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Effect of Using Different Blends of Non-Saccharomyces Yeast Isolated from Italia and Negra Criolla Grapes on the Aromatic Diversity and Sensory Profile of Piscos

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Abstract: The objective of this study was to investigate the impact of co-fermentation of Italia and Negra Criolla grape musts using non-*Saccharomyces* yeast strains (NSYSs) isolated from both grape varieties, on the major volatile compounds and sensory characteristics of Piscos (distilled spirits). Native NSYSs previously isolated from Italia (*Pichia terricola*, *Metschnikowia pulcherrima*, and *Naganishia vaughanmartiniae*) and Negra Criolla (*Vishniacozyma carnescens*, *Vishniacozyma heimaeyensis*, and *Aureobasidium pullulans*) grapes' skins were inoculated at the beginning of grape must fermentation. A centroid simplex design was applied in order to obtain 10 representative yeast blends for use as mono- (n = 3), bi- (n = 3), and ternary (n = 4) inoculations. Additionally, a control sample without inoculum was also set up. For each yeast blend, the volatile composition and sensory characteristics of Piscos were evaluated. Results showed that mono-inoculation using specific NSYSs, such as *P. terricola*, *M. pulcherrima*, and *N. vaughanmartiniae*, led to a notable predominance of some terpenes such as α-terpineol, citronerol, and geraniol in Pisco from Italia grapes compared to the control Pisco. Conversely, in Pisco from Negra Criolla grapes, where *V. carnescens*, *V. heimaeyensis*, and *A. pullulans* were used in a similar mono-inoculation process, a higher presence of phenylethyl alcohol and 2-phenylethyl acetate compared to the control was observed. The sensory analysis revealed that citrus, floral, alcohol, and syrup descriptors had a higher intensity in mono-inoculated Pisco Italia, whereas spice, herbaceous, and cooked vegetable descriptors had the highest intensity in Negra Criolla Piscos produced with ternary NSYS inoculum inoculations. This study demonstrates that the use of native non-*Saccharomyces* yeast strains in the co-fermentation of grape musts can significantly influence the volatile profile and sensory characteristics of Pisco. These findings will allow us to establish new inoculation strategies to impact the overall sensory and aromatic profile of the Piscos produced with different grape varieties.

Keywords: aroma profile; co-starter yeast cultures; *Metschnikowia pulcherrima*; *Naganishia vaughanmartiniae*; *Pichia terricola*; sensory profile

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1. Introduction

The wine microbiota is a complex ecosystem that plays a direct role in the production and synthesis of many oenologically relevant metabolites, having profound effects on the final composition of the wine [1,2]. Non-*Saccharomyces* yeast strains (NSYSs) are dominant on the grape surface and at early stages in the winemaking process, playing a crucial

role in the fermentation, which includes the conversion of sugar to alcohol and many other secondary metabolites [3]. Additionally, certain native grape yeast strains, related to a geographical region, might represent an important factor in the typicity and sensory characteristics of wine and its distilled derivatives [4,5].

In practice, wineries commonly inoculate *Saccharomyces cerevisiae* yeast starter cultures into the grape must to suppress wild microorganisms and due to its good fermentation performance. However, the role of NSYSs as potential co-initiators to positively modulate some relevant chemical parameters (lactic acid, ethanol, glycerol, volatile compounds, etc.) and to improve the sensory complexity of wine is increasingly being studied [6–8]. Local and regional climate, soil, grape variety, geographical location and aspects related to the vineyard (terroir), vine growing practices, and vineyard management influence the diversity and abundance of NSYSs [9–11]. In fact, the grape hosts a great diversity of NSYSs, such as the genera *Hanseniospora*, *Pichia*, *Candida*, *Metschnikowia*, *Cryptococcus*, and *Issatchenkia* [12,13]. Currently, co-fermentation with *S. cerevisiae* and NSYSs by simultaneous or sequential inoculation is an interesting strategy to avoid wine fermentation defects [8,14]. In all cases, co-fermentation has also been shown to improve the flavor and quality of the wine due to the lower volatile acidity and higher content of volatile aroma compounds of these wines than the wines produced with mono-inoculation [15,16]. Although NSYSs seem to positively influence the volatile profile and sensory properties of the wines, the combination of different types of NSYSs (pure cultures) as fermentation starters has been little studied, due to the complex interaction of strains, which makes it difficult to understand or predict their effects [17]. For example, it has been shown that NSYSs belonging to the genera *Pichia*, *Hanseniaspora*, and *Wickerhamomyces* have a high production of enzymes such as β-glucosidase and β-xylosidase involved in the release of terpenes into the wine, which are responsible for the improvement of its aromatic profile [18]. Other NSYSs, such as the *Metschnikowia* genus, exert a moderate fermentative power, but they are associated with enzymatic activities involved in the formation of favorable wine aroma and color precursors, and also show potential antimicrobial activity against undesirable yeasts and fungi [19]. Therefore, the combination of NSYSs represents a promising technique to improve not only the quality of wine, but also of its distillates, which concentrate most volatile compounds of a fermented beverage [18,20]. However, studies on the influence of NSYSs on the volatile and sensory profiles of distilled beverages are still limited, and absent in the case of Pisco.

Pisco, Peru's flagship product, is the distillate of freshly fermented grape must from Italia, Quebranta, Torontel, or Negra Criolla grape varieties. Moquegua is one of the five regions of Peru with a Pisco Denomination of Origin [21,22]. Previous studies in commercial samples showed that Pisco Negra Criolla is characterized by a low level of volatile compounds, being dominated by β-phenylethanol [23]. However, Piscos produced from aromatic grapes such as Italia exhibited a higher intensity of varietal aromas, such as terpenes [24], which have been related to fruity and positive sensorial attributes in other distilled spirits [25,26]. In addition, these compounds are responsible for the typicity and differentiation of this distillate [23,27]. Considering this background, the aim of the present study was to evaluate the volatile and sensory characteristics of Piscos produced from micro-vinifications with 10 yeast blends composed of the major NSYSs previously isolated from the grape skin surfaces of Italia and Negra Criolla grapes inoculated in the must following different yeast blending strategies (mono- and co-inoculation).

2. Materials and Methods

2.1. Grape Harvest

Grapes were collected from the agro-industrial company "Antonio Biondi e Hijos S.A.C." (Moquegua Valley, Perú), an area within the Origin Designation of Pisco. Three hundred kilograms of grapes of the Italia and 300 kg of the Negra Criolla varieties were harvested during the 2022 harvest campaign. All grape samples were aseptically transferred into a 25 kg plastic container. Only healthy and undamaged grapes were harvested from

the vineyards and stored at −20 °C until analysis. The grapes harvested for the Italia variety had the following characteristics: sugar content of 21.5 ± 0.15° Brix, total acidity of 6.15 ± 0.11 g/L, and pH of 3.4 ± 0.01. Meanwhile, the red variety Criolla had the following characteristics: sugar content of 22.5 ± 0.10° Brix, total acidity of 4.15 ± 0.17 g/L, and pH of 3.6 ± 0.04.

2.2. Yeast Strains and Viable Cell Counts

The native NSYSs used in this study were isolated from the grape surface, selected and identified in a previous study [24]. Briefly, in that study, NSYSs were isolated from Italia and Negra Criolla grapes surface by using selective media, such as Sabouraud dextrose agar with chloramphenicol (SCD) and dicloran rose Bengal agar with chloramphenicol (DRBC). The more abundant species identified from the Italia variety were *Pichia terrícola* (P), *Metschnikowia pulcherrima* (M), and *Naganishia vaughanmartiniae* (N). In the case of the Negra Criolla variety, the most abundant native yeast species identified were *Vishniacozyma carnescens* (C), *Vishniacozyma heimaeyensis* (V), and *Aureobasidium pullulans* (A). Each isolated yeast strain was kept freeze-dried as previously shown [24].

