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Impact of Stabilization Method and Filtration Step on the Ester Profile of "Brandy de Jerez"

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Abstract: Brandy stabilization is an important step aimed at decanting the suspended organic and inorganic particles that may cause undesirable turbidity (cloudiness or haze) in brandies, affecting the physico-chemical stability, the organoleptic characteristics, and the consumer's quality perception of the brandy. This phenomenon originates from insoluble salts, volatile compounds (higher alcohols, fatty acid esters, and others), and ethanol-soluble lignins. Among them, ethyl esters of long-chain fatty acids are considered the main cause of haze formation, due to a decrease in their solubility when brandies are stored at low temperatures. For this reason, producers are recommended to intentionally encourage the formation of haze and then to remove it before releasing the brandy to the market. The purpose of this work was to study the influence of two methods of stabilization, the traditional method at room temperature for 1 year, and cold stabilization for 7 days at −10 °C, on the ester profile of "Brandy de Jerez". The results were compared with non-stabilized samples, to determine the main changes in the volatile composition. The use of multivariate statistical analyses made it possible to identify the esters (potential markers) most impacted by the stabilization process. It was observed that traditional stabilization yielded the most distinct ester profile, while brandies stabilized at cold temperature displayed a lower impact on their volatile composition. Furthermore, both stabilization processes produced a significant decrease in ethyl esters of long-chain fatty acids, which are the compounds responsible for haze formation.

Keywords: esters; brandy; HS-SPME/GC-MS; cold stabilization; multivariate statistics

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1. Introduction

Brandy is a matured spirit obtained by distillation of wine. Its consumption is extensive throughout the world, and Spain is one of the world's leading producers, with the so-called "Brandy de Jerez". This product is elaborated in the Southern Spanish area known as *Marco de Jerez* under a protected geographical indication [1], following the specification provided by the Technical File [2]. "Brandy de Jerez" is produced from *holandas* (<70% *v*/*v* of ethanol), spirits (70–86% *v*/*v* of ethanol) or wine distillates (86–94.8% *v*/*v* of ethanol), and its organoleptic equilibrium is reached by ageing in American oak (*Quercus alba*) casks of a capacity lower than 1000 L and previously seasoned with Sherry wines. This ageing process follows the traditional dynamic system known as *Criaderas* and *Solera* [3]. Three quality categories can be distinguished according to the regulation of the "Brandy de Jerez" and their ageing: *Solera* brandy (minimum 6 months of ageing), *Solera Reserva* brandy (minimum 1 year), and *Solera Gran Reserva* brandy (more than 3 years).

The final characteristics of this product are determined by the different steps followed during the production process, such as the selection of the raw material, the winemaking, the distillation system (alquitara, charentais alembic, or distillation column) used to distil

the wine, the ageing or maturation in American sherry casks, and finally the stabilization of the drink before bottling [4]. Brandy stabilization is an important step aimed at decanting the suspended organic and inorganic particles that may cause undesirable turbidity (commonly referred to as cloudiness or haze) in brandies. This turbidity can change the physico-chemical properties of the brandy once bottled, and it affects the organoleptic characteristics of the final drink and the perception of consumers about the quality of this product. Cloudiness often appears in fruit brandies when the alcohol content is below 45% v/v, as well as at temperatures below 7 °C, usually reached during storage and transportation [5]. In addition, haze can slowly form in the final product after bottling. For this reason, producers are recommended to intentionally encourage the formation of haze and then to remove it before releasing the brandy to the market [6]. Haze formation originates from insoluble salts (potassium bitartrate, calcium tartrate, calcium oxalate), volatile compounds (including esters), and ethanol-soluble lignins [6]. Among the volatile metabolites, ethyl and isoamyl esters of long-chain fatty acids, phenylethanol, and ethyl lactate have been described to be linked to haze formation [4,7,8].

There are several ways to reduce the cloudiness in spirit beverages, such as the use of activated carbon, an adsorbent and hydrophobic material that is able to trap volatile compounds and adsorb organic compounds [9], or based on filtration materials (cellulose, carbon, diatomaceous earth, or candle filters) that need to be carefully chosen to prevent loss of flavour compounds [7]. A common practice for removing the haze formed in alcoholic beverages (particularly red, sparkling, and sweet fortified wines and brandies) is a stabilization process at room temperature (around 25 °C) for 7–30 days, followed by filtration before bottling. However, this method may not complete all the physicochemical reactions and insolubilizations that can occur in brandies, and the haze may not be completely removed after filtration. Additionally, new insolubilizations may appear in brandies stabilized by means of this method when exposed to low temperatures, due to destabilization of colloidal equilibriums. This can be a problem for those brandies that are exported to regions with cold climates, since during shipping bottles may remain at low temperatures for long periods of time, creating insolubilizations in the final product. In this sense, a common alternative to prevent haze formation is cold stabilization [4,10], by freezing the spirit at temperatures below −5 °C for a few days, followed by subsequent filtration before bottling [7,11,12].

