applied sciences

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# Beneficial Effects of *Pistacia terebinthus* Resin on Wine Making

**Michalis Kallis [1], Konstantina Boura [2,*], Ioannis K. Karabagias [3], Maria Kanellaki [2,*] and Athanasios A. Koutinas [2]**

[1] Department of Chemical Engineering, Cyprus University of Technology, 30 Archbishop Kyprianou Street, Limassol 3036, Cyprus
[2] Food Biotechnology Group, Department of Chemistry, University of Patras, 26504 Patras, Greece
[3] Department of Food Science and Technology, School of Agricultural Sciences, University of Patras, G. Seferi 2, 30100 Agrinio, Greece
* Correspondence: konstantinaboura@gmail.com (K.B.); m.kanellaki@upatras.gr (M.K.)

**Abstract:** In this work we studied the use of *Pistacia terebinthus* resin as carrier of a psychrotolerant and alcohol resistant yeast strain *Saccharomyces cerevisiae* AXAZ-1 for 27 repeated fermentation batches of white must (12.5 °Be) at 28, 21, 14 and 7 °C. The immobilized biocatalyst showed high operational stability during this process. Regarding the repeated fermentation batches with free cells, the fermentation time proved to be higher and so ethanol productivity was lower. Extracted terpenes, terpenoids and polyphenols from *P. terebinthus* resin were detected in the produced wines contributing to their preservation for at least 35 days at room temperature and 95 days at 4 °C without any addition of potassium metabisulfite. Those extracted compounds from resin gave also a particular pleasant aroma to the produced wines.

**Keywords:** *Pistacia terebinthus* resin; immobilization; *Saccharomyces cerevisiae* AXAZ-1; antioxidant; phytochemicals; winemaking



**Citation:** Kallis, M.; Boura, K.; Karabagias, I.K.; Kanellaki, M.; Koutinas, A.A. Beneficial Effects of *Pistacia terebinthus* Resin on Wine Making. *Appl. Sci.* **2022**, *12*, 9097. https://doi.org/10.3390/app12189097

Academic Editor: Monica Gallo

Received: 8 August 2022
Accepted: 6 September 2022
Published: 9 September 2022



## 1. Introduction

The phytochemicals are non-nutrient plant bioactive compounds that have beneficial effects on human health and prevent diseases. Polyphenols, phytoestrogens, terpenes, terpenoids and carotenoids are some of the major phytochemicals with antioxidant properties [1,2]. *Pistacia* is a genus of flowering plants which belongs to the Anacardiaceae family rich in terpenoids and widely distributed in the Mediterranean basin [3,4]. Different species of *Pistacia* genus are used as food, medicinal, ornamentals [5]. *P. terebinthus* (also known as pissa pafos in Cyprus) has also been used as antidotal, aphrodisiac, stimulant, and diuretic and is suitable to treat leprosy since ancient times [6].

The characteristic taste combined with the beneficial properties of *Pistacia* resins has attracted the interest of the food industries. *Pistacia* resins are the main ingredient or are used as additives in many foods. In particular, the resin and its by-products are used in a wide variety of products, such as bakeries, traditional and gourmet sweets, snacks, chewing gum, alcoholic beverages, flavored wines and filter coffees [3]. The oil from *P. terebinthus* seeds is used as cooking oil as well as for soap production [7].

*P. terebinthus* resin was used for the immobilization of *L. casei* during novel probiotic yoghurt production giving fine organoleptic traits, and no pathogenic microorganism was detected [8]. Furthermore, the addition of *P. terebinthus* L. into cake formulation led to the production of a new functional cake with high nutritional value [9].

Due to the complex mixture of different bioactive groups of *Pistacia* resins such as terpenes and polyphenols, these resins can be applied to the production of functional foods and drugs. A high number of terpenes and terpenoids from the essential oils of *P. terebinthus* were detected using GC-MS [4,10]. Andrikopoulos et al. 2003 [11] reported that the highest total polyphenol content (1120 mg/kg) was extracted from *P. terebinthus* among other resins

extracted from *P. lentiscus*, plants belonging to the family *Dipterocarpaceae*, *Acacia* species, genus *Astragalus.*

Fermentations using immobilized cells has gained high attention in food bioprocessing. Cell protection, operation stability, higher fermentation rate, higher fermentation productivity are some of the advantages of the immobilized cells compared to free cells. Food grade materials such as fruits, alginates, resins, delignified cellulosic material (tubular cellulose) have been used as immobilization carriers of cells [8,12,13]. It is worth noting that the operational stability of using the immobilized biocatalyst in repeated fermentation batches makes the process even more cost-effective as it occurred in the case of winemaking [14] and brewing [15].

The concept of this work is based on the use of the edible *P. terebinthus* resin, which is rich in phytochemicals, as carrier for immobilization of the cryotolerant and alcohol resistant S. cerevisiae AXAZ-1 strain in order to promote the alcoholic fermentation of the must (12.5 °Be) and enhance the organoleptic traits and preservation of the produced wines through its terpene and polyphenolic content compared to free cells. The application of univariate and multivariate statistical analysis highlighted the effect of fermentation temperature on fermentation time, ethanol content, ethanol productivity, residual sugar, sugar conversion, volatile composition, volatile acidity, polyphenolic content, and antioxidant activity of the produced white wines (Figures S1–S6).

## 2. Materials and Methods

### 2.1. Yeast Strain

The alcohol-resistant and psychrotolerant yeast strain *Saccharomyces cerevisiae* AXAZ-1, previously isolated from the agricultural area of North Achaia (Greece) [16] and sequenced by Kopsahelis et al., 2009 [17], was selected for the fermentation process. The culture medium, the growth and the harvest of the yeast were prepared referring to the method reported by Kallis et al., 2019 [12].

### 2.2. Harvesting of Pistacia terebinthus Resin

*Pistacia terebinthus* resin was obtained from trees located in Paphos district in Cyprus during summer (July–August). The resin was collected by causing minor damage to the tree by making a hole into the trunk puncturing the vacuoles and letting the resin exit the tree in a procedure known as tapping. The tree damage was then repaired by filling the wound with resin. The excess resin was then collected. The procedure was repeated twice. The resin was then diligently cleaned and stored in a cool and dry place.