The Tournas method [28] was applied to each strain to determine the viable cell count (CFU/g) of the lyophilized yeast. To do so, 0.1 g of each freeze-dried yeast strain was sampled and diluted up to $1{:}10^{10}$. From this dilution, surface seeding by extension using a volume of 0.15 mL in Petri dishes with malt extract agar (Amyl Media Dandenong, Australia) was performed. The microorganisms were incubated at 28 °C for 48 h. Once the colonies were grown, colony-forming units (CFUs) were counted and their concentration per gram (CFU/g) was calculated.

2.3. Yeast Blends

To establish the different combinations of freeze-dried yeast strains of both grape varieties to be inoculated in the must, a simplex centroid mixture design with three components was implemented [29]. As detailed in Tables 1 and 2, the combined percentage of each strain in each mixture totals 100%. Ten yeast blends and a control (spontaneous fermentation without yeast inoculum) were formulated. The simplex centroid allows for an even distribution of the yeast mixtures across the plot, resulting in individual yeast (3 treatments), binary mixtures (3 treatments), and tertiary mixtures (4 treatments). This type of mixture design was selected because of the equal spacing between the product locations in the ternary plot and the total number of mixtures produced. The 11 samples were sufficient to obtain adequate analytical and sensory understanding while minimizing the economic costs mainly related to the sensory analysis.

Table 1. Design of centroid simplex mixtures for yeast formulations with the most abundant yeast strains isolated from the Italia grape variety.

Yeast Formulation (Code) *	M *Metschnikowia pulcherrima*		P *Pichia terrícola*		N *Naganishia vaughanmartiniae*	
	Grams	%	Grams	%	Grams	%
I0M100P0N	0	0	2.4	100	0	0
I0M50P50N	0	0	1.2	50	1.2	50
I33M33P33N	0.8	33.3	0.8	33.3	0.8	33.3
I100M0P0N	2.4	100	0	0	0	0
I16M66P16N	0.4	16.7	1.6	66.6	0.4	16.7
I50M50P0N	1.2	50	1.2	50	0	0
I50M0P50N	1.2	50	0	0	1.2	50
I66M16P16N	1.6	66.6	0.4	16.7	0.4	16.7
I0M0P100N	0	0	0	0	2.4	100
I16M16P66N	0.4	16.7	0.4	16.7	1.6	66.6
Control	0	0	0	0	0	0

* Sample code refers to the grape variety (I—Italia) and the percentage of each yeast (%M, %P, and %N) in the inoculum. All the treatments were prepared once except the mono-inoculated samples (I0M100P0N, I100M0P0N, I0M0P100N), which were prepared in duplicate.

Table 2. Design of simplex centroid mixtures for yeast formulations with the most abundant yeast strain isolated from the Negra Criolla grape variety.

Yeast Formulation (Code) *	V *Vishniacozyma heimaeyensis*		C *Vishniacozyma carnescens*		A *Aureobasidium pullulans*	
	Grams	%	Grams	%	Grams	%
N0V100C0A	0	0	2.4	100	0	0
N0V50C50A	0	0	1.2	50	1.2	50
N33V33C33A	0.8	33.3	0.8	33.3	0.8	33.3
N100V0C0A	2.4	100	0	0	0	0
N16V66C16A	0.4	16.7	1.6	66.6	0.4	16.7
N50V50C0A	1.2	50	1.2	50	0	0
N50V0C50A	1.2	50	0	0	1.2	50
N66V16C16A	1.6	66.6	0.4	16.7	0.4	16.7
N0V0C100A	0	0	0	0	2.4	100
N16V16C66A	0.4	16.7	0.4	16.7	1.6	66.6
Control	0	0	0	0	0	0

* Sample code refers to the grape variety (N—Negra Criolla) and the percentage of each yeast (%V, %C, and %A) in the inoculum. All the treatments were prepared once except the mono-inoculated samples (N0V100C0A, N100V0C0A, N0V0C100A), which were prepared in duplicate.

2.4. Grape Must Inoculation and Production of Pisco

The micro-vinifications were carried out on fresh musts without the addition of sulfur dioxide (SO_2) obtained from destemming and crushing by hand. The grapes were macerated for 24 h, then, the skins were removed, and the must was poured into 4 L glass containers up to ¾ of their capacity. After 24 h, freeze-dried yeasts P, M, and N, isolated from Italia grapes, and freeze-dried yeasts C, V, and A, isolated from Negra Criolla grapes, were inoculated, according to the experimental design shown in Tables 1 and 2. Thirty grams of freeze-dried yeasts, containing approximately 10^{10} viable cells/g, were resuspended in 300 mL of chemically pure water at 35 °C for 20 min [20]. The fermentation was carried out at 22 ± 2 °C. To verify the implantation of the inoculated yeasts, samples of grape must were taken at three different points during vinification: 24 h after yeast inoculation, on the 6th day, and on the 12th day of fermentation. Samples from each of the treatments were taken in sterile tubes, and then stored under refrigeration until analysis. Samples were serially diluted with sterile peptone: water in a concentration of 1 in 10 (w/v). For yeast counts, 0.1 mL of each dilution was spread in triplicate on GPYA (glucose, peptone, yeast extract) culture medium. Plates were incubated at 28 °C for 48 h for colony development [30]. Alcoholic fermentation was considered finished when a concentration of 1 g/L residual sugar was reached in the different must samples. The freshly fermented musts were distilled in a 20 L copper still. The head cut was made based on 0.80% of the total volume of the fermented must, and for the tail cut, 42%, (v/v) was considered as the final degree of the body. The alcoholic strength was determined at 20 °C by a hydrostatic scale using 100 mL of the distillate (Piscos from the Negra Criolla grape variety and Piscos from the Italia grape variety). At the end of this stage, each treatment of Pisco was poured into glass containers for further characterization of volatile compounds.

2.5. Quantitative Analysis of Major Volatile Compounds

The volatile compounds in the Pisco obtained from the I and NC grape varieties were analyzed by gas chromatography–mass spectrometry (GC-MS) using the procedure previously described [24]. Briefly, a Solid Phase extraction (SPE) on a VacMaster 10 station (Biotage, Uppsala, Sweden) using Supelclean Envi-Carb SPE Tube 200 mg cartridges (total volume 3 mL) was used. After cartridge conditioning, each sample (diluted Pisco 12% ethanol/water) was passed through the cartridge, dried under vacuum (−0.6 bar for 20 min), and eluted with 1.6 mL of dichloromethane. The eluted sample was mixed with the internal standards 4-hydroxy-4-methyl-2-pentanone and 2-octanol dissolved in dichloromethane (400 mg/L). One microliter of the Pisco aroma extract was injected using a CTC autosampler in a Bruker 436 GC coupled to a Bruker EVOQ GC-TQ MS detector

(Bruker, Billerica, MA, USA). The GC and MS conditions were the same as already described [24]. Volatile compounds were identified by comparing the retention times and mass spectra of reference compounds injected under the same chromatographic conditions with those available in the MS library (NIST 2.0). Standard curves of volatile compounds were prepared in dichloromethane and injected under the same conditions to carry out the quantitative analysis (Table S1).

2.6. Sensory Evaluation

Sensory analysis was carried out by a panel of 10 expert tasters (4 women and 6 men between 30 and 50 years old) from the National Association of Official Tasters—PISCO. This analysis was carried out in two sessions, one for each Pisco type (session 1—Pisco Italia, session 2—Pisco Negra Criolla).