Previous works carried out on fruit brandies (apricot, plum, and rye) studied different conditions of stabilization, such as the temperature and filtration systems [4,5,7,12]. The results showed the influence of these factors on the final turbidity of the samples, obtaining the best results with intermediate values of temperature (from −1 to −4 °C) and filter pore size (filter sheets with a higher nominal retention rate, >0.7 µm, and membranes with 800 nm pore size). However, most of the studies focused on the effect of the stabilization and filtration processes on the physical characteristics of the brandies, ignoring their effect on the aroma quality. In this sense, esters are one of the most important families of volatile compounds impacting the aroma of brandies, being the main known contributors to the flower and fruity notes [13]. In brandies, these compounds are mainly originated during wine fermentation and are modulated during wine maturation, distillation, and brandy ageing [14]. Their relevance for the organoleptic properties of brandies comes from their low perception threshold and their contribution to the brandy aroma through synergistic interactions, even at concentrations below their perception thresholds [15].

Thus, the objective of the present work was to compare the influence of two methods of stabilization on the ester profile of "Brandy de Jerez": the traditional method at room temperature for 1 year, to assure that all physicochemical reactions and insolubilizations that may occur during stabilization have been completed, followed by filtration; and cold stabilization for 7 days at −10 °C, followed by filtration. The ester profile obtained with both stabilization processes was also compared with the ester profile of brandies without stabilization.

2. Materials and Methods

2.1. Brandy Samples

The brandies used in this work were from Jerez de la Frontera (Spain), from the protected geographical indication (PGI) [1] "Brandy de Jerez". The samples were elaborated following the traditional dynamic system called *Criaderas* and *Solera* as is established in the regulations of the Regulatory Council of "Brandy de Jerez" [16]. A total of 27 brandies were prepared: 9 non-stabilized (NonStab), 9 stabilized at cold temperature (ColdT), and 9 stabilized at room temperature (RoomT).

First, 1 brandy *Solera* (36% *v*/*v* of alcohol and 6–9 months of ageing), 3 *Solera Reserva* (36% *v*/*v* and 12–18 months) and 5 *Solera Gran Reserva* (40% *v*/*v* and 6–12 years) were elaborated at Bodegas Fundador, corresponding to the non-stabilized brandies. The formulations of the younger scales of the *Criaderas* and *Solera* from the dynamic ageing systems of the brandies included two or more of the following distillates: holandas (65% *v*/*v*), column spirits (77% *v*/*v*), and high-grade wine distillates (94.7% *v*/*v*). The hydrations of the final *Solera* and *Solera Reserva* brandies were adjusted to 36% *v*/*v* and the *Solera Gran Reserva* to 40% *v*/*v* in stainless steel tanks.

Then, a total of 50 L from industrially elaborated brandies were taken, to prepare brandies stabilized using two static methods (using 20 L of brandy in each method), which were carried out in darkness and repeated in two consecutive years. The stabilization methods consisted of:

- Traditional stabilization: room temperature (20 ± 5 °C) for 1 year, followed by filtration with PALL CORPORATION plates (New York, NY, USA) Seitz K-200 series.
- Cold stabilization: low temperature (-10 ± 1 °C) stabilization for 7 days and filtration using Seitz PALL CORPORATION plates (New York, NY, USA) Seitz K-200 series at -10 °C.

2.2. Chemicals

High-performance liquid chromatography (HPLC)-grade ethanol was obtained from J.T. Baker Chemicals B.V. (Denventer, The Netherlands). Milli-Q water was produced by a Milli-Q Plus water system (Millipore, Madrid, Spain). Ethylenediaminetetraacetic acid (EDTA) was supplied by Panreac Applichem (Barcelona, Spain). Sigma-Aldrich (Madrid, Spain) supplied the following: sodium chloride ACS reagent grade (purity ≥99.8%), and the standard compounds ethyl butyrate (≥99%), ethyl hexanoate (≥99%), ethyl octanoate (≥99%), ethyl decanoate (≥99%), ethyl dodecanoate (≥99%), ethyl myristate (≥99%), ethyl palmitate (≥99%), ethyl stearate (≥99%), propyl acetate (≥99%), isobutyl acetate (≥99%), isoamyl acetate (≥99%), hexyl acetate (≥99%), phenylethyl acetate (≥99%), ethyl isobutyrate (98%), ethyl 2-methylbutyrate (≥99%), ethyl isovalerate (≥99%), ethyl phenylacetate (98%), ethyl cinnamate (98%), ethyl dihydrocinnamate (98%), methyl hexanoate (≥99%), methyl octanoate (≥99%), methyl decanoate (≥99%), isoamyl butyrate (98%), ethyl heptanoate (98%), ethyl nonanoate (98%), ethyl propanoate (≥99%), isobutyl hexanoate (≥99%), ethyl valerate (≥99.7%), and acetaldehyde (≥99.5%). The deuterated internal standards [$^2H_3$]-ethyl butyrate, [$^2H_{11}$]-ethyl hexanoate, [$^2H_{15}$]-ethyl octanoate, [$^2H_5$]-ethyl trans-cinnamate, [$^2H_{23}$]-ethyl dodecanoate, [$^2H_{27}$]-ethyl myristate, [$^2H_{31}$]-ethyl palmitate, and [$^2H_{35}$]-ethyl stearate were supplied by CDN isotopes (Pointe-Claire, QC, Canada).