### 2.3. Yeast Cells Immobilization on Pistacia terebinthus Resin

The immobilized biocatalyst was prepared by mixing 8 g of wet biomass of AXAZ-1 cells and 100 g of crude pieces of *Pistacia terebinthus* resin per 400 mL of synthetic medium. Specifically, the priory harvested wet biomass of *S. cerevisiae* (Section 2.1) was added in each flask containing the synthetic medium and pieces of *P. terebinthus* resin and the mixtures were incubated at 28 °C for 24 h to allow cell immobilization by natural adsorption and entrapment [8,18]. The immobilization process was carried out by addition of *P. terebinthus* resin and yeast biomass into a sterile synthetic medium consisting of 120 g/L of glucose, 4 g/L of yeast extract, 1 g/L $(NH_4)_2SO_4$, 1 g/L $KH_2PO_4$, and 5 g/L $MgSO_4$ (Merck, Darmstadt, Germany). In order to obtain the optimum conditions for production of the most effective immobilized biocatalyst, experiments were performed according to previous work by Kallis et al., 2019 [12]. The fermented liquid was decanted, and the immobilized biocatalyst was washed twice with the sterile synthetic medium for the removal of free cells.

### 2.4. Repeated Fermentation Batches of Must (12.5 °Be) at Different Temperatures

The best immobilized biocatalyst prepared by mixing 8 g of wet biomass of yeast cells and 100 g of resin were introduced into 400 mL of must of 12.5 °Be and repeated fermenta-

tion batches were carried out without agitation at 28, 21, 14, and 7 °C [12]. Towards the end of each batch, the fermented liquid was decanted and preserved, and the immobilized biocatalyst was washed twice with the sterile synthetic medium (400 mL) for the removal of free cells. Fermentations were monitored by determination of the °Be density at various time intervals until a final density of 0–0.5 °Be. For comparative reasons, repeated fermentation batches with free yeast cells were also carried out at the same temperatures. Samples were collected at the end of fermentations and analyzed for ethanol, residual sugar, total phenolic content, and volatile by-products.

### 2.5. Ethanol, Residual Sugar and Major Volatiles Analyses

Ethanol, residual sugar, and major volatiles (acetaldehyde, ethyl acetate, 1-propanol, isobutanol, methanol, and amyl alcohols) were determined as described by previous study [12]. Ethanol productivity was calculated as g of ethanol per liter liquid volume produced per day (g/L/d). All analyses were conducted in triplicate (three independent samples) and the mean values are presented (max deviation for all values was about ±5%).

### 2.6. Phenolic Content Determination

The determination of the total phenol content of the fermented wines was based on the method reported in Kallis et al., 2019 [12]. A calibration curve was obtained with gallic acid solutions (concentration range 50–500 mg/L) and the results were expressed as gallic acid equivalents (GAE). All analyses were conducted in triplicate (three independent samples) and the mean values are presented (max deviation for all values was about ±5%).

### 2.7. DPPH Free Radical Scavenging Activity

Wines produced were evaluated for antioxidant potential using DPPH (1,1-diphenyl-2-picryl hydrazil) radical scavenging assay. The DPPH free-radical scavenging activity was determined by the methods described by Chen et al. (2013) and Liu et al. (2008), with modifications [19,20]. The DPPH· solution was prepared by mixing 0.025 g of DPPH· in 1 L of MeOH. Each sample analyzed was diluted four times with MeOH at a final volume of 100 µL and the final samples contained 10, 20, 50 and 100 µL of wine. Each of the samples was then diluted using 3.9 mL of DPPH solution and the absorbance was measured at 515 nm until we reached a curve plate. The final absorptions along with the standard curve were used to calculate the remaining DPPH concentration (g/L). The percentage %DPPH·REM and $EC_{50}$ were then calculated. The linearity of the method used was performed by using ascorbic acid with different dilutions (20, 30, 50, 80, 130 and 180 g) of ascorbic acid/kg of DPPH. The standard curve included 5 concentrations of DPPH· in MeOH (0.025, 0.021875, 0.01875, 0.0125 and 0.00625 g of DPPH·/L of MeOH).

### 2.8. Head Space (HS) Solid Phase Microextraction (SPME) Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Samples of the produced wines were studied for volatile by-products composition and terpenoids content using HS-SPME GC-MS analysis according to the method reported by Kallis et al., 2019 [12].

### 2.9. Preliminary Sensory Evaluation of the Produced Wines

Sensory evaluation of the produced wines was carried out after 30 days of storage by 10 (5 males and 5 females) active aged adults (untrained laboratory members and wine consumers) familiar with wine tastes, using the triangle test. The tasters were asked to give scores on a 0–10 scale using locally approved protocols in our laboratories regarding aroma characteristics [21]. The wine evaluation was done at the 30th day of their storage among wines produced by: (a) immobilized cells on *P. terebinthus* resin at 14 °C and storage at 22–28 °C; (b) immobilized cells on *P. terebinthus* resin at 14 °C and storage at 4 °C; and (c) by free cells at 14 °C and storage at 4 °C. Wines were assessed for their sensory characteristics such as appearance, flavor, texture, acidity, mastic odor, wine odor, and overall acceptability.

Taste tests were conducted at ambient room temperature using the appropriate ISO wine tasting glasses. Mouthwashes with water were performed between tests [22].

### 2.10. Statistical Analysis

All fermentations were carried out in triplicate (three independent samples) and the mean values are presented (standard deviation for all values was about ±5% in most cases). The statistical analysis of data included Paired-samples *t*-test, One-way Analysis of Variance (ANOVA), Linear Discriminant Analysis (LDA), and Factor Analysis (FA).

The paired-samples *t*-test was applied in order to check the statistically significant differences in the studied parameters (i.e., volatile acidity) when immobilized and free cells were used in different time intervals (0, >30, and >90 days) with respect to the fermentation temperature (28, 21, 14, and 7 °C). For the ANOVA the fermentation temperature comprised the factor variable at four levels (28, 21, 14, and 7 °C), whereas the dependent variables were the fermentation time (h), ethanol (%, *v*/*v*), ethanol productivity (g/L/d), residual sugar (g/L) and sugar conversion (%). Complementary to ANOVA, Bonferroni's *Post-hoc* analysis was applied to investigate the multiple testing of significance between the average values of the studied parameters with respect to the factor variable. The least significance difference was $p < 0.05$.

To build linear classification models related to the impact of fermentation temperature on the studied parameters, LDA (a supervised statistical technique) was applied. For the LDA analysis the fermentation temperature (28, 21, 14, and 7 °C) comprised the group variable, while the fermentation time (h), ethanol (%, *v*/*v*), ethanol productivity (g/L/d), residual sugar (g/L) and sugar conversion (%) comprised the independent variables. In LDA the classification rate is usually provided by the original and cross-validation method, while the Wilks' Lambda index (ranges between 0–1) provides how well each level of the independent variables contributes to the classification model. The effectiveness of the prediction ability of the LDA models was evaluated by the cross-validation method [23].