This test was conducted following the method of Rabitti [31]. To do so, 22 Pisco type samples were evaluated (11 Piscos from Italia and 11 Piscos from Negra Criolla). In this test, a focus group with expert tasters first defined the most significant sensory attributes of the Piscos. For this task, the attributes previously identified [23,27] and new attributes suggested by the experts were considered. The focus group selected 10 aroma descriptors deemed most suitable to characterize the Italia and Negra Criolla Piscos: fruity (Fr), citrus (Ci), aniseed (An), floral (Fl), herbaceous (He), spicy (Sp), oily (Oi), buttery (Bu), syrupy (Sy), alcohol (Al), olive (Ol), nutty (Nu), and cooked vegetable (Cv) notes. Subsequently, the same expert panel assessed the Piscos. For this purpose, 25 mL of Pisco was poured into AFNOR glasses, labeled with three-digit codes, and covered with Petri dishes to prevent the loss of volatile compounds. Each panelist was given a tasting sheet listing the 10 descriptors and asked to evaluate the intensity of each attribute retronasally using a five-point linear scale. The evaluations were conducted under white lighting and performed in duplicate. Between tastings, panelists cleansed their palates by rinsing with mineral water and consuming unsalted crackers. The Piscos were served at a controlled room temperature of 22 °C. The sequence of sample presentations was systematically rotated among the panelists. All participants were instructed to refrain from smoking, eating, or drinking anything other than water for at least one hour before each tasting session. They were fully informed of the study's purpose and provided written consent to participate.

2.7. Statistical Analyses

ANOVA and Tukey tests were conducted to analyze mean differences in the volatile profiles of the Piscos produced from different yeast combinations using a significance level set at $p < 0.05$. Principal component analysis (PCA) was applied to better understand the most significant relationships of volatile compounds and aroma descriptors with the yeast mixtures used in the production of Italia and Negra Criolla Piscos. All statistical analyses and PCA were conducted using the R program (version 4.2.1).

3. Results and Discussion

3.1. Implementation of the NSYSs in the Musts

The NSYSs used as inoculum were those with the highest abundance previously isolated from the skin surface of Italia and Negra Criolla grapes, which were *Pichia terricola*, *Metschnikowia pulcherrima,* and *Naganishia vaughanmartiniae* in the Italia variety, and *Vishniacozyma carnescens*, *Vishniacozyma heimaeyensis*, and *Aureobasidium pullulans* in the Negra Criolla variety [24]. The implementation of each of these yeast strains in the grape musts was first checked. High cell counts (>10^{10} CFU/g) in the musts from both grape varieties (Italia and Negra Criolla) during the first 24 h of the fermentation process were found (Figure S1). Moreover, the growth dynamics of the different NSYS mixtures (Tables 1 and 2) in each must was also tested. For this, yeast inoculation was performed at the beginning of the fermentation in the musts of both grape varieties, and viable cell content analyses were performed at different points during the fermentation process (days 1, 6, and 12). In general, results (Figure S2) confirmed a gradual growth, increasing from day 1 to day 12,

and from here, a progressive decrease was observed in the yeast population in all musts. In all cases, musts were considered ready for distillation when residual sugar concentrations of 1 g/L were reached.

3.2. Influence of the Different NSYS Blends on the Volatile Composition of the Piscos

As previously shown, the population of the NSYS blends exhibited differences during the fermentation process, which could affect the volatile composition of the Piscos produced from these musts. To investigate this, the major volatile compounds of the Italia and Negra Criolla Piscos produced with the different NSYS blends were determined (Figure 1).

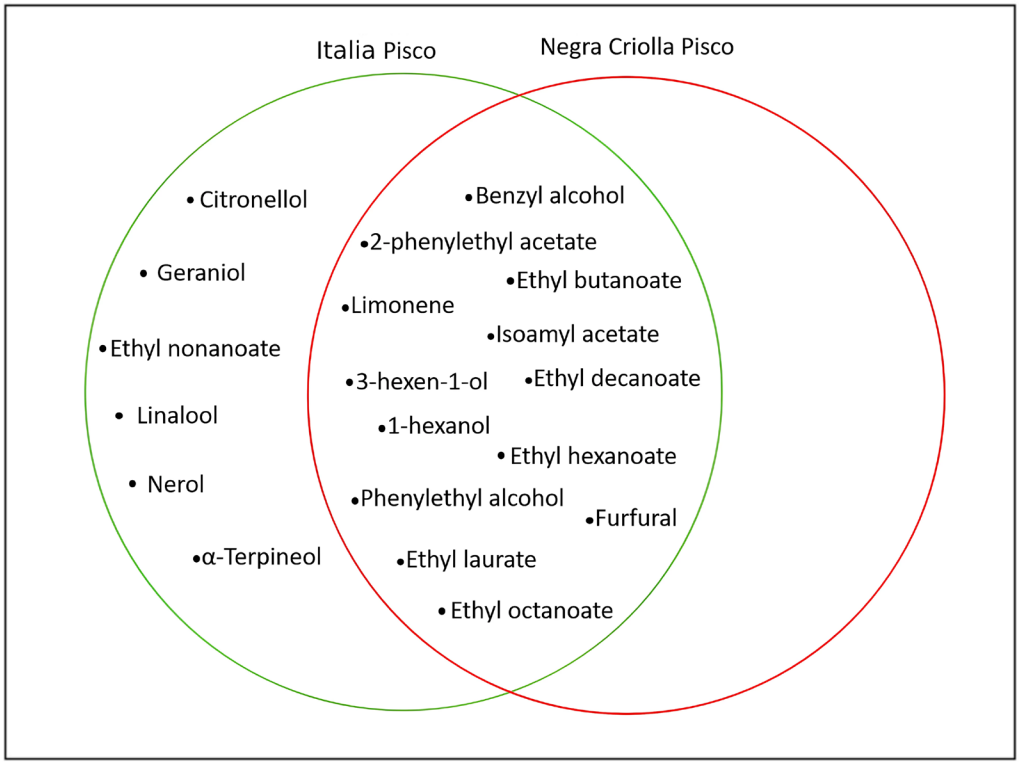


Figure 1. Volatile compounds of Italia and Negra Criolla Piscos. Green and red color refers to Italia and Negra Criolla Piscos respectively.

In the case of Piscos from the Italia grape variety (Table 3), 19 major volatile compounds from different chemical families were quantified. These included terpenes (citronellol, linalool, limonene, nerol, α-terpineol, geraniol), higher alcohols (1-hexanol, 3-hexen-1-ol), acetate esters (isoamyl acetate), ethyl esters (ethyl butanoate, ethyl decanoate, ethyl hexanoate, ethyl laurate, ethyl octanoate), phenylethyl esters (benzyl alcohol, phenylethyl acetate, phenylethyl alcohol), and aldehydes (furfural). As shown in Table 3, there were significant differences ($p < 0.05$) in the contents of volatile compounds in the Piscos from the different yeast mixtures compared to the control (without inoculation). Interestingly, all Piscos from the Italia grape variety exhibited a higher phenylethyl alcohol content, between 4.87 and 16.0 mg/L, compared to the values reported in previous studies in commercial Piscos [27]. Regarding ester compounds (ethyl and acetate esters), a significantly lower content ($p \leq 0.05$) of these compounds was found in the Piscos produced with NSYSs than in the control, except for ethyl nonanoate (Table 3). The opposite effect was observed in a previous study [32], where co-inoculation of a grape must with different ratios of *Saccharomyces* and *Pichia fermentans* yeasts produced a higher content of ethyl esters than the control (without inoculation). Nonetheless, in the abovementioned study, the concentrations of some specific ethyl esters (such as ethyl isovalerate, ethyl octanoate, ethyl decanoate, and ethyl laurate) were not different from the control.