2.3. HS-SPME-GC-MS

Sample Preparation and Extraction Conditions

A previously developed methodology was used to determine the esters in the brandies [17]. Briefly, 25 mL of brandy sample was spiked with 20 µL of an internal standard mix solution of 8 deuterated compounds at 200 mg/L. The peak integration of each ester was normalized using specific deuterated standards, as follows: ethyl isobutyrate, ethyl 2-methylbutyrate, ethyl isovalerate, ethyl propanoate, ethyl butyrate, isoamyl acetate, ethyl valerate, propyl acetate, and isobutyl acetate were normalized with [$^2H_3$]-ethyl butyrate. Ethyl hexanoate, methyl hexanoate, methyl octanoate, isoamyl butanoate,

isobutyl hexanoate, ethyl heptanoate, and hexyl acetate were normalized with [$^2H_{11}$]-ethyl hexanoate. [$^2H_{15}$]-Ethyl octanoate was used to normalize ethyl octanoate, methyl decanoate. Phenylethyl acetate, ethyl dihydrocinnamate, and ethyl cinnamate were normalized with [$^2H_5$]-ethyl trans-cinnamate; while ethyl dodecanoate, ethyl decanoate, ethyl nonanoate, and ethyl phenylacetate were normalized with [$^2H_{23}$]-ethyl dodecanoate. Finally, [$^2H_{27}$]-ethyl myristate, [$^2H_{31}$]-ethyl palmitate, and [$^2H_{35}$]-ethyl stearate were used to normalize ethyl myristate, ethyl palmitate, and ethyl stearate, respectively. Then, the spiked samples were diluted in EDTA solution (200 mM and pH adjusted to 7 with NaOH 1 M), since this chemical prevents oxidation of the compounds [18]. Afterwards, the brandy samples were diluted to reach 7.2% v/v ethanol, and 10 mL of this solution was transferred to a 20 mL SPME vial containing 3.5 g of NaCl, to increase the ionic strength of the analytes and release more volatiles into the headspace [18,19]. The vials were capped, and the solution was homogenized using a vortex shaker for 30 s and placed in a Combipal autosampler tray (CTC Analytics, Zwingen, Switzerland).

A 100 µm PDMS fiber (Supelco, Bellefont, PA, USA), previously conditioned according to the supplier recommendation, was used for extraction of esters via HS-SPME. For the equilibrium step, the vials were incubated at 500 rpm for 2 min at 33 °C, while the extraction was performed at the same temperature and agitation conditions for 55 min.

The conditions of the gas chromatograph were as follows: the desorption time and temperature were set at 15 min and 250 °C, respectively, performed in a Trace GC ultragas chromatograph (Thermo Fisher Scientific S. p.A., Rodano, Milan, Italy). The desorbed volatiles passed to an ISQ Single MS spectrometer (Thermo Fisher Scientific, Austin, TX, USA). The injection was performed in splitless mode, and the column used for volatile separation was a BP21 column of 50m × 0.32 mm and 0.25 µm film thickness (SGE Analytical Science, Milton Keynes, UK). The carrier gas was helium at a constant flow rate of 1.7 mL/min. The oven temperature program was set at 40 °C for 5 min, raised to 220 °C at 3 °C/min, and held for 30 min. The MS operated in electron ionization mode at 70 eV using selected-ion-monitoring (SIM) mode. The transfer line and source temperature of the MS were set at 230 °C and 200 °C, respectively. The identification procedure was performed by comparing the retention times and mass spectra with those of the pure standards. All the samples were analyzed in duplicate by repeating the extraction procedure two times for each sample, in order to reduce the contribution of experimental and analytical variability to the final concentration measurements.

2.4. Statistical Analysis

Quantification of the samples was performed by fitting calibration curves with the commercial standard of each ester. Then, to check the structure of the data and to look for grouping of the brandy samples, a principal component analysis (PCA) was fitted [20]. Afterwards, the main differences between samples related to the stabilization process were studied by means of a multilevel partial least squares discriminant analysis (ML-PLS-DA), due to the paired structure of the data [21]. Multilevel decomposition was applied, due to the paired structure of the data, with three measures for the same samples: before and after cold stabilization and filtration, allowing focusing on the effect of this treatment, regardless the initial composition of each brandy. The model was optimized and validated by means of a described previously double cross-validation scheme [22], using leave-one-out cross validation of both nested loops and the balanced error rate (BER) as diagnostic statistics. The p-value of the model was obtained from a permutation test (n = 1000, since this was large enough to sample the tails of the distribution and to attain a p-value up to 0.001) for BER, area under the receiver operating characteristic curve (AUROC), and the average number of misclassified (NMC). The p-value of the model was calculated as follows:

$$p\text{-value} = \frac{1 + (\text{Diagnostic}_P \leq \text{Diagnostic})}{N} \quad (1)$$

where ($Diagnostic_P \leq Diagnostic$) is the number of elements in the H_0 distribution that are smaller or equal to the diagnostic (BER, AUROC, or NMC) of the original data.