Finally, FA as a dimension reduction technique (a non-supervised statistical technique) was applied alternatively when the number of independent variables was slightly increased (i.e., volatile compounds) in order to find the principal components that show the best correlations of data in the multi-dimensional space, and check at the same time the sample adequacy based on the Keiser–Meyer–Olkin (KMO) criterion (it should be >0.50). The extraction method during FA was principal component analysis (PCA) [24]. Statistical analysis of data was computed using the SPSS software (v. 27.0, IBM Corp., Armonk, NY, USA).

## 3. Results and Discussion

### 3.1. Selection of the Appropriate Quantity of Yeast/Resin

A criterion for the selection of the *P. terebinthus* resin as carrier for yeast immobilization was its antimicrobial action due to contained terpenes and phenolics [18]. The immobilized biocatalyst produced from 8 g/100 g yeast/resin (wet form) was selected as the most suitable because it exhibited operational stability and lower fermentation time in 400 mL of the synthetic medium compared to 4 g/50 g and 16 g/200 g according to the study of Kallis et al., 2019 [9]. After freeze-drying, 1.724 g of dry yeast (*S. cerevisiae* AXAZ-1) were immobilized on 100 g of the resin.

Although increasing the quantity of yeast/resin (16 g/200 g) to promote the fermentation, the fermentation ability was decreased probably because higher amounts of extracted terpenes and polyphenols inhibited the fermentation action of the yeast partially. Thus, the immobilized biocatalyst produced from 8 g/100 g (yeast/resin) was selected as the most suitable.

### 3.2. Repeated Fermentation Batches of Must by Immobilized and Free Yeast Cells

Repeated fermentation batches of white must (12.5 °Be) for winemaking at 28, 21, 14 and 7 °C were performed by immobilized and free yeast cells in order to examine the

fermentation time, operation stability, productivity, volatile by-products, total phenolics, antioxidant capacity, and preservation of the produced white wines for comparative reasons.

The fermentation time using immobilized cells was lower than that for free cells at all fermentation temperatures (28, 21, 14 and 7 °C) as shown in Figure 1, Tables 1 and 2 (total results in Tables S1 and S2). This effect has also been confirmed in other immobilized systems [14,15,25]. The immobilized biocatalyst was used for 27 repeated fermentation batches of the must (12.5 °Be) from 28 °C down to 7 °C gradually (Table 2). During these batches the fermentation time, produced ethanol, ethanol productivity, and sugar conversion remained about stable at each temperature due to the immobilized biocatalyst stability. As the fermentation temperature was decreased, the fermentation time was increased as also the residual sugar [15,26]. Regarding repeated fermentation batches with free cells, although a higher amount of yeast (2 g) was used against 1.724 g of immobilized cells in each batch of 400 mL, the fermentation time and residual sugars were higher, and so the ethanol productivity was lower. Ethanol concentration was at similar levels in wines produced by immobilized or free cells (Tables 1 and 2).

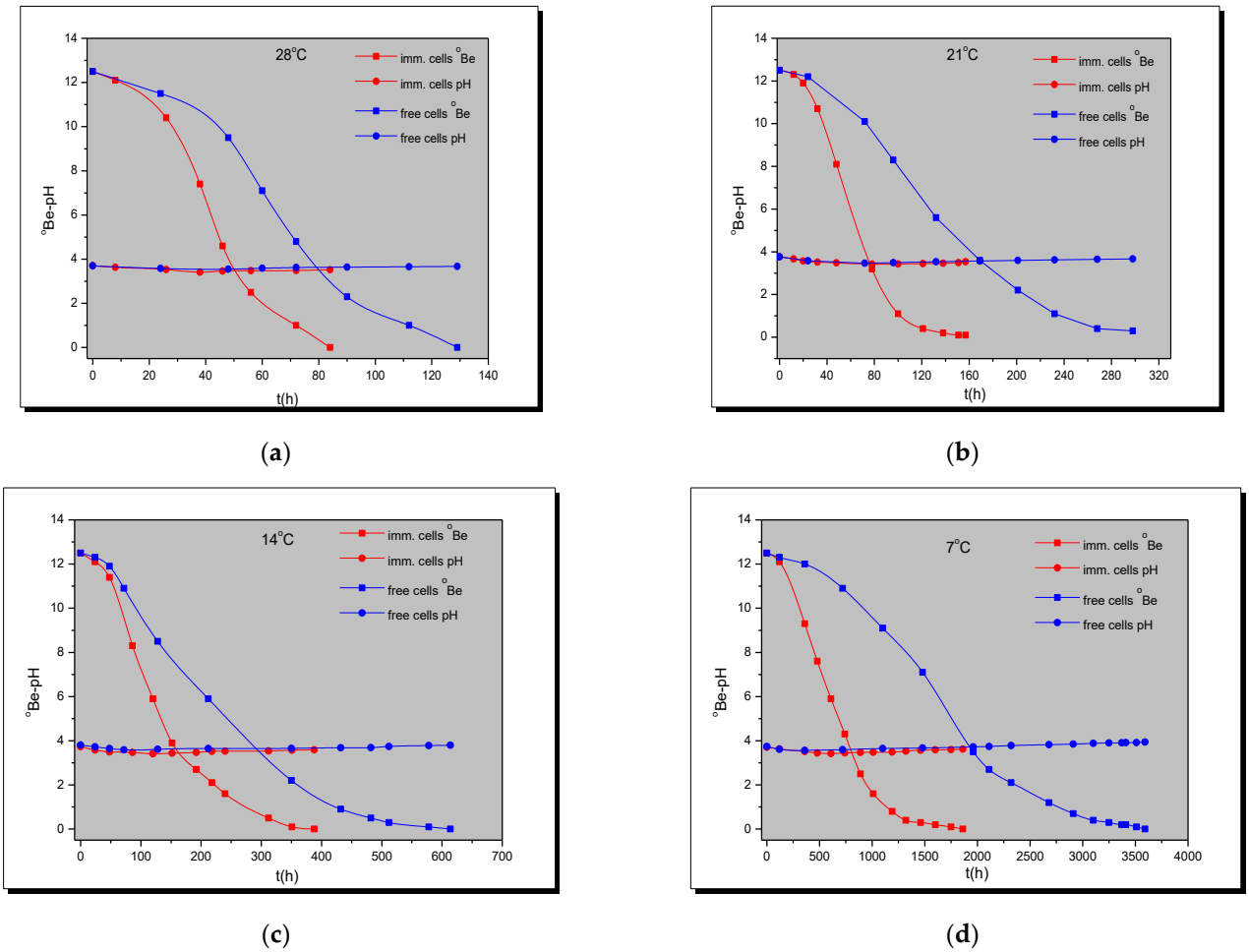


**Figure 1.** Fermentation kinetics of must (12.5 °Be) using immobilized and free cells at (**a**) 28 °C; (**b**) 21 °C; (**c**) 14 °C; (**d**) 7 °C.