Table 3. Volatile compounds (mean ± SD μg/L) in Pisco Italia.

Compound	Control	I0M0P100N	I0M100P0N	I0M50P50N	I100M0P0N	I16M16P66N	I16M66P16N	I33M33P33N	I50M0P50N	I50M50P0N	I66M16P16N
Terpenes											
Linalool	$1.25 \times 10^3 \pm 10^{ab}$	$1.34 \times 10^3 \pm 90^{ab}$	$3.58 \times 10^3 \pm 600^{a}$	837 ± 8.97^{ab}	$1.87 \times 10^3 \pm 20^{ab}$	$1.18 \times 10^3 \pm 10^{ab}$	988.72 ± 4.57^{ab}	608.12 ± 2.27^{b}	$1.84 \times 10^3 \pm 10^{ab}$	$1.53 \times 10^3 \pm 100^{ab}$	927.46 ± 6.53^{ab}
Limonene	20.00 ± 0.11^{ab}	21.96 ± 3.49^{ab}	2.94 ± 00^{b}	2.94 ± 00^{b}	2.94 ± 00^{b}	2.94 ± 00^{b}	34.95 ± 3.02^{bc}	2.94 ± 00^{b}	67.98 ± 6.34^{c}	50.41 ± 3.48^{ac}	2.94 ± 00^{b}
Citronellol	233.0 ± 4.91^{a}	265.14 ± 3.99^{a}	251.62 ± 2.15^{a}	208.33 ± 8.87^{a}	496.64 ± 8.25^{a}	99.53 ± 2.69^{a}	194.72 ± 4.94^{a}	182.5 ± 1.88^{a}	403.09 ± 3.94^{a}	318.66 ± 8.17^{a}	179.26 ± 4.61^{a}
Nerol	116.74 ± 7.28^{a}	186.93 ± 8.19^{a}	186.17 ± 3.31^{a}	162.29 ± 5.12^{a}	331.81 ± 5.79^{a}	116.69 ± 2.67^{a}	161.92 ± 2.7^{a}	138.47 ± 1.0^{a}	275.17 ± 5.3^{a}	226.91 ± 6.76^{a}	139.07 ± 3.88^{a}
α-Terpineol	252.18 ± 8.93^{ab}	236.14 ± 1.29^{ab}	600.38 ± 0.52^{a}	179.14 ± 6.92^{ab}	378.52 ± 7.32^{ab}	209.97 ± 5.54^{ab}	166.82 ± 4.87^{ab}	130.41 ± 1.77^{b}	348.56 ± 7.63^{ab}	275.73 ± 28.38^{ab}	162.0 ± 13.68^{ab}
Geraniol	162.31 ± 2.46^{a}	293.67 ± 8.71^{a}	334.32 ± 5.1^{a}	294.94 ± 13.36^{a}	691.38 ± 26.98^{a}	140.8 ± 12.85^{a}	208.12 ± 3.98^{a}	243.68 ± 4.19^{a}	496.55 ± 6.5^{a}	364.4 ± 30.96^{a}	167.12 ± 21.86^{a}
C6 alcohols											
1-Hexanol	191.92 ± 3.46^{ab}	230.06 ± 7.61^{ab}	544.0 ± 16.56^{c}	348.5 ± 5.09^{bc}	460.52 ± 47.08^{ac}	106.54 ± 14.89^{b}	291.18 ± 26.35^{bc}	312.49 ± 5.44^{bc}	301.55 ± 8.16^{bc}	332.86 ± 6.57^{bc}	145.5 ± 0.3^{b}
3-Hexen-1-ol	54.66 ± 0.08^{ab}	70.03 ± 7.29^{a}	118.36 ± 5.91^{ab}	160.64 ± 10.34^{ab}	204.25 ± 26.71^{b}	60.31 ± 1.7^{ab}	88.67 ± 8.27^{ab}	166.44 ± 0.5^{ab}	92.35 ± 11.5^{ab}	156.35 ± 16.32^{ab}	55.70 ± 1.33^{ab}
Esters											
Ethyl butanoate	74.39 ± 10.86^{a}	6.60 ± 0.64^{b}	n.d	23.34 ± 1.03^{c}	13.09 ± 1.15^{bc}	n.d	n.d	24.63 ± 1.76^{c}	n.d	n.d	n.d
Ethyl decanoate	1780 ± 130^{a}	158.71 ± 6.73^{b}	179.11 ± 9.86^{b}	122.98 ± 5.66^{b}	276.42 ± 8.13^{b}	135.20 ± 4.93^{b}	130.55 ± 3.13^{b}	115.4 ± 6.19^{b}	163.86 ± 3.88^{b}	243.53 ± 8.72^{b}	195.26 ± 7.06^{b}
Ethyl hexanoate	317.78 ± 14.24^{a}	25.47 ± 2.65^{bc}	n.d	n.d	20.48 ± 2.66^{bc}	n.d	n.d	n.d	38.01 ± 5.06^{c}	26.62 ± 1.64^{bc}	21.82 ± 0.34^{bc}
Ethyl laurate	2730 ± 190^{a}	265.77 ± 3.18^{b}	649.69 ± 5.64^{b}	226.42 ± 11.22^{b}	339.67 ± 1.94^{b}	224.04 ± 7.55^{b}	197.02 ± 7.02^{b}	213.43 ± 2.47^{b}	244.32 ± 3.17^{b}	338.12 ± 3.64^{b}	281.47 ± 4.43^{b}
Ethyl octanoate	740 ± 120^{a}	83.95 ± 5.56^{b}	240.62 ± 6.03^{c}	67.45 ± 2.86^{bc}	125.15 ± 8.96^{bc}	62.92 ± 3.02^{bc}	97.56 ± 1.08^{bc}	61.97 ± 0.82^{bc}	114.30 ± 5.2^{bc}	106.59 ± 12.63^{bc}	85.38 ± 9.75^{bc}
Ethyl nonanoate	$4.60 \times 10^6 \pm 0.19 \times 10^{6\,a}$	$8.07 \times 10^6 \pm 0.00^{a}$	$3.28 \times 10^6 \pm 0.00^{a}$	$7.98 \times 10^6 \pm 0.00^{a}$	$4.96 \times 10^6 \pm 0.00^{a}$	$12.62 \times 10^6 \pm 00^{a}$	$12.62 \times 10^6 \pm 00^{a}$	$7.02 \times 10^6 \pm 0.00^{a}$	$3.79 \times 10^6 \pm 0.00^{a}$	12.62 ± 00^{b}	$5.21 \times 10^6 \pm 0.00^{a}$
Isoamyl acetate	556.08 ± 8.69^{a}	47.27 ± 2.24^{bc}	111.17 ± 0.44^{bc}	140.45 ± 7.39^{b}	90.00 ± 9.0^{bc}	17.00 ± 2.72^{c}	19.40 ± 2.4^{c}	89.90 ± 0.33^{bc}	63.92 ± 1.34^{bc}	24.09 ± 2.3^{bc}	46.52 ± 1.06^{bc}
2-Phenylethyl acetate	222.01 ± 1.76^{ab}	181.26 ± 3.2^{b}	218.82 ± 3.91^{ab}	161.36 ± 1.97^{b}	235.36 ± 4.16^{a}	162.03 ± 2.26^{b}	172.50 ± 1.7^{ab}	153.65 ± 0.31^{b}	214.34 ± 6.22^{ab}	190.12 ± 7.13^{ab}	158.24 ± 2.09^{b}
Aldehydes											
Furfural	46.96 ± 2.26^{ab}	43.02 ± 1.18^{ab}	36.84 ± 00^{a}	52.61 ± 2.04^{bc}	50.47 ± 2.56^{bc}	54.76 ± 2.08^{b}	43.33 ± 0.02^{ab}	48.08 ± 0.06^{ab}	48.05 ± 1.38^{ab}	49.17 ± 0.74^{bc}	40.55 ± 0.05^{ac}
Alcohols											
Benzyl alcohol	99.59 ± 0.59^{ab}	89.34 ± 9.02^{ac}	96.88 ± 7.73^{ab}	126.80 ± 3.47^{bc}	125.02 ± 4.35^{bc}	72.91 ± 00^{a}	92.62 ± 1.19^{ab}	122.60 ± 0.16^{ab}	111.10 ± 0.23^{ab}	134.79 ± 1.48^{b}	82.50 ± 0.97^{ab}
Phenylethyl alcohol	$6.1 \times 10^3 \pm 50^{a}$	$8.04 \times 10^3 \pm 750^{b}$	$24.1 \times 10^3 \pm 700^{b}$	$11.20 \times 10^3 \pm 60^{c}$	$17.60 \times 10^3 \pm 270^{bc}$	$5.98 \times 10^3 \pm 90^{b}$	$9.88 \times 10^3 \pm 30^{b}$	$11.9 \times 10^3 \pm 190^{c}$	$11.8 \times 10^3 \pm 270^{b}$	$16.0 \times 10^3 \pm 1730^{b}$	$4.87 \times 10^3 \pm 480^{b}$