The most discriminatory metabolites were selected using an iterative process based on previously described variable importance in projection (VIP) scores [23]. Briefly, the method was based on selecting the variables with a VIP value above a changing threshold during an iterative procedure: as long as the model improved, the threshold was increased and a new model with a reduced number of variables was fitted. This process was performed within a double-cross validation scheme repeated 100 times. Finally, the variables with high stability (those that remained in the final model) above 70% were chosen. The statistical analyses were performed using the software R version 4.0.3 and the package *mixOmics* [24].

3. Results and Discussion

Esters in brandies are mainly derived from the initial distillate, which depends on the base wine used for distillation (where its conservation in the presence or absence of lees, has an important influence), although they are modulated during ageing [25,26]. The most abundant family of esters measured in the brandy samples was the ethyl esters of fatty acids, with concentrations ranging from 11.6 to 48.8 mg/L, being in agreement with those found in previous studies with similar samples [27,28]. Among them, ethyl octanoate (that ranged from 9 to 18 mg/L) followed by ethyl decanoate (from 0.034 to 20.1 mg/L) displayed the overall highest concentrations. These compounds are considered important contributors to the aroma of distilled beverages and have been identified as odor active compounds in distillates [27,29]. Meanwhile, higher alcohol acetates and ethyl esters of branched acids were found in concentrations of 1.27–3.81 mg/L and 0.14–1.76 mg/L, respectively, with isoamyl acetate being the most abundant acetate ester, in accordance with the literature [27].

3.1. Effect of Ageing

A principal component analysis (PCA) was carried out to study the main variation sources in the data and to determine the grouping of samples. The first two principal components of the PCA accounted for the 61% of the total sample variability (Figure 1), the main variation source being explained by PC1 (39%), which was related with the ageing of the brandies (Figure 1A). It was observed that *Solera* and *Solera Reserva* brandies with a more similar period of ageing, averaging 6–9 and 12–18 months, respectively (Figure 1A), displayed a similar ester profile, characterized by higher levels of isoamyl acetate, hexyl acetate, isobutyl acetate, ethyl butyrate, methyl hexanoate, propyl acetate, ethyl octanoate, phenylethyl acetate, ethyl valerate, isobutyl hexanoate, methyl octanoate, isoamyl octanoate, ethyl dihydrocinnamate, and ethyl hexanoate (Figure 2A). Meanwhile, the ester profile of the *Solera Gran Reserva* brandies (averaging 6–12 years of ageing) displayed a more differentiated ester profile, with an overall increase of the rest of the esters analyzed, including ethyl 2-methylbutyrate, ethyl nonanoate, ethyl phenylacetate, ethyl isovalerate, ethyl isobutyrate, ethyl decanoate, ethyl dodecanoate, ethyl propanoate, ethyl heptanoate, isoamyl butanoate, ethyl myristate, and ethyl cinnamate. Among these, ethyl isobutyrate, ethyl 2-methylbutyrate and ethyl decanoate have been described as olfactive markers of a similar product (Calvados), as well as isoamyl acetate [30], which, conversely, displayed lower concentrations in the *Solera Gran Reserva* brandies. These results demonstrated the distinctive and exclusive character of the brandies with a longer ageing period. Another study [31] also found that "Brandies de Jerez" *Solera Gran Reserva* displayed a clear difference in phenolic and furanic derivative profile from other brandies produced in different regions, indicating their highly specific character. The higher concentration of esters observed for the *Solera Gran Reserva* brandies may be related to the continuous esterification of fatty acids and ethanol, and losses of water and ethanol through evaporation effects and perspiration through the pores of the wood during ageing [26,32].

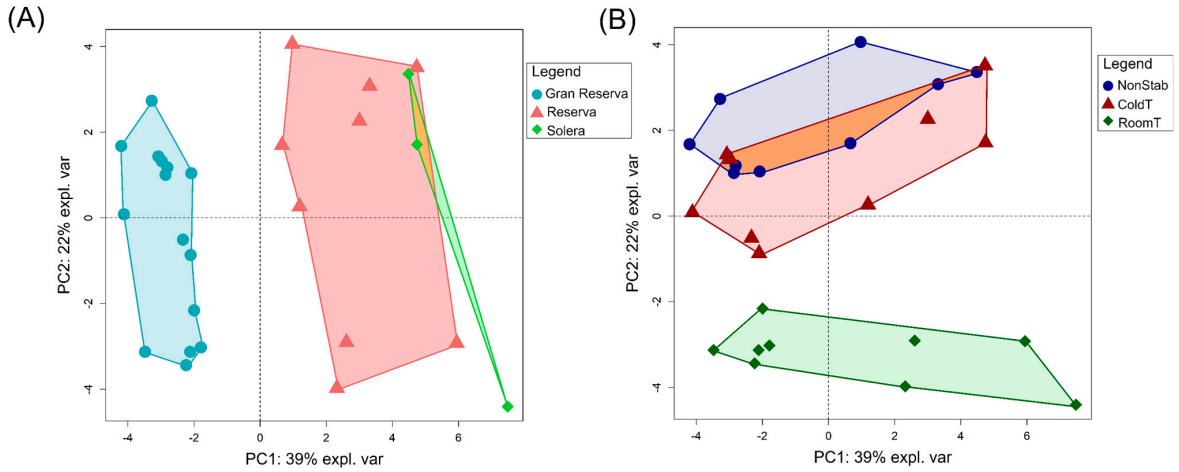

Figure 1. Principal component analysis (PCA) carried out on the brandy samples. Scores plot for the first two principal component highlighting brandies according to the ageing factor (**A**), and before and after cold stabilization and filtration treatment (**B**). NonStab: non-stabilized brandy; ColdT: brandy stabilized at cold temperature; RoomT: brandy stabilized at room temperature.