**Table 1.** Kinetic parameters of must repeated fermentation batches (12.5 °Be) using free cells at 28, 21, 14 and 7 °C.

| Fermentation Temperature (°C) | Fermentation Batch | Fermentation Time (h) | Ethanol (%*v*/*v*) | Ethanol Productivity (g/L/d) | Residual Sugar (g/L) | Sugar Conversion (%) |
|---|---|---|---|---|---|---|
| 28 | 1–9 | 124.4 ± 6.3 a | 11.89 ± 0.23 e | 18.14 ± 1.10 g | 23.2 ± 5.0 k | 89.16 ± 2.34 m |
| 21 | 10–15 | 286.3 ± 14.3 b | 11.08 ± 0.48 f | 7.35 ± 0.56 h | 26.0 ± 4.2 k | 87.85 ± 1.99 m |
| 14 | 16–19 | 611.5 ± 12.6 c | 10.88 ± 0.28 f | 3.37 ± 0.15 i | 27.4 ± 1.9 k | 87.18 ± 0.87 m |
| 7 | 20–22 | 3600.3 ± 48.7 d | 10.70 ± 0.20 f | 0.56 ± 0.01 j | 40.2 ± 1.4 l | 81.17 ± 0.66 n |

Different letters in each column indicate statistically significant differences at the confidence level $p < 0.05$.

**Table 2.** Kinetic parameters of must repeated fermentation batches (12.5 °Be) using immobilized biocatalyst at 28, 21, 14 and 7 °C.

| Fermentation Temperature (°C) | Fermentation Batch | Fermentation Time (h) | Ethanol (%*v*/*v*) | Ethanol Productivity (g/L/d) | Residual Sugar (g/L) | Sugar Conversion (%) |
|---|---|---|---|---|---|---|
| 28 | 1–10 | 80.9 ± 3.14 a | 11.98 ± 0.39 e | 28.09 ± 1.37 f | 12.0 ± 2.1 j | 94.39 ± 0.96 l |
| 21 | 11–17 | 154 ± 5.54 b | 11.72 ± 0.52 e | 14.42 ± 0.86 g | 19.3 ± 2.2 k | 90.97 ± 1.03 m |
| 14 | 18–23 | 362.3 ± 16.12 c | 11.64 ± 0.25 e | 6.09 ± 0.27 h | 20.7 ± 1.6 k | 90.34 ± 0.74 m |
| 7 | 24–27 | 1794.3 ± 108.6 d | 11.51 ± 0.11 e | 1.22 ± 0.07 i | 23.9 ± 3.5 k | 88.83 ± 1.65 mn |

Different letters in each column indicate statistically significant differences at the confidence level $p < 0.05$.

### *3.3. Major Volatile By-Products in Wines*

As shown in Tables 3 and 4 (total results in Tables S3 and S4) the major volatile by-products in wines (methanol, acetaldehyde, ethyl acetate, propanol, isobutyl alcohol, amyl alcohols) were determined as they affect the flavor and quality of the wines [23,24].

**Table 3.** Major volatile by-products of must repeated fermentation batches (12.5 °Be) using immobilized biocatalyst at 28, 21, 14 and 7 °C.

| Fermentation Temperature (°C) | Fermentation Batch | Methanol (mg/L) | Acetaldehyde (mg/L) | Ethyl Acetate (mg/L) | 1-Propanol (mg/L) | Isobutyl Alcohol (mg/L) | Amyl Alcohol (mg/L) |
|---|---|---|---|---|---|---|---|
| 28 | 1–10 | 101.39 ± 17.75 a | 94.12 ± 4.92 c | 23.75 ± 2.75 e | 35.60 ± 4.54 i | 73.11 ± 5.01 l | 99.07 ± 6.08 o |
| 21 | 11–17 | 119.68 ± 20.46 a | 89.68 ± 8.26 c | 52.31 ± 4.61 f | 40.38 ± 8.34 i | 68.49 ± 7.49 l | 72.35 ± 10.40 p |
| 14 | 18–23 | 121.37 ± 21.90 a | 98.67 ± 8.43 c | 66.72 ± 5.31 g | 55.14 ± 3.72 j | 44.07 ± 6.46 m | 64.23 ± 12.01 p |
| 7 | 24–27 | 139.46 ± 13.59 ab | 104.62 ± 6.37 cd | 36.85 ± 1.67 h | 19.71 ± 1.64 k | 12.01 ± 1.83 n | 30.00 ± 0.80 q |

Different letters in each column indicate statistically significant differences at the confidence level $p < 0.05$.

**Table 4.** Major volatile by-products of must repeated fermentation batches (12.5 °Be) using free cells at 28, 21, 14 and 7 °C.

| Fermentation Temperature (°C) | Fermentation Batch | Methanol (mg/L) | Acetaldehyde (mg/L) | Ethyl Acetate (mg/L) | 1-Propanol (mg/L) | Isobutyl Alcohol (mg/L) | Amyl Alcohol (mg/L) |
|---|---|---|---|---|---|---|---|
| 28 | 1–9 | 112.14 ± 27.56 a | 97.85 ± 6.97 c | 67.75 ± 6.80 d | 26.90 ± 3.74 g | 92.93 ± 4.32 j | 107.00 ± 8.34 n |
| 21 | 10–15 | 120.84 ± 30.86 a | 104.47 ± 9.31 c | 26.97 ± 3.61 e | 40.62 ± 4.04 h | 80.79 ± 9.93 k | 95.07 ± 7.25 o |
| 14 | 16–19 | 143.85 ± 6.00 ab | 105.24 ± 11.23 c | 34.73 ± 3.60 e | 38.20 ± 2.68 h | 58.10 ± 8.62 l | 73.50 ± 4.16 p |
| 7 | 20–22 | 176.13 ± 4.55 b | 107.67 ± 4.84 c | 9.68 ± 1.53 f | 18.84 ± 1.35 i | 19.11 ± 1.86 m | 36.2 ± 4.31 q |

Different letters in each column indicate statistically significant differences at the confidence level $p < 0.05$.