Values with different letters in the same the line are significantly different according to the Tukey test (95%). Codes for the samples are shown in Table 1.

Additionally, it is worth noting that isoamyl alcohols, which have been associated with fruity aroma notes in Piscos [33], showed significantly ($p < 0.05$) higher concentrations in the control (Table 3) compared to other native yeast blends.

Regarding the volatile composition of the Pisco from the Negra Criolla grape produced with the different NSYSs, thirteen major volatile compounds were identified and quantified (Table 4). Unlike Pisco from the Italia grape, five terpenic compounds (citronerol, linalool, nerol, α-terpineol, and geraniol) were not detected. These results are in agreement with the varietal origin of these volatiles from glucoside precursors, which are practically absent from Negra Criolla grapes [34]. As shown in Table 4, in Pisco from Negra Criolla grapes, where *V. carnescens*, *V. heimaeyensis*, and *A. pullulans* were used in a similar mono-inoculation process, a higher presence of phenylethyl alcohol and 2-phenylethyl acetate was observed compared to the control. Only the concentration of ethyl decanoate and ethyl laurate was significantly ($p < 0.05$) higher in the Pisco control without co-inoculation, while the concentration of benzyl alcohol was higher ($p < 0.05$) in the Piscos from the different yeast blends compared to the control.

Table 4 also shows that the most abundant volatile compound in Pisco Negra Criolla was phenylethyl alcohol (2-phenyl ethanol), which was found at higher concentrations (10.9–35.9 mg/L) than those found in Pisco Italia (4.87–24.1 mg/L) (Table 4). This compound is associated with olfactory descriptors such as roses and flowers [35]. The next six major volatile compounds identified in the Pisco Negra Criolla from all the yeast treatments and control samples were benzyl alcohol, isoamyl acetate, ethyl laurate, phenylethyl acetate, 1-hexanol, and ethyl decanoate, which have been previously linked to desirable descriptors in wine [32]. The abundance of benzene volatile compounds, such as phenylethyl alcohol and benzyl alcohol in Piscos from the Negra Criolla grape variety, is in agreement with previous studies performed in Pisco from Quebranta grape variety [36]. Some volatile compounds, such as phenylethyl alcohol, benzyl alcohol, isoamyl acetate, phenylethyl acetate, and 1-hexanol were found at higher concentrations in Piscos from co-inoculation treatments compared to the control but, except for benzyl alcohol, these results were not statistically significant. A similar effect was observed in previous studies [37,38], where the co-inoculation tends to increase aromatic alcohols such as phenylethyl alcohol, benzyl alcohol, and phenyl ethyl acetate. However, this effect was dependent on factors such as the inoculation strategy, grape variety, and type of strain used.

It is noteworthy to observe the contrast in the Pisco made from Negra Criolla grapes, where *V. carnescens*, *V. heimaeyensis*, and *A. pullulans* were used in a similar mono-inoculation process; a higher presence of phenylethyl alcohol and 2-phenylethyl acetate was observed compared to the control. However, in the Piscos from the Negra Criolla grape variety, the co-inoculation treatments with NSYSs did not significantly improve the content of other ethyl esters, such as ethyl butanoate, ethyl hexanoate, and ethyl octanoate.

As can also be seen in Table 4, ethyl butanoate, ethyl hexanoate, ethyl octanoate, furfural, 3-hexen-1-ol, and limonene were the volatile compounds present at lower concentrations in Piscos from Negra Criolla; all of them exhibited concentrations lower than 174 µg/L (Table 4). Previously, in commercial Negra Criolla Piscos, the concentrations reported for some of these compounds, such as furfural and ethyl octanoate, were lower [27], although the different processing conditions can affect the final concentration of these compounds. The concentrations of these compounds (furfural and ethyl octanoate) did not show significant differences ($p < 0.05$) among any of the yeast treatments, or with the control (Table 4).

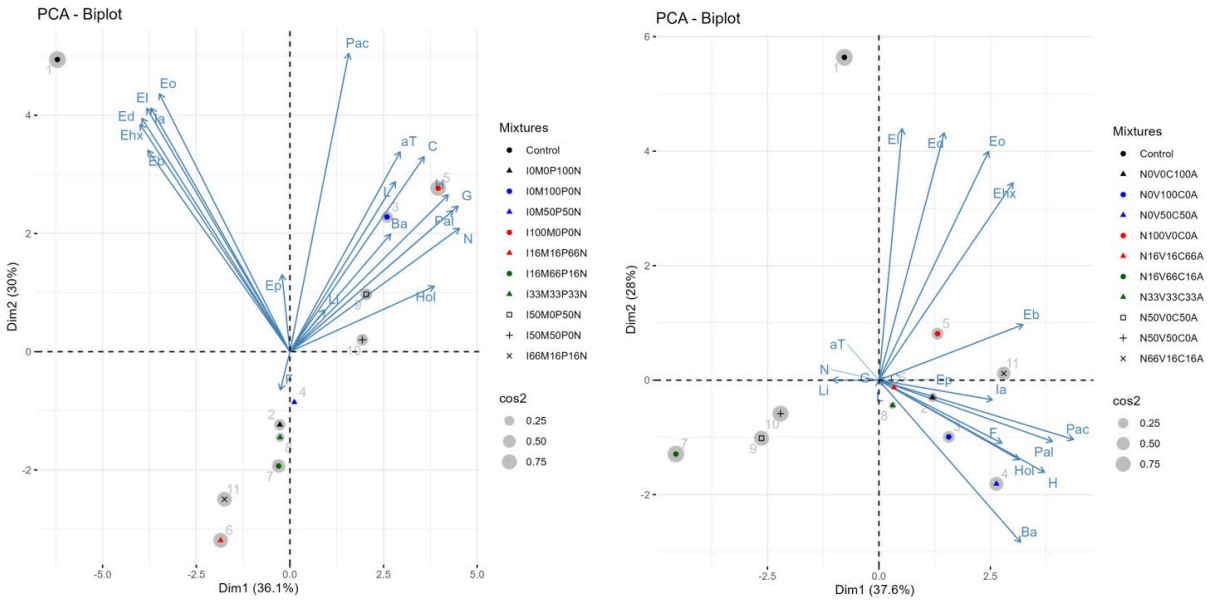
Table 4. Volatile compounds quantified (mean ± SD µg/L) in Pisco Negra Criolla.