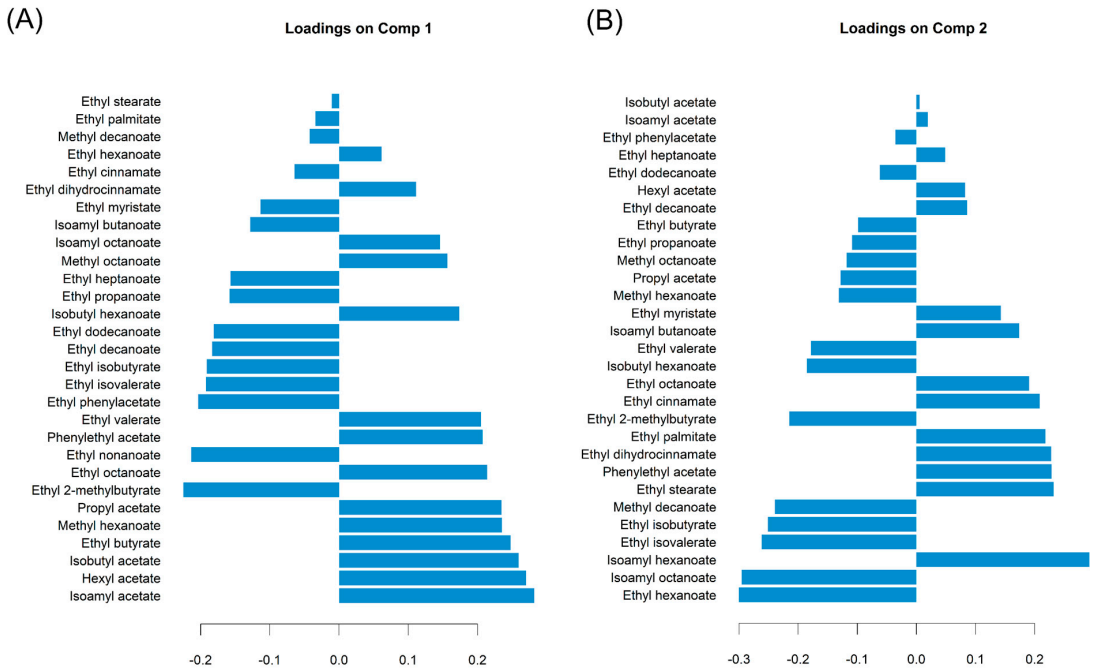

Figure 2. Loadings bar plot on component 1 (**A**) and component 2 (**B**) of the principal component analysis carried out on the brandy samples.

3.2. Effect of the Stabilization Process

Brandies before and after both stabilization processes are highlighted in the scores plot of Figure 1B. The most distinct ester profile was observed for the brandies submitted to room temperature stabilization, as is shown by a clear separation in component 2 of the PCA (Figures 1B and 2B). However, due to the large differences observed between brandies, predominantly related to the ageing and the paired structure of the data [21] with repeated measurements of the same samples (before stabilization, after cold stabilization, and after room temperature stabilization), differences related to stabilization were not clearly highlighted, especially for the samples stabilized at cold temperature. Therefore, a multilevel decomposition was applied, to focus on the effect of the treatment, and a partial least squares discriminant analysis (ML-PLS-DA) was performed for the three

classes: brandies before stabilization, after cold stabilization and filtration, and after room temperature stabilization and filtration (Figure 3).