Methanol does not contribute to the organoleptic impact of the wine when it ranges from 0.1 to 0.2 g/L [25]. In all cases the average value of methanol concentrations was in acceptable levels and specifically higher using free cells for wine making compared to those using immobilized biocatalyst. In both fermentation methods the methanol concentrations were increased, thereby decreasing the fermentation temperature. The concentrations of methanol in the wines derived from fermentation with immobilized biocatalyst were lower than those with free cells and were increased as the temperature was decreased. The methanol content in wines is strictly regulated by the International Office of Vine and Wine (OIV) at <400 mg/L for red wines and <250 mg/L for white or rose wine (International Organisation of Vine and Wine, 2015).

The concentration of acetaldehyde in the wines derived from fermentation with immobilized biocatalyst was slightly lower than that with free cells and lower than 120 mg/L. A range of acetaldehyde concentrations in wine can be found; concentrations range from 30 to 130 mg/L [26]. This compound in low levels can give the wine fruit notes, but at higher concentrations it is reminiscent of nuts [27], and at still higher levels produces a green, grassy or apple-like off flavor [28].

The concentration of ethyl acetate in wines is below 50 to 100 mg/L [29]. The produced wines using both methods (Tables 3 and 4) exhibited low concentrations providing them with a pleasant aroma. Regarding propanol-1 and ethyl acetate their concentrations in the immobilized cells were higher than those in the free cells. Ethyl acetate is perceived as the odour of nail polish remover and has a reported sensory threshold of 12 mg/L. Ethyl acetate is the major ester produced by yeast and at low levels can contribute 'fruity' aroma properties and add complexity to wine.

Quantitatively, the most important higher alcohols are the straight-chain alcohols 1-propanol, 2-methyl-1-propanol (isobutyl alcohol), 2-methyl-1-butanol, and 3-methyl-1-butanol (isoamyl alcohol). Most straight-chain higher alcohols have a strong pungent odor. At low concentrations (∼0.3 g/L or less), they generally add an aspect of complexity to the bouquet. At higher levels they increasingly overpower the fragrance [25].

The concentrations of isobutanol and amyl alcohols (2-methyl-1-butanol and 3-methyl-1-butanol) in the produced wines using immobilized biocatalyst were lower than those with free cells and were decreased as the temperature was decreased. While regarding the 1-propanol, its concentration was increased decreasing the temperature using either free cells or immobilized biocatalyst except in the fermentation temperature of 7 °C. Either using free or immobilized biocatalyst for the wine making in all conditions, the average total higher alcohol concentration that was detected in the produced wines (isobutanol, amyl alcohols and 1-propanol) was in acceptable levels (140–420 mg/L) with the produced wines using the immobilized biocatalyst exhibiting lower total higher alcohols concentrations.

### *3.4. Volatile by Products Detected by HS-SPME GS-MS in Wines*

The technique of headspace solid phase microextraction/gas chromatography-mass spectrometry (HS-SPME/GC-MS) was used for the qualitative determination of the volatile by-products in wines produced by fermentation of must by immobilized and free cells at 28, 21, 14 and 7 °C (Table 5). A total of 125 compounds was detected in the wines, mainly alcohols, esters, ketones, aldehydes, and acids, many of which belong to terpenes (Table 6). Their detection depends on the presence or not of the resin, fermentation time and the fermentation temperature. The detected terpenes are monoterpenes (α-pinene, ocimene, β-pinene, limonene, careen, etc.) monoterpenoids (1,8 cineole, linalool oxide, linalool, bornyl acetate, etc.), and sesquiterpenoids (spathulenol and farnesol).

**Table 5.** Volatile by-products detected by SPME/GC-MS analysis in must fermentation batches (12.5 °Be) using immobilized and free cells at 28, 21, 14 and 7 °C.

| No | Compound | R.I | R.I.B | 28 °C | | 21 °C | | 14 °C | | 7 °C | | Identification Method |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | I/W | F/W | I/W | F/W | I/W | F/W | I/W | F/W | |
| 1 | Ethyl Acetate | 879 | 885 | + | + | + | + | + | + | + | + | a,b |
| 2 | Ethanol | 885 | 883 | + | + | + | + | + | + | + | + | a,b |
| 3 | 2,5-Hexanediol | 969 | 907 | + | - | + | - | - | - | - | - | b |
| 4 | α-Pinene | 994 | 1017 | + | - | + | - | - | - | - | - | b |
| 5 | Toluene | 1015 | 1043 | + | + | - | - | - | - | - | - | b |
| 6 | Z-β-Ocimene | 1019 | 1035 | + | - | + | - | - | - | - | - | b |
| 7 | 2-Fluoro-1-propene | 1063 | 1079 | + | + | + | + | + | - | - | - | b |