Compound	Control	N0V0C100A	N0V100C0A	N0V50C50A	N100V0C0A	N16V16C66A	N16V66C16A	N33V33C33A	N50V0C50A	N50V50C0A	N66V16C16A
Terpenes											
Limonene	14.41 ± 1.3 ^{a}	9.1 ± 1.39 ^{a}	7.61 ± 1.4 ^{a}	16.35 ± 0.18 ^{a}	7.64 ± 0.45 ^{a}	2.94 ± 00 ^{a}	12.85 ± 0.11 ^{a}	13.07 ± 1.14 ^{a}	17.27 ± 1.57 ^{a}	15.55 ± 2.27 ^{a}	16.08 ± 0.76 ^{a}
C6 alcohols											
1-Hexanol	191.18 ± 5.99 ^{a}	339.59 ± 27.75 ^{a}	291.34 ± 41.68 ^{a}	387.54 ± 12.07 ^{a}	252.21 ± 34.49 ^{a}	210.14 ± 25.47 ^{a}	125.2 ± 1.16 ^{a}	303.78 ± 5.43 ^{a}	255.2 ± 25.75 ^{a}	231.21 ± 13.12 ^{a}	297.92 ± 2.59 ^{a}
3-Hexen-1-ol	26.65 ± 0.0 ^{a}	26.65 ± 0.0 ^{a}	36.72 ± 1.65 ^{ab}	55.6 ± 5.03 ^{b}	34.8 ± 4.81 ^{ab}	26.65 ± 0.0 ^{a}	26.65 ± 0.0 ^{a}	26.65 ± 0.0 ^{a}	26.65 ± 0.0 ^{a}	26.65 ± 0.0 ^{a}	40.68 ± 2.76 ^{ab}
Esters											
Ethyl butanoate	21.39 ± 2.59 ^{a}	5.99 ± 0.37 ^{a}	25.28 ± 3.27 ^{a}	25.1 ± 0.25 ^{a}	32.12 ± 3.88 ^{a}	19.3 ± 0.6 ^{a}	8.28 ± 0.68 ^{a}	26.48 ± 0.93 ^{a}	n.d	17.34 ± 1.78 ^{a}	31.66 ± 0.56 ^{a}
Ethyl decanoate	536.58 ± 52.45 ^{a}	217.79 ± 4.01 ^{b}	223.71 ± 29.38 ^{b}	215.81 ±15.91 ^{b}	229.03 ± 10.89 ^{b}	202.67 ± 2.25 ^{b}	70.36 ± 8.07 ^{b}	236.2 ± 7.17 ^{b}	127.39 ± 13.45 ^{b}	152.88 ± 8.21 ^{b}	220.22 ± 7.81 ^{b}
Ethyl hexanoate	93.68 ± 7.42 ^{a}	61.32 ± 5.01 ^{a}	43.8 ± 5.78 ^{a}	43.2 ±2.51 ^{a}	82.17 ± 8.37 ^{a}	47.58 ± 4.02 ^{a}	5.5 ± 0.54 ^{a}	44.21 ± 0.81 ^{a}	27.94 ± 0.06 ^{a}	24.46 ± 2.16 ^{a}	79.97 ± 1.45 ^{a}
Ethyl laurate	1.2 × 10^{3} ± 50 ^{a}	442.77 ± 29.03 ^{b}	447.24 ± 45.07 ^{b}	418.4 ± 2.51 ^{b}	362.61 ± 33.06 ^{b}	484.76 ± 18.39 ^{b}	220.74 ± 1.53 ^{b}	473.15 ± 8.26 ^{b}	303.24 ± 4.18 ^{b}	407.33 ± 13.22 ^{b}	423.76 ± 10.86 ^{b}
Ethyl octanoate	173.85 ± 21.1 ^{a}	112.18 ± 13.25 ^{a}	90.65 ± 13.34 ^{a}	88.16 ± 0.38 ^{a}	134.25 ± 16.38 ^{a}	93.97 ± 4.09 ^{a}	41.41 ± 0.7 ^{a}	90.72 ± 1.59 ^{a}	67.31 ± 0.31 ^{a}	65.52 ± 0.05 ^{a}	118.75 ± 2.23 ^{a}
Isoamyl acetate	376.24 ± 49.38 ^{a}	882.59 ± 46.36 ^{a}	430.5 ± 51.97 ^{a}	441.84 ± 5.76 ^{a}	458.38 ± 18.66 ^{a}	819.12 ± 24.56 ^{a}	306.15 ± 17.66 ^{a}	481.18 ± 21.5 ^{a}	460.35 ± 6.68 ^{a}	398.96 ± 6.36 ^{a}	895.0 ± 25.39 ^{a}
2-Phenylethyl acetate	222.56 ± 10.15 ^{ab}	356.41 ± 42.75 ^{ab}	362.73 ± 43.99 ^{ab}	400.9 ± 2.04 ^{ab}	375.58 ± 45.54 ^{ab}	310.86 ± 0.47 ^{ab}	179.73 ± 1.82 ^{a}	281.78 ± 5.05 ^{ab}	259.19 ± 3.38 ^{ab}	243.36 ± 3.95 ^{ab}	474.09 ± 11.08 ^{b}
Aldehydes											
Furfural	64.62 ± 2.84 ^{ab}	78.0 ± 7.6 ^{ab}	147.55 ± 13.54 ^{a}	108.52 ± 0.21 ^{ab}	80.61 ± 4.19 ^{ab}	60.26 ± 1.63 ^{ab}	51.01 ± 1.37 ^{b}	101.91 ± 4.45 ^{ab}	50.69 ± 0.13 ^{b}	63.07 ± 1.81 ^{ab}	64.8 ± 2.35 ^{ab}
Alcohols											
Benzyl alcohol	117.51 ± 3.79 ^{ab}	489.48 ± 9.55 ^{ab}	632.97 ± 10.62 ^{ab}	734.82 ± 1.25 ^{ab}	439.47 ± 9.22 ^{ab}	513.76 ± 12.32 ^{ab}	255.23 ± 1.16 ^{a}	685.26 ± 8.58 ^{bc}	312.15 ± 5.37 ^{ab}	332.79 ± 6.84 ^{ab}	445.82 ± 7.18 ^{b}
Phenylethyl alcohol	15.8 × 10^{3} ± 1790 ^{a}	34.7 × 10^{3} ± 3400 ^{a}	26.2 × 10^{3} ± 3900 ^{a}	29.9 × 10^{3} ± 280 ^{a}	25.0 × 10^{3} ± 3680 ^{a}	33.9 × 10^{3} ± 1700 ^{a}	10.9 × 10^{3} ± 150 ^{a}	21.1 × 10^{3} ± 60 ^{a}	20.1 × 10^{3} ± 490 ^{a}	19.7 × 10^{3} ± 330 ^{a}	35.9 × 10^{3} ± 130 ^{a}

Values with different letters in the same line are significantly different according to the Tukey test (95%). Codes for the samples are shown in Table 2.

Overall, comparing the volatile composition of Italia and Negra Criolla Piscos inoculated with different yeast starters, it can be concluded that Piscos from the Italia grape variety showed a higher amount of varietal volatile compounds such as geraniol, linalool, nerol, and citronellol, compared to Piscos from the Negra Criolla variety, in which these compounds are practically absent (Table 3) and (Table 4). Additionally, co-inoculation with the major native NSYSs isolated from Italia and Negra Criolla grapes affected the volatile composition of Piscos compared to the control in a different way depending on the specific yeast blend.

