detected, except for the compounds 2-methyl-1-butanol, 2-phenylethanol, and methanol, whose amounts were similar. Although the amount of all individual alcohols was not higher than the respective threshold levels, the overall content of the higher alcohols influences the organoleptic quality of the wines and, if not more than 300 mg/L, contributes to a positive impact on the aroma and flavor of wine [46,47]. Additionally, the higher alcohols can be esterified with acetic acid to produce low-threshold aromatic esters (e.g., 2-phenylethyl acetate, isoamyl acetate, isobutyl acetate), most of them with floral or fruity descriptors [48]. As regards terpenic compounds, in the wines produced in FT-C and FT-D were detected higher amounts of geraniol, linalool, nerol, nonanol, and 4-terpineol than in the wines obtained by spontaneous fermentation (FT-A) and using *S. cerevisiae* as a single starter (FT-B). This relation was also reported by

other authors [49] and could be attributable to the metabolism of *H. uvarum* that through its β-glucosidase activity can favor the increase in amounts of volatile terpenes [7,19]. Linanol was the only terpene found in concentrations above the olfactory threshold level and our data confirm that this compound particularly characterizes Aglianico wines [50]. The amounts of the other terpenic compounds were not higher than their olfactory thresholds. However, the aroma of the wine is the sum of all the volatile compounds, and as a consequence of the synergistic character, some volatile compounds can be potentiated by the presence of others [51]. Therefore, even terpenic alcohols, that have concentrations below their odor threshold, could contribute to aromatic complexity.

Table 2. Volatile composition of the wines obtained by different fermentation treatments: FT-A (spontaneous fermentation); FT-B (*S. cerevisiae* FE); FT-C (*H. uvarum* AS27); FT-D (*H. uvarum* AS27 + *S. cerevisiae* FE).

Compounds	FT-A	FT-B	FT-C	FT-D	Threshold (μg/L)	Odor Descriptor	Ref.
Acetaldehyde **	11.5 ± 0.1 ^{b}	9.61 ± 0.12 ^{a}	9.52 ± 0.31 ^{a}	9.27 ± 0.12 ^{a}	500	Green leaves, fruity	[52]
Ethyl acetate **	118 ± 1 ^{d}	44.8 ± 1.1 ^{a}	68.5 ± 1.8 ^{c}	55.5 ± 1.9 ^{b}	18,000	Solvent, fruity, sweetish	[53]
1-Heptanol	186 ± 11 ^{c}	111 ± 7 ^{b}	82.4 ± 5.1 ^{a}	109 ± 12 ^{b}	200–300	Lemon, orange, copper	[54]
2-Methyl propanol	24.4 ± 0.1 ^{a}	27.8 ± 0.1 ^{c}	26.6 ± 0.2 ^{b}	24.5 ± 0.4 ^{a}	40,000	Wine, solvent, bitter	[55]
1-Octanol	237 ± 2 ^{c}	207 ± 2 ^{b}	198 ± 2 ^{a}	212 ± 3 ^{b}	820	Coconut, walnut, oily	[52]
1-Pentanol	63.1 ± 1.1 ^{c}	35.1 ± 0.9 ^{a}	47.8 ± 1.2 ^{b}	58.9 ± 2.6 ^{c}	64,430	Alcohol, medicinal	[52]
1-Propanol	18.9 ± 1.3 ^{b}	31.8 ± 1.2 ^{d}	13.7 ± 1.5 ^{a}	22.8 ± 1.6 ^{c}	830	Pungent, harsh, ripe fruit	[55]
2,3-Butanediol	412 ± 12 ^{a}	598 ± 14 ^{c}	551 ± 12 ^{b}	618 ± 17 ^{c}	120,000	Butter, creamy	[54]
2-Methyl-1-butanol	156 ± 12 ^{a}	173. ± 12 ^{a}	164 ± 14 ^{a}	203 ± 13 ^{b}	32,000	Alcohol, banana	[52]
2-Pentanol	46.4 ± 1.1 ^{a}	55.4 ± 1.1 ^{b}	82.4 ± 1.6 ^{c}	81.4 ± 1.6 ^{c}	-	Green	[56]
2-Phenylethanol	89.8 ± 11.3 ^{a}	69.1 ± 4.1 ^{a}	78.7 ± 14.2 ^{a}	112 ± 12 ^{b}	14,000	Roses	[57]
3-Methyl-1-butanol	86.8 ± 1.2 ^{a}	108 ± 11 ^{a}	101 ± 12 ^{a}	119 ± 12 ^{b}	30,000	Cheese	[57]
Hexanoic acid	64.8 ± 1.6 ^{a}	83.2 ± 1.6 ^{b}	66.4 ± 1.9 ^{a}	78.6 ± 2.7 ^{b}	420	Cheese, rancid	[54]
Methanol	119 ± 1	112 ± 13	107 ± 4	105 ± 6	100,000	Sweet	[58]
Methionol	163 ± 2 ^{b}	104 ± 2 ^{a}	99.2 ± 3.6 ^{a}	98.4 ± 6.1 ^{a}	500	Boiled potato, rubber	[59]
Linalool	32.2 ± 0.3 ^{b}	23.2 ± 1.4 ^{a}	69.3 ± 0.7 ^{c}	68.6 ± 0.8 ^{c}	25.2	Muscat, flowery, fruit	[54]
4-Terpineol	19.7 ± 0.1 ^{a}	28.5 ± 0.3 ^{b}	45.8 ± 0.6 ^{d}	43.5 ± 0.5 ^{c}	110–400	Light aroma, wood, soil	[54]
α-Terpineol	8.4 ± 0.7 ^{a}	14.9 ± 0.8 ^{b}	37.5 ± 0.4 ^{d}	32.8 ± 0.8 ^{c}	250	Anise	[57]
Nerol	34.5 ± 0.7 ^{a}	42.6 ± 1.1 ^{b}	77.8 ± 0.5 ^{c}	98.6 ± 1.0 ^{d}	400	Rose-like aromas	[60]
Nonanol	12.5 ± 0.9 ^{a}	29.53 ± 1.4 ^{b}	68.2 ± 1.5 ^{c}	66.5 ± 0.9 ^{c}	310	Coconut, walnut, oily	[52]
Geraniol	10.4 ± 0.2 ^{b}	5.62 ± 0.13 ^{a}	10.9 ± 0.2 ^{b}	12.5±0.2 ^{c}	130	Floral	[60]