**Table 5.** *Cont.*

| No | Compound | R.I | R.I.B | 28 °C | | 21 °C | | 14 °C | | 7 °C | | Identification Method |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | I/W | F/W | I/W | F/W | I/W | F/W | I/W | F/W | |
| 8 | β-Pinene | 1085 | 1100 | + | - | + | - | - | - | - | - | b |
| 9 | Isoamyl Acetate | 1110 | 1114 | + | + | + | + | + | - | - | - | b |
| 10 | 4-Methyl-2-Pentanol | 1138 | E.Σ | + | + | + | + | + | + | + | + | - |
| 11 | δ-3-Carene | 1143 | 1141 | + | - | + | - | - | - | - | - | b |
| 12 | Limonene | 1167 | 1200 | + | - | + | - | + | - | + | - | b |
| 13 | Dehydro-1,8-Cineole | 1179 | 1195 | + | - | + | - | - | - | - | - | b |
| 14 | 1-Butanol | 1182 | 1179 | + | + | + | + | - | - | - | - | b |
| 15 | 1,8-Cineole | 1205 | 1208 | + | - | + | - | - | - | - | - | b |
| 16 | Isoamyl Alcohol | 1206 | 1200 | + | + | + | + | + | + | + | + | b |
| 17 | Ethyl Hexanoate | 1231 | 1258 | + | + | + | + | + | + | + | + | b |
| 18 | p-menth-1-en-9-al | 1235 | 1228 | + | - | + | - | - | - | - | - | b |
| 19 | p-Cymene | 1246 | 1275 | + | - | + | - | - | - | - | - | b |
| 20 | Terpinolene | 1252 | 1283 | + | - | + | - | - | - | - | - | b |
| 21 | 3-Penten-1-ol | 1264 | 1305 | + | - | + | - | - | - | - | - | b |
| 22 | o-Cymene | 1270 | 1291 | + | - | - | - | - | - | - | - | b |
| 23 | Furfuryl Ether | 1277 | - | - | + | - | + | + | + | + | + | c |
| 24 | 4-Penten-1-ol | 1294 | 1312 | + | + | + | + | - | - | - | - | b |
| 25 | p-Menth-2-ene | 1303 | - | + | - | - | - | - | - | - | - | c |
| 26 | 6-Methyl-5-Hepten-2-One | 1305 | 1319 | + | + | + | + | - | - | - | - | b |
| 27 | 3-Methyl-2-Buten-1-ol | 1309 | 1320 | + | + | + | + | - | - | - | - | b |
| 28 | 4-Methyl-1-pentanol | 1322 | 1329 | + | - | + | - | - | - | - | - | b |
| 29 | 2-Heptanol | 1329 | 1334 | + | - | + | - | - | - | - | - | b |
| 30 | 1-Hexanol | 1332 | 1354 | + | - | + | - | - | - | - | - | b |
| 31 | Verbenyl Ethyl Ether | 1333 | 1371 | + | - | + | - | + | - | + | - | b |
| 32 | Ethyl Lactate | 1340 | 1344 | + | + | + | + | + | + | + | + | b |
| 33 | p-1,3,8-Menthatriene | 1346 | - | + | - | + | - | + | - | - | - | c |
| 34 | Nonanal | 1357 | 1390 | - | + | + | - | - | - | - | - | b |
| 35 | o-Methylanisole | 1369 | 1393 | + | - | + | - | - | - | - | - | b |
| 36 | Ethyl Octanoate | 1402 | 1424 | + | + | + | + | + | + | + | + | b |
| 37 | 3-Ethoxy-1-propanol | 1415 | 1419 | - | + | - | + | + | + | + | + | b |
| 38 | Heptanol | 1416 | 1443 | + | + | + | + | + | + | - | - | b |
| 39 | Furfural | 1427 | 1452 | + | + | + | + | - | - | - | - | b |
| 40 | 2-Octanol | 1435 | 1430 | - | + | - | + | - | + | - | + | b |
| 41 | Dehydro-p-Cymene | 1438 | 1432 | + | - | + | - | + | - | - | - | b |
| 42 | 2-Ethyl-1-Hexanol | 1448 | 1492 | + | - | + | + | + | + | + | + | b |
| 43 | Acetic Acid | 1450 | 1445 | + | + | + | + | + | + | + | + | b |
| 44 | Linalool Oxide | 1464 | 1460 | + | - | + | - | - | - | - | - | b |
| 45 | Camphor | 1467 | 1495 | + | - | + | - | - | - | - | - | b |
| 46 | Benzaldehyde | 1480 | 1514 | + | + | + | + | - | - | - | - | b |
| 47 | α-Campholenal | 1484 | 1482 | + | - | - | - | - | - | - | - | b |
| 48 | Pinocamphone | 1495 | - | + | - | + | - | - | - | - | - | c |
| 49 | trans-Chrysanthenyl Acetate | 1511 | 1582 | + | - | + | - | - | - | - | - | b |
| 50 | 2-nonanol | 1513 | 1528 | - | + | - | + | - | + | - | + | b |
| 51 | Pinocarvone | 1521 | 1561 | + | - | + | - | - | - | - | - | b |
| 52 | Linalool | 1536 | 1552 | + | - | + | - | + | - | + | - | b |
| 53 | Fenchol | 1541 | 1574 | + | - | + | - | - | - | - | - | b |
| 54 | 2,3-Butanediol | 1547 | 1559 | + | + | + | + | + | + | + | + | b |
| 55 | Bornyl Acetate | 1550 | 1565 | + | - | + | - | + | - | + | - | b |
| 56 | 3-Methylcamphenilol | 1551 | 1592 | + | - | - | - | - | - | - | - | b |