Thus, in order to have a better knowledge of the differences in the volatile composition of the Piscos produced with the NSYS blends, a PCA was run. Figure 2a,b show the PCA biplots obtained with the first two principal components (PCs), built with the quantitative data of the volatile compounds determined in Piscos from Italia and Negra Criolla grape varieties (Tables 3 and 4).



(**a**) (**b**)

Figure 2. PCA biplots: (**a**) Italia Piscos; (**b**) Negra Criolla Piscos. Volatile compounds' labels: benzyl alcohol (Ba), citronellol (C), ethyl butanoate (Eb), ethyl decanoate (Ed), ethyl hexanoate (Ehx), ethyl laurate (ethyl dodecanoate) (El), ethyl octanoate (Eo), ethyl nonanoate (Ep), furfural (F), geraniol (G), 1-hexanol (H), 3-hexen-1-ol (Hol), isoamyl acetate (Ia), linalool (L), limonene (Li), nerol (N), 2-phenylethyl acetate (Pac), phenylethyl alcohol (Pal), α-terpineol (aT). Codes for the samples are shown in Tables 1 and 2.

As can be seen in Figure 2a, the first two PCs accounted for 60.8% of the variance in the data in the case of Piscos from the Italia grape variety. Here, it was possible to identify the relationships of different volatile compounds with each NSYS blend. As shown in Figure 2a, Pisco produced without inoculation (control) was related to some ethyl esters, such as ethyl butanoate, ethyl decanoate, ethyl hexanoate, ethyl laurate, ethyl nonanoate, and ethyl octanoate, which are produced during the fermentation process. Furthermore, Piscos produced with mono-inoculums of *M. pulcherrima* (I100M0P0N) and *P. terrícola* (I0M100P0N) were highly related to the terpenes citronellol, linalool, limonene, nerol, α-terpineol, and geraniol, and also with the alcohols 1-hexanol and 3-hexen-1-ol. On the contrary, Pisco inoculated with *N. vaughanmartiniae* (I0M0P100N) was not related to any of the volatile compounds identified in the samples. Therefore, the choice of some of these mono-inoculums might be a strategy to achieve distinctive and desirable aromatic profiles

in Pisco, since these terpene compounds are linked with floral and pleasant aromas in grape distilled spirits [26]. In the case of the binary co-inoculations, I50M0P50N and I50M50P0N, the effect was very similar, although lower, to that observed with the mono-inoculation with *M. pulcherrima* and *P. terrícola* (Figure 2a). Finally, in Piscos produced by tertiary co-inoculation, such as I66M16P16N, I16M66P16N, I16M16P16N, and I16M16P66N, a lower content of esters and, in general, of most volatile compounds compared to the control was observed (Figure 2a).

Figure 2b shows the PCA biplot for Piscos from Negra Criolla grapes. The two first principal components accounted for 62.8% of the variance in the data. Here, the differentiation between the control Pisco and Piscos inoculated with NSYSs is also quite evident. As shown in Figure 2b, the mono-inoculation with *V. carnescens* (N0V100C0A) and *A. pullulans* (N0V0C100A) was related to a higher concentration of some volatile compounds, such as isoamyl acetate, phenylethyl alcohol, 1-hexanol, and 2-phenylethyl acetate. Nonetheless, Pisco from the mono-inoculum of *V. heimaeyensis* (N100V0C0A) was also related to some esters, such as ethyl butanoate. Among binary co-inoculations, only Pisco N0V50C50A showed a strong association with certain volatile compounds, mainly alcohols, such as benzyl alcohol, furfural, 3-hexen-1-ol, 1-hexanol, phenylethyl alcohol, and 2-phenylethyl acetate. Interestingly, Piscos from the combination of both yeasts (*V. carnescens* and *A. pullulans*) exhibited higher amounts of these compounds compared to the Piscos from mono-inoculated yeast (N0V100C0A, N0V0C100A). Additionally, according to Figure 2b, from all the different yeast combinations, only the control Pisco and Pisco from the mono-inoculum of *V. heimaeyensis* (N100V0C0A) were related to ethyl esters. In fact, as previously commented, most co-inoculation treatments exhibited a lower content of this group of compounds compared to the control (Table 4). This effect might be related to the specific dynamics of spontaneous fermentation, where the diversity and succession of wild yeasts could promote a more varied enzymatic metabolism and a more active ester synthesis. It has been described that uncontrolled spontaneous fermentations allow multiple yeast strains to interact freely, which can lead to greater biodiversity of the enzymes [39]. These enzymes play a crucial role in the esterification of acids and alcohols, generating a wider range and perhaps a greater quantity of esters [40], as confirmed by the results from the control Pisco in this study. In contrast, directed mono-inoculation with selected yeasts can stabilize the fermentation process and restrict interspecific competition, which could result in a more homogeneous and predictable enzymatic production [39], and consequently, in lower levels of esters compared to spontaneous fermentation. Moreover, yeasts selected for co-inoculation may consume or modify ester precursors, such as fatty acids and higher alcohols, in a different way compared to wild yeasts, thus affecting the final concentration of these volatile compounds.

3.3. Sensory Evaluation

In order to investigate how the use of NSYSs could affect the sensory characteristics of Pisco, the intensity of the most characteristics aroma descriptors of the Italia and Negra Criolla Piscos previously selected by an expert sensory panel was assessed and compared to the control (spontaneous fermentation). The average intensity values reported by the expert panel for Italia and Negra Criolla Piscos are shown in Table S2 and Table S3, respectively. As shown in Table S2, citric was the sensory descriptor rated with the highest intensities (from 2.1 to 4.1) in all the Italia Piscos, while the lowest intensity rates were found for butter and oily flavor descriptors (from non-detected to 2). In the case of Piscos from Negra Criolla varieties (Table S3), the highest intensity scores were for syrup (from 2.7 to 4.5), alcohol (from 3.2 to 4.4), and nutty (from 2.8 to 4), while the lowest corresponded to butter (from 1.8 to 3.1) and aniseed (from 1 to 3.3). As shown in both tables, the use of different yeast treatments induced larger differences in the intensity scores of Piscos from both grape varieties.

For a better understanding of these differences and to check the relationships with the yeast blends used as inoculum, a PCA with the average intensity data for both types of

Piscos (from Tables S2 and S3) was performed. Figure 3a shows the graphic representation of the PCA for the Italia Piscos. The control Pisco was related to descriptors such as nutty, syrup, and, to a lesser extent, to floral aroma attributes. However, as previously commented, the co-inoculation treatments produced Piscos with quite different sensory profiles. For instance, Pisco I100M0P0N, produced with *Metschnikowia pulcherrima*, was very related to the citric attribute, which agrees with the higher number of terpene compounds quantified in this Pisco (Table 3, Figure 3a). Additionally, Pisco I0M0P100N, produced with a mono-inoculum of *Naganishia vaughanmartiniae*, was much related to butter, aniseed, and cooked vegetable attributes and very little related to oily, nutty, and spicy attributes. Interestingly, Pisco I0M100P0N, from *Pichia terrícola*, produced Piscos more similar to the control, which means, more related to floral, syrup, and nutty attributes, but contrary to the control, this Pisco also was related to oily aroma notes. According to previous studies [8,32], the floral, fruity, and nutty aroma attributes might be associated with certain ethyl esters such as ethyl decanoate and ethyl laurate, which in the present study have been positively identified in Piscos from the Italia variety (Table 3). As shown in Figure 3a, some co-inoculation treatments with several strains (binary and tertiary mixtures), such as I33M33P33N, I0M50P50N, I50M50P0N, and I16M66P16N, exhibited a poor expression of most aroma attributes, with the exception of I66M16P16N, which was related to some sensory attributes such as butter, anise, and cooked vegetables. Pisco Italia produced with 100% *Metschnikowia pulcherrima* (I100M0P0N) (Table 3 and Figure 3a) was also related to the herbaceous attribute, which, in general, is an undesirable aroma note associated with some compounds, such as 1-hexanol and 3-hexen-1-ol [8,32]. This agrees with the high amount of these volatile compounds detected in the Piscos from this yeast blend (Table 3).