Data are expressed as mean values ± standard deviations (n = 3); a–d: within a row, different letters indicate significant differences ($p \leq 0.05$). All data are expressed as μg/L, except where otherwise indicated (** value was expressed in mg/L).

Several authors reported the ability of non-*Saccharomyces* yeasts to enhance the quality and aromatic complexity of wines, affecting both the primary and the secondary aroma [48,61]. The wide variety of aromas is greatly demanded and desired in traditional red wine [29,38]. In this context, it should be considered that the exclusive use of *S. cerevisiae* strains as fermentative yeasts could flatten the aromatic profiles. Conversely, the co-inoculum or sequential inoculum of *Hanseniaspora* spp. and *Saccharomyces* yeasts could

highly affect the wine quality, releasing varietal aromas from precursors such as glycosylated terpenes or bonded thiols employing β-glucosidase or C-S-lyase activities [48,62,63].

Our results eliminate several doubts related to the use of *Hanseniaspora* selected strains in winemaking, confirming, as also reported by other authors, that the use of *Saccharomyces* and non-*Saccharomyces* in sequential inoculum produces wines with aromatic complexity greater than that of wines obtained with the use of *S. cerevisiae* as a single starter [3]. However, greater aromatic complexity does not axiomatically mean a better sensory quality of wines. Therefore, sensory analysis is important to evaluate the wine bouquet [64].

3.3. Sensory Analysis

Chemical analysis, while allowing the recognition of the individual components responsible for sensory characteristics, fails to provide information on the real sensory perception as a whole, which is also the result of synergistic or antagonistic effects of the various chemical compounds contained in the wine [65].

Therefore, to assess the impacts of all compounds, a sensory evaluation of the wines was carried out with a group of sensory experts. The results of the sensory analysis of the four wines are reported in Figure 2. Significant differences were obtained in the scores awarded in the evaluation of acidity, spiciness, astringency, softness, ro-red fruits, ro-spiciness, and overall judgment (Table S1; supplementary material).