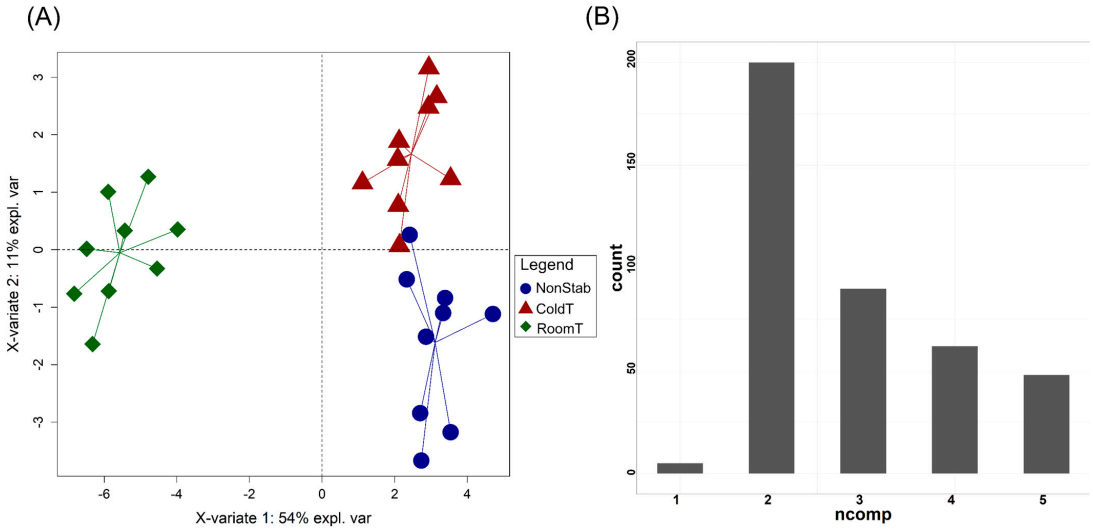

Figure 3. Graphical outputs of the multilevel partial least squares discriminant analysis (ML-PLS-DA) carried out to discriminate between the brandy samples before and after cold stabilization and filtration. (**A**) Scores plot for component 1 (X-variate 1) and component 2 (X-variate 2), and (**B**) histogram of the number of components optimized for the sub-models during the double-cross validation. NonStab: non-stabilized brandies; ColdT: brandy stabilized at cold temperature; RoomT: brandy stabilized at room temperature.

The model was optimized and validated following a double cross-validation scheme. The sub-models were predominantly optimized into two components, revealing the low complexity of the model (also shown during the PCA assessment) and reducing the risk of overfitting (Figure 3B). The ML-PLS-DA displayed a good performance, with a BER of 0.09 ± 0.05 (Table 1). Brandies stabilized at room temperature were correctly assigned in all the cases, supporting a more distinct ester profile, while the error of the model was due to incorrect assignment between brandies without stabilization and after cold stabilization. Therefore, this error suggested a lower impact on the ester profile when cold stabilization was applied. Then, the model was successfully validated using a permutation test. The null distributions calculated from the class label permutation are shown in Figure 4 for the three diagnostics statistics: BER, AUROC and NMC, which displayed an expected Gaussian shape (Figure 4). The average performance of the model fell in the tail of the null distribution for the three diagnostics, obtaining a *p*-value of the model below 0.05 in all the cases (Table 1). All these results supported the robustness of the ML-PLS-DA, being the most model suitable for analyzing and interpreting the dataset.

Table 1. Multilevel partial least squares discriminant analysis (ML-PLS-DA) performance (from a double cross-validation scheme).

Mean BER	Class	Mean Class Error	Predicted as NonStab	Predicted as ColdT	Predicted as RoomT	*p*-Value (Model)
0.09 ± 0.05	NonStab	0.15 ± 0.11	765	135	0	BER, AUROC, NMC: 0.001
	ColdT	0.14 ± 0.10	122	778	0	
	RoomT	0.00 ± 0.00	0	0	900	

BER: balanced error rate. NonStab: brandies before stabilization. ColdT: brandies with cold stabilization. RoomT: brandies with room temperature stabilization. *p*-value of the model obtained from a permutation test ($n = 1000$) for the balanced error rate (BER), area under the receiver operating characteristic curve (AUROC), and the average number of misclassified (NMC).