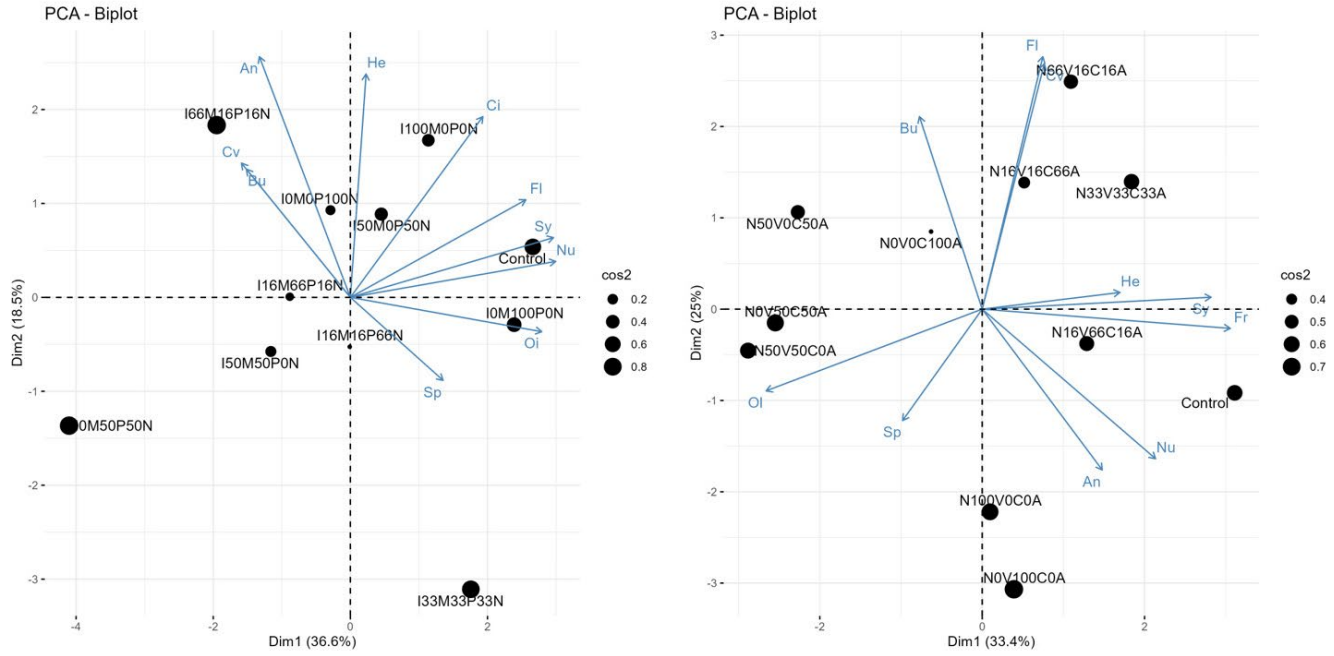


Figure 3. PCA biplots of sensory descriptors in control Pisco and in Piscos inoculated with different NSYSs: (**a**) Pisco Italia; (**b**) Pisco Negra Criolla. Sensory descriptors: alcohol (Al), aniseed (An), butter (Bu), citric (Ci), cooked vegetables (Cv), floral (Fl), fruity (Fr), herbaceous (He), nutty (Nu), oily (Oi), olive (Ol), spicy (Sp), syrup (Sy).

In the case of Negra Criolla Piscos, the PCA biplot (Figure 3b) shows that the control Pisco (N0V0C0A) was related to descriptors such as fruity, nutty, and syrup, followed by herbaceous and aniseed attributes. On the contrary, it was little related to butter, oily, and spicy flavor descriptors. Furthermore, the mono-inoculation with *Aureobasidium pullulans*

(N0V0C100A) was associated with an increase in the butter descriptor. Pisco produced from this inoculum was very little related to other sensory attributes characteristic of the control Pisco. In the case of Piscos N0V100C0A and N100V0C0A, from the mono-inoculums with *Vishniacozyma carnescens* and *Vishniacozyma heimaeyensis*, respectively, a positive relationship with some sensory attributes such as aniseed, nutty, and spicy was observed (Figure 3b). Interestingly, these Piscos exhibited a very different profile compared to the control. Co-fermentations with two and three NSYS species also tended to have a variable sensory profile compared to the control sample. For example, the floral and cooked vegetable attributes were higher in relation to N16V16C66A, N66V16C16A, and N33V33C33A Piscos, while the oily descriptor was mostly associated with N50V50C0A and N0V50C50A treatments, while herbaceous, syrup, and fruity attributes showed a higher association to N16V66C16A (Figure 3b).

4. Conclusions

Results from this study corroborate that Italia Piscos exhibited higher concentrations of varietal-specific volatile compounds, such as geraniol and linalool, which were almost absent in Negra Criolla Piscos. Additionally, this research has proven for the first time the large effect of co-inoculation with the major yeasts isolated from the grape skin of Italia and Negra Criolla grapes in the volatile and sensory profile of Piscos produced from these grape varieties. The mono-inoculation with *Metschnikowia pulcherrima*, or the co-inoculation of this yeast with *Naganishia vaughanmartiniae*, contributes to the increase in some terpene compounds such as citronellol, geraniol, linalool, and limonene, which have been shown to significantly enhance the citric notes and decrease the perceived intensity of some off-flavors (cooked vegetables) in Piscos from the Italia grape variety. In the case of Piscos from the Negra Criolla grape variety, mono-inoculation with the major yeasts isolated from the skin (*Aureobasidium pullulans*, *Vishniacozyma carnescens*, and *Vishniacozyma heimaeyensis*) increases the concentration of some volatile compounds (phenylethyl alcohol, benzyl alcohol, isoamyl acetate, phenylethyl acetate, and 1-hexanol). Additionally, Piscos produced from *V. carnescens* and *V. heimaeyensis* exhibit a significant enhancement of the aniseed and alcohol aroma notes and a reduction in the cooked vegetable descriptor compared to the control. Although new studies will be necessary in more controlled conditions in order to discard the contribution of the potential indigenous yeasts, the findings of this study emphasize the potential of using autochthonous NSYS blending for modulating the aroma characteristics of Pisco, indicating that this might be a promising tool for oenological innovation in Pisco production.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/beverages10040126/s1, Table S1. Reference compounds and calibration curves used for quantification of Pisco volatiles. Table S2. Intensity values (mean ± SD) for characteristic flavor attributes of Pisco Italia. Table S3. Intensity values (mean ± SD) for characteristic flavor attributes of Pisco Negra Criolla. Figure S1. Viable cell counts found in the grape musts (first 24 h of fermentation) inoculated with the different NSYSs isolated from the grape surface. Grape Italia (*Metschnikowia pulcherrima*—M; *Pichia terrícola*—P; *Naganishia vaughanmartiniae*—N). Grape Negra Criolla (*Vishniacozyma heimaeyensis*—V); *Vishniacozyma carnescens*—C; *Aureobasidium pullulans*—A). Figure S2. Total yeast population count (CFU/g of must) in Italia and Negra Criolla grape musts during 12 days of fermentation. The values reported in the graph at each sampling time represent the mixtures of inoculated yeast and the control sample (uninoculated). Codes for the samples are shown in Tables 1 and 2.

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