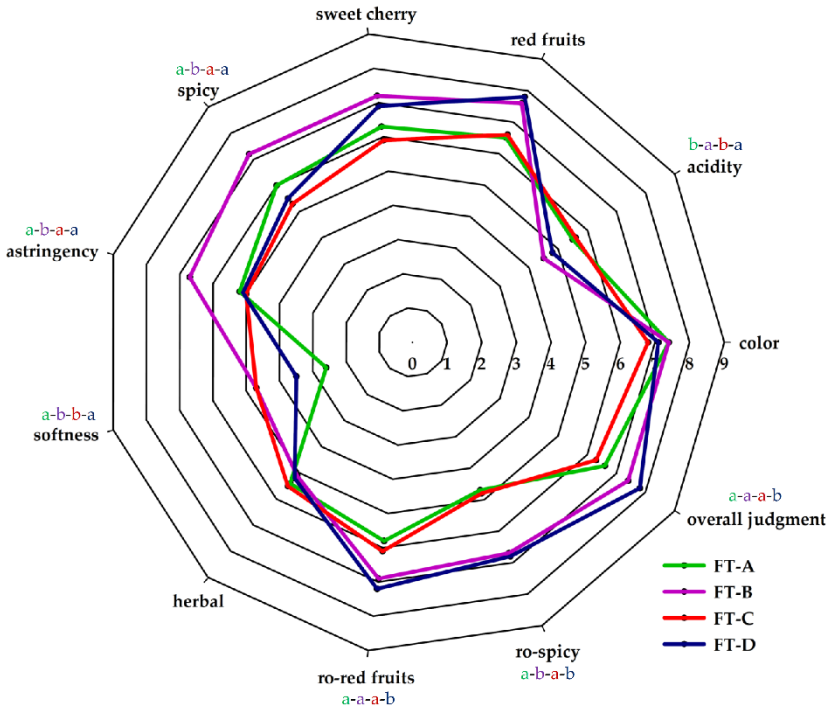

Figure 2. Sensory profiles of Aglianico wines obtained by different fermentation treatments: FT-A (spontaneous fermentation); FT-B (*S. cerevisiae* FE); FT-C (*H. uvarum* AS27); FT-D (*H. uvarum* AS27 + *S. cerevisiae* FE).

The wines obtained in FT-B and FT-D scored best in overall judgment. The score of this parameter has been awarded on the basis of an overall evaluation of the wine that includes the visual, taste, and olfactory aspects of the wine. The sensory color analysis did not show significant differences between the four wines produced, although chemical analyses had detected significant differences in the amounts of total polyphenols, anthocyanins, catechins, and in IC and CT values. The sensory evaluation of color is based on the perception of the following characters: intensity, hue, vivacity, clarity; therefore, it is not entirely related to chemical–physical analysis. The assessment of astringency was significantly different between the wine produced in FT-B and the other wines. It is not

easy to justify this result because astringency is influenced by the concentration of several compounds. The class of molecules that contributes most significantly to overall red wine astringency is tannins [66]. However, the astringent sensation can also be modulated by other compounds contained in wine such as acids, sugars, ethanol, anthocyanins, and polysaccharides [67–69]. The fraction of anthocyanin, unlike polysaccharides, can increase perceived astringency, along with the presence of some tannins [70,71]. Different studies have revealed that several polysaccharide families can interact with tannins, so they could reduce astringency by limitation of available proanthocyanidins [72,73]. Some polysaccharides, such as mannoproteins, are released from yeast cell walls during fermentation and later, during contact of wine with yeast lees [74]. In some research, it has been shown that the non-*Saccharomyces* yeasts have a greater capacity to release polysaccharides compared to *S. cerevisiae* and that this feature is also strain-dependent [75,76]. On the basis of this consideration, the ability to release these compounds by the *H. uvarum* AS27 strain should also be investigated in the future.

Polysaccharides, and especially mannoproteins, also have an important sensory level on softness sensory perception. The softness is a tactile sensation related to the presence of various substances such as glycerol, alcohols, and polysaccharides. It is perceived as an embracing and roundness sensation on the tongue [77]. Glycerol is a non-volatile compound that has no aromatic properties, but which significantly contributes to wine quality by providing sweetness, fullness, and softness [78]. Therefore, the amounts of polysaccharides and glycerol contained in the four different wines may have influenced this sensory parameter. In the sensory panel, the judges attributed significantly higher scores in sensory perception of acidity to wines obtained in FT-A and FT-C; the reason may be due to the higher volatile acidity values found in these wines. Some acids in lower concentrations such as succinic acid, pyruvic acid, citric acid, etc., without significant repercussions on pH values, may affect sensory perception [48]. Besides, as far as the acidity is concerned, the indirect impact of the yeast strain is linked to its ability to produce and release polysaccharides, which increase the softness of the wines, causing their acidic sensation to decrease. Among the other parameters examined by the taster judges, significant differences were found in spiciness, ro-red fruits and ro-spiciness. These sensory perceptions are mainly due to compounds originating from the grape varieties [79].

4. Conclusions

The results showed that the use of *H. uvarum* AS27 as the selected strain, in sequential inoculum with a commercial *Saccharomyces* yeast, could be a biotechnological resource to enhance Aglianico wine. The use of mixed starters represents an alternative both to spontaneous fermentation and to the use of *Saccharomyces* strains as a single starter, taking advantage of the positive role that non-*Saccharomyces* yeasts, suitably selected, can have in the definition of the chemical and organoleptic characteristics of wine [80]. Future investigations will be carried out on the vinification of Aglianico and other grape varieties using *H. uvarum* AS27 in co-culture with *S. cerevisiae* strains.

Moreover, using better performing analytical techniques such as liquid or gas chromatography/mass spectrometry, it will be possible to have more information on the composition of the wines produced and their evolution during ripening and aging.

Supplementary Materials: The following are available online at https://www.mdpi.com/2227-9717/9/2/326/s1. Table S1. Sensory analysis data of Aglianico wines.

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