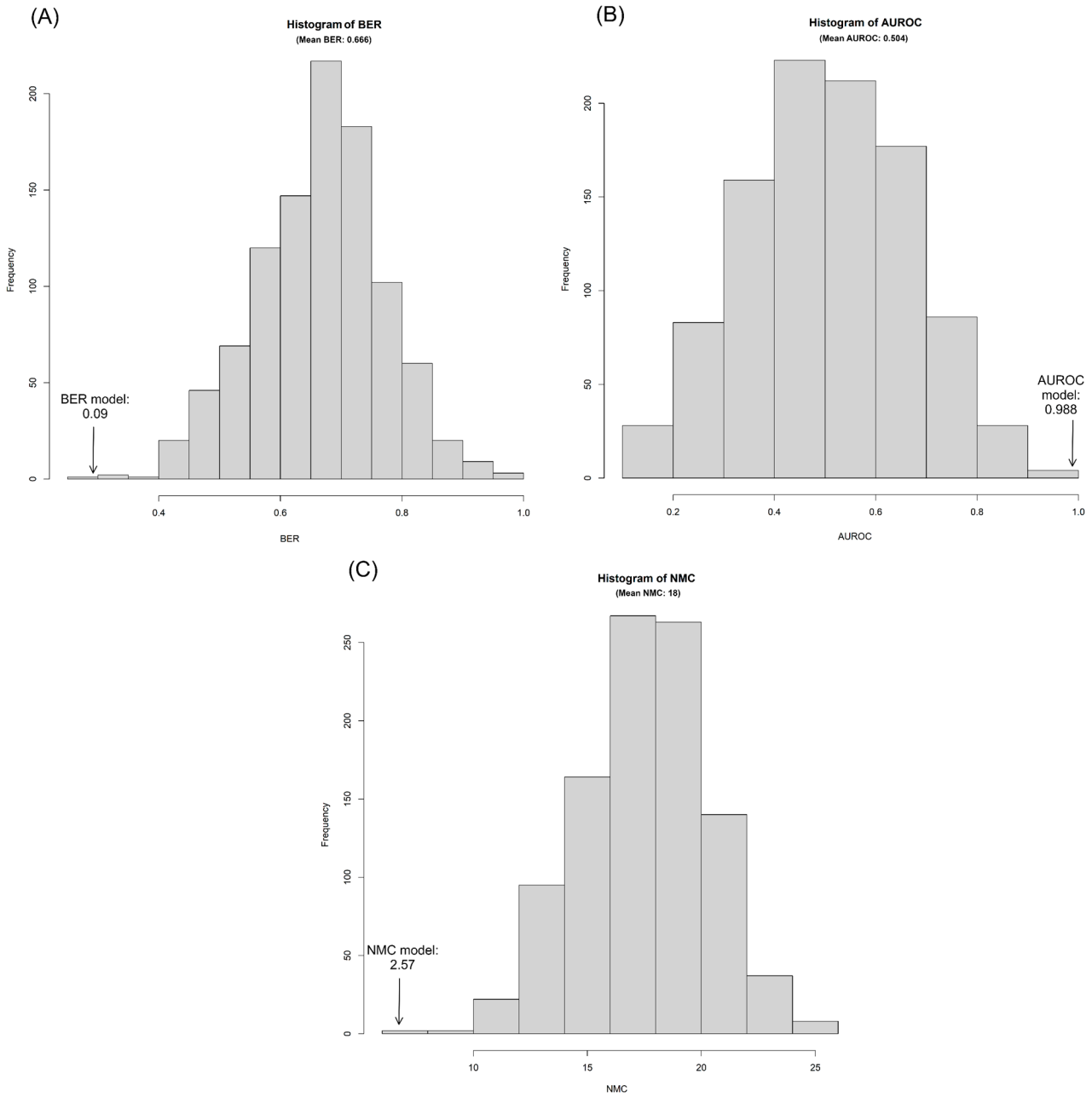

Figure 4. Results from the permutation test (n = 1000) performed on the multilevel partial least squares discriminant analysis (ML-PLS-DA). Histogram obtained from the permutation test for (**A**) the balanced error rate (BER), (**B**) area under the receiver operating characteristic curve (AUROC), and (**C**) the average number of misclassified (NMC). The real average performance of the model for the three statistics obtained from the double cross-validation is shown in each plot.

Differences in the ester profile of the brandies related to the stabilization process are shown in the scores and loadings plots of the ML-PLS-DA model (Figures 3A and 5). As was expected from the previous PCA, the brandies submitted to room temperature stabilization and filtration displayed the most distinct ester profile, being separated from the rest in component 1 (X-variate 1), which explained a high 54% of the variance. This stabilization process resulted in higher contents of many esters, such as isoamyl octanoate, ethyl isovalerate, ethyl isobutyrate, isobutyl hexanoate, ethyl 2-methylbutyrate, propyl acetate, ethyl butyrate, isoamyl acetate, methyl hexanoate, ethyl hexanoate, ethyl valerate, methyl decanoate, and isobutyl acetate. The percentage of change related to the control

brandy (before stabilization) is shown in Supplementary Table S1. Meanwhile, other esters such as isoamyl hexanoate, isoamyl butanoate, ethyl octanoate, hexyl acetate, phenylethyl acetate, ethyl cinnamate, ethyl decanoate, ethyl heptanoate, ethyl palmitate, ethyl dihydrocinnamate, ethyl stearate, ethyl nonanoate, and ethyl myristate displayed a significant decrease in their concentration.