**Table 5.** *Cont.*

| No | Compound | R.I | R.I.B | 28 °C | | 21 °C | | 14 °C | | 7 °C | | Identification Method |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | I/W | F/W | I/W | F/W | I/W | F/W | I/W | F/W | |
| 57 | 6-Methyl-3,5-Heptadien-2-one | 1554 | 1602 | + | - | + | - | - | - | - | - | b |
| 58 | Isopinocamphone | 1557 | 1562 | + | - | + | - | - | - | - | - | b |
| 59 | 4-Terpineol | 1562 | 1593 | + | - | + | - | + | - | + | - | b |
| 60 | 1-Octanol | 1565 | 1570 | - | + | - | + | - | + | - | + | b |
| 61 | Dihydrocarvone | 1569 | 1600 | + | - | + | - | - | - | - | - | b |
| 62 | 2-Undecanol | 1573 | - | + | + | + | + | + | + | + | + | c |
| 63 | 5-Methyl-Furfural | 1580 | 1578 | - | - | - | + | + | + | + | + | b |
| 64 | Myrtenal | 1588 | 1602 | + | - | + | - | - | - | - | - | b |
| 65 | 1,3-Butanediol | 1592 | 1590 | - | + | - | + | + | + | + | + | b |
| 66 | Ethyl Decanoate | 1597 | 1591 | + | + | + | + | + | + | + | + | b |
| 67 | p-Isopropyl-Cyclohexanol | 1603 | - | + | - | - | - | - | - | - | - | c |
| 68 | L-trans-Pinocarveol | 1618 | 1632 | + | - | + | - | + | - | + | - | b |
| 69 | α-Phellandren-8-ol | 1626 | 1710 | + | - | + | - | + | - | + | - | b |
| 70 | Pinocarvyl Acetate | 1631 | 1661 | + | - | + | - | + | - | - | - | b |
| 71 | δ-Terpineol | 1633 | 1668 | + | - | - | - | - | - | - | - | b |
| 72 | Succinic Acid Diethyl Ester | 1638 | 1658 | + | + | + | + | + | + | + | + | b |
| 73 | Acetophenone | 1643 | 1643 | + | + | - | - | - | - | - | - | b |
| 74 | 2-Bornylene | 1645 | - | + | - | + | - | + | - | + | - | c |
| 75 | Ethyl-4-decenoate | 1651 | 1692 | + | - | + | - | + | - | + | - | b |
| 76 | estragole | 1657 | 1661 | - | - | - | - | + | + | + | + | b |
| 77 | α-Terpineol | 1665 | 1669 | + | + | + | + | + | + | + | + | b |
| 78 | Borneol | 1668 | 1677 | + | - | + | - | + | - | + | - | b |
| 79 | trans-Verbenol | 1679 | 1679 | + | - | + | - | + | - | - | - | b |
| 80 | Methionol | 1683 | - | + | + | + | + | + | + | + | + | c |
| 81 | Verbenone | 1705 | 1695 | + | - | + | - | + | - | + | - | b |
| 82 | Carvone | 1707 | 1711 | + | - | + | - | + | - | - | - | b |
| 83 | Carvotanacetol | 1711 | - | + | - | + | - | + | - | - | - | c |
| 84 | Ethyl 9-Decenoate | 1715 | 1711 | + | + | + | + | + | + | + | - | b |
| 85 | exo-2-Hydroxycineol | 1719 | 1723 | + | - | + | - | + | - | - | - | b |
| 86 | 1-Decanol | 1727 | 1783 | + | + | - | + | - | + | - | - | b |
| 87 | p-Methyl-Acetophenone | 1754 | 1763 | + | + | + | + | + | + | + | - | b |
| 88 | β-Phellandren-8-ol | 1757 | - | + | - | + | - | + | - | + | - | c |
| 89 | Myrtenol | 1777 | 1788 | + | - | + | - | + | - | + | - | b |
| 90 | 9-Decenol | 1783 | - | - | + | - | + | - | + | - | - | c |
| 91 | β-Citronellol | 1792 | 1790 | + | + | + | + | + | + | + | + | b |
| 92 | 2-Phenylethyl Acetate | 1794 | 1795 | + | + | + | + | + | + | + | + | b |
| 93 | Anethole | 1806 | 1815 | - | - | + | + | + | + | + | + | b |
| 94 | trans-Carveol | 1812 | 1825 | + | - | + | - | + | - | - | - | b |
| 95 | Ethyl Dodecanoate | 1824 | 1833 | + | + | + | + | + | + | + | - | b |
| 96 | p-cymen-8-ol | 1828 | 1833 | + | - | + | - | + | - | + | - | b |
| 97 | Hexanoic Acid | 1839 | 1829 | - | + | - | + | + | + | + | + | b |
| 98 | cis-Carveol | 1845 | 1848 | + | - | + | - | + | - | + | - | b |
| 99 | trans-Myrtanol | 1854 | 1856 | + | - | + | - | - | - | - | - | b |
| 100 | Benzyl alcohol | 1857 | 1889 | + | + | + | + | + | - | - | - | b |
| 101 | p-cymen-9-ol | 1878 | - | + | - | - | - | - | - | - | - | c |
| 102 | 3-Methylbutyl Pentadecanoate | 1885 | 1889 | + | - | + | - | - | - | - | - | b |
| 103 | Ethyl Pentadecanoate | 1895 | 1897 | + | - | + | - | + | + | + | + | b |
| 104 | Phenylethyl Alcohol | 1896 | 1908 | + | + | + | + | + | + | + | + | b |
| 105 | Piperitenone | 1917 | 1918 | + | - | - | - | - | - | - | - | b |

**Table 5.** *Cont.*

| No | Compound | R.I | R.I.B | 28 °C | | 21 °C | | 14 °C | | 7 °C | | Identification Method |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | I/W | F/W | I/W | F/W | I/W | F/W | I/W | F/W | |
| 106 | 1-Tridecanol | 1947 | 1954 | - | - | + | - | + | - | + | - | b |
| 107 | 1-Dodecanol | 1953 | 1969 | + | - | + | - | + | - | + | + | b |
| 108 | Ethyl 9-Hexadecenoate | 1974 | 1977 | + | + | + | + | + | + | + | + | b |
| 109 | Octanoic Acid | 2056 | 2056 | + | + | + | + | + | + | + | + | b |
| 110 | p-Cresol | 2071 | 2076 | + | - | + | - | - | - | - | - | b |
| 111 | Ethyl Myristate | 2084 | 2094 | + | - | + | - | - | + | - | + | b |
| 112 | Spathulenol | 2103 | 2104 | + | - | - | - | - | - | - | - | b |
| 113 | Nonanoic Acid | 2164 | 2192 | + | - | + | - | + | - | - | - | b |
| 114 | Ethyl Palmitate | 2243 | 2251 | + | - | + | - | + | + | + | + | b |
| 115 | Capric Acid | 2263 | 2256 | + | + | + | + | + | + | + | + | b |
| 116 | Ethyl-9-Hexadecanoate | 2275 | 2292 | + | - | + | - | + | + | + | + | b |
| 117 | Undecylenic Acid | 2289 | - | - | + | - | + | + | - | + | - | c |
| 118 | 2,4-Bis-(1,1-dimethylethyl) Phenol | 2298 | 2312 | + | + | - | - | - | - | - | - | b |
| 119 | Farnesol | 2323 | 2343 | + | - | + | - | + | + | + | + | b |
| 120 | Hexadecanol | 2347 | 2359 | + | - | + | - | + | - | - | - | b |
| 121 | Ethyl-9-octadecenoate | 2458 | 2435 | + | + | + | + | + | + | + | + | b |
| 122 | Lauric Acid | 2516 | 2517 | + | + | + | + | + | + | + | + | b |
| 123 | Myristic Acid | 2647 | 2670 | + | + | + | + | + | + | + | + | b |
| 124 | Palmitic Acid | > | 2700 | + | + | + | + | + | + | + | + | b |
| 125 | Oleic Acid | > | 2700 | + | + | + | + | + | + | + | + | b |

a: Positive identification from mass spectra data and retention time of standard compounds; b: Identification from retention time and mass spectra from bibliography; c: Mass spectrum with degree of uncertainty. +: Detected compound. -: Non detected compound. R.I: Kovats Index. R.I.B: Kovats Index from bibliography. I/W: Wine produced from must (12.5 °Be) using immobilized cells on *P. terebinthus* resin. F/W: Wine produced from must (12.5 °Be) using free cells.