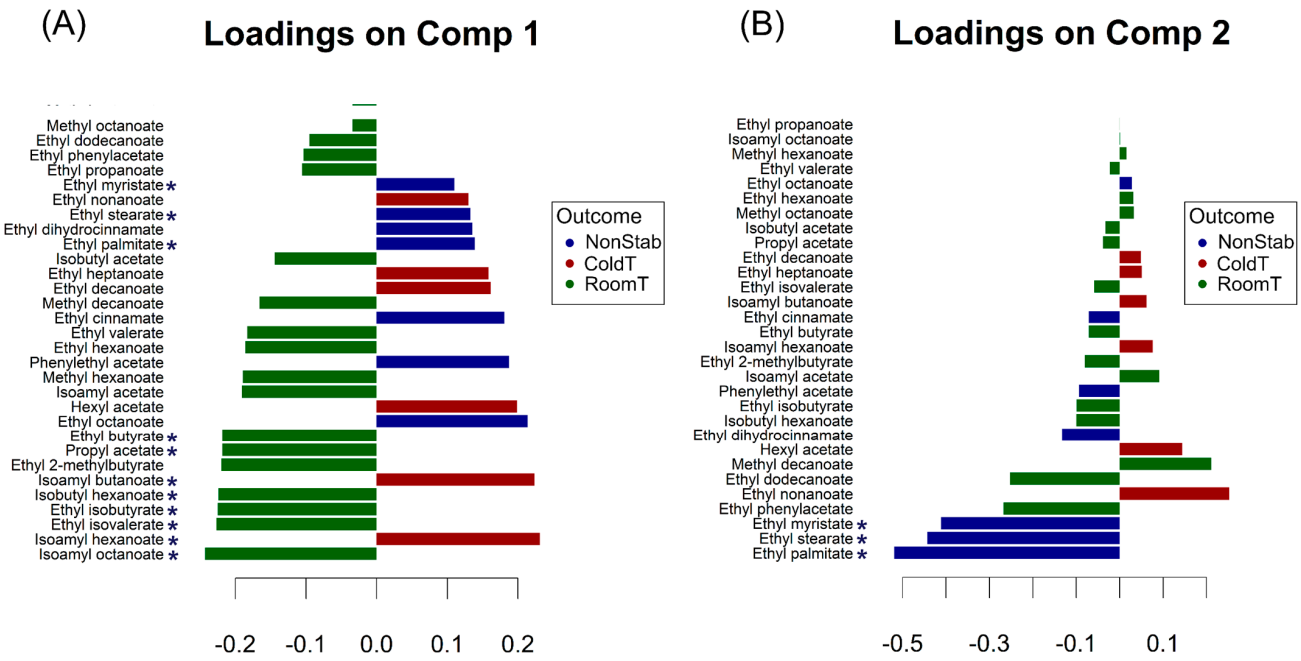

Figure 5. Loadings contribution barplot on component 1 (**A**) and component 2 (**B**) of the multilevel partial least squares discriminant analysis (ML-PLS-DA) carried out to discriminate between brandy samples before and after cold stabilization and filtration. Color indicates the class for which the compound had a maximal mean value. Bar length represents the multivariate regression coefficient, with either a positive or negative sign for that particular feature of each component, i.e., the importance of each variable in the model. NonStab: non-stabilized brandies; ColdT: brandy stabilized at cold temperature; RoomT: brandy stabilized at room temperature. An asterisk highlights the most discriminative compounds selected during a variable reduction procedure (i.e., potential markers related with the stabilization method).

The brandies submitted to cold stabilization were separated from those before stabilization in component 2 (X-variate 2), which explained 11% of the total variance (Figures 3A and 4B). This meant that the volatile profile of these brandies was less impacted by the stabilization process. This was mainly driven by a decrease in ethyl esters of long-chain fatty acids: ethyl palmitate, ethyl stearate, and ethyl myristate, with this method being effective for reducing these undesirable compounds, since turbidity of brandies is associated with ethyl esters of long-chain fatty acids due to decreases in their solubility, mainly related to low storage temperatures [7]. Therefore, a reduction of these compounds is beneficial in terms of ensuring the stability of brandies [33]. However, these ethyl esters displayed a greater decrease in brandies stabilized at room temperature, especially for ethyl stearate (decreasing below the detection limit in all the brandies, Supplementary Table S1). Meanwhile, both stabilization procedures yielded decreases of ethyl palmitate between 70–98% of the initial values and ethyl myristate between 11–87% (Supplementary Table S1). A previous study showed a similar reduction of ethyl myristate after brandy stabilization [7]. These results (lower content of ethyl esters of long-chain fatty acids and similar profile to the rest of the esters present in samples before stabilization) indicated a lower impact on the ester profile when cold stabilization was used, compared to room temperature stabilization.

A variable reduction procedure was performed to determine the esters most impacted by the stabilization process, i.e., the markers of this process. This procedure was repeated 100 times, and the compounds selected in at least the 70% of the iteration are highlighted in Figure 4 with an asterisk. Ethyl esters of long chain fatty acids were among the most impacted esters. The ester compounds selected as markers of room temperature stabilization were isoamyl octanoate, isoamyl hexanoate, ethyl isovalerate, ethyl isobutyrate, isobutyl hexanoate, isoamyl butanoate, propyl acetate, ethyl butyrate, ethyl palmitate, ethyl stearate, and ethyl myristate. As previously addressed, changes in these compounds could not be properly controlled; thus, this stabilization method may not be adequate for obtaining brandies with high quality standards. Meanwhile, none of the esters were selected as markers for the brandies stabilized at cold temperature, providing evidence of the minor impact of this process on the volatile profile of the brandies.

4. Conclusions

This study showed the impact of the cold and room temperature stabilization and subsequent filtration on the ester profile of brandies. Both methods achieved a drastic reduction of the long-chain fatty acids ethyl esters (ethyl palmitate, myristate, and stearate), with these compounds being the major cause of turbidity in brandies that meet consumer specifications. The traditional stabilization was slightly more effective in reducing these undesirable ethyl esters. However, this stabilization method produced important changes to the ester profile of the brandies, with significant increases of isoamyl octanoate, ethyl isovalerate, ethyl isobutyrate, isobutyl hexanoate, propyl acetate, and ethyl butyrate. Thus, using this stabilization method may lead to non-controlled aromatic changes. Meanwhile, cold stabilization produced less changes in the ester profile of brandies, being postulated as method for stabilizing brandies with a reduced impact on its aromatic quality, while achieving a successfully reduction in long-chain fatty acids.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app13063428/s1, Table S1: Percentage of change of the esters in brandies after stabilization with respect to the brandy before stabilization.

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