**Table 6.** Number of volatile by-products detected by SPME/GC-MS analysis in must fermentation batches (12.5 °Be) using immobilized and free cells at 28, 21, 14 and 7 °C.

| Compound | 28 °C | | 21 °C | | 14 °C | | 7 °C | | Total |
|---|---|---|---|---|---|---|---|---|---|
| | I/W | F/W | I/W | F/W | I/W | F/W | I/W | F/W | |
| Alcohols | 46 | 20 | 40 | 20 | 29 | 17 | 22 | 15 | 53 |
| Terpenoids | 25 | 2 | 20 | 2 | 16 | 3 | 12 | 3 | 25 |
| Esters | 21 | 12 | 21 | 12 | 18 | 15 | 16 | 13 | 21 |
| Terpenoids | 3 | - | 3 | - | 2 | - | 1 | - | 3 |
| Organic acids | 8 | 9 | 8 | 9 | 10 | 8 | 9 | 8 | 10 |
| Terpenoids | - | - | - | - | - | - | - | - | - |
| Aldehydes | 5 | 3 | 5 | 3 | 1 | 1 | 1 | 1 | 7 |
| Terpenoids | 3 | - | 2 | - | - | - | - | - | 3 |
| Ketones | 12 | 3 | 10 | 2 | 3 | 1 | 2 | - | 12 |
| Terpenoids | 9 | - | 8 | - | 2 | - | 1 | - | 9 |
| Hydrocarbons | 13 | 1 | 10 | - | 4 | - | 2 | - | 13 |
| Terpenoids | 12 | - | 10 | - | 4 | - | 2 | - | 12 |
| Other compounds | 5 | 3 | 6 | 4 | 6 | 4 | 5 | 4 | 8 |
| Terpenoids | 2 | - | 2 | - | 1 | - | 1 | - | 2 |
| Total compounds | 110 | 51 | 100 | 50 | 71 | 46 | 57 | 41 | 124 |
| Total terpenoids | 54 | 2 | 45 | 2 | 25 | 3 | 17 | 3 | 54 |

I/W: Wine produced from must (12.5 °Be) using immobilized cells on *P. terebinthus* resin. F/W: Wine produced from must (12.5 °Be) using free cells.

### 3.5. Polyphenolic Content of the Produced Wines and Their Antioxidant Activity

Polyphenols exhibit important antioxidant activity [27]. Generally, the polyphenolic content in the produced white wines ranges from 305.4 to 392.7 mg GAE/L (average value) for free cells and from 544.2 to 654.2 mg GAE/L (average value) for immobilized cells as shown in Tables 7 and 8 (total results in Tables S7 and S8). Sánchez-Moreno et al., 1999 reported polyphenolic content in white wines ranging from 178.3 to 292.79 mg GAE/L, with an average value of 240.9 mg GAE/L [28]. Fernandez-Pachon et al., (2004) reported polyphenolic content in white wines ranging from 200 to 400 mg GAE/L, with an average value of 256 mg GAE/L [29]. As shown in Table 7 the average phenolic content of the wines fermented by immobilized biocatalyst was higher than that by free cells at each fermentation temperature. The difference in polyphenolic content between wines fermented by immobilized biocatalyst and free ones is 74.5, 126.6, 81.4 and 83.7 mg GAE/L at 28, 21, 14 and 7 °C. The wines fermented by immobilized biocatalyst presented higher average phenolic content at 21 °C as it occurred in the case of glucose, fructose and sucrose fermentation by yeast cells immobilized on resin [12]. Regarding fermentation by free cells the average phenolic content was decreased as the temperature was decreased. Temperature and solvent play an important role in polyphenol extraction [30] and the extraction rate is controlled by the internal resistance of the solid phase [31].

**Table 7.** Polyphenolic content of wines produced from must (12.5 °Be) using immobilized and free cells at 28, 21, 14 and 7 °C.

| Fermentation Temperature (°C) | Fermentation Using Immobilized Cells | | Fermentation Using Free Cells | |
|---|---|---|---|---|
| | Fermentation Batch | Polyphenolic Content (mg GAE/L) | Fermentation Batch | Polyphenolic Content (mg GAE/L) |
| 28 | 1, 3, 5, 7, 9 | 377.2 ± 25.3 a | 1, 3, 5, 7, 8 | 302.7 ± 29.2 A |
| 21 | 10, 12, 14, 16, 17 | 392.7 ± 15.1 a | 9, 10, 11, 13, 15 | 266.1 ± 23.9 A |
| 14 | 19, 20, 21, 23 | 324.3 ± 11.4 b | 16, 17, 18, 19 | 242.9 ± 9.7 AB |
| 7 | 25, 26, 27 | 305.4 ± 15 b | 20, 21, 22 | 221.7 ± 7.2 AB |

Different lower and uppercase letters in each column indicate statistically significant differences at the confidence level $p < 0.05$.

**Table 8.** Antioxidant activity of wines produced from must (12.5 °Be) using immobilized and free cells at 28, 21, 14 and 7 °C.

| Fermentation Temperature (°C) | Fermentation Using Immobilized Cells | | Fermentation Using Free Cells | |
|---|---|---|---|---|
| | Fermentation Batch | $EC_{50}$ (mL Wine/g $DPPH^{\cdot}$) | Fermentation Batch | $EC_{50}$ (mL Wine/g $DPPH^{\cdot}$) |
| 28 | 1, 3, 5, 7, 9 | 544.2 ± 0.6 a | 1, 3, 5, 7, 8 | 700.5 ± 1.1 A |
| 21 | 10, 12, 14, 16, 17 | 537.8 ± 1.1 b | 9, 10, 11, 13, 15 | 761 ± 0.7 B |
| 14 | 19, 20, 21, 23 | 597.5 ± 0.8 c | 16, 17, 18, 19 | 918.4 ± 3 C |
| 7 | 25, 26, 27 | 654.2 ± 0.8 d | 20, 21, 22 | 990.1 ± 1 D |

Different lower and uppercase letters in each column indicate statistically significant differences at the confidence level $p < 0.05$.

The antioxidant activity expressed as Efficient Concentration ($EC_{50}$) value is inversely related to the phenolic content and generally follows the order red wines < rose wines < white wines. As shown in Table 8, 537.8 mL of wine (average value) produced by immobilized cells on resin at 21 °C are required to scavenge DPPH free radicals, in relation to 544.2, 597.5 and 654.2 mL of wine (average values) at 28, 14 and 7 °C. Regarding wines produced by free cells, 700.5, 761.0, 918.4 and 990.1 mL of wine (average value) are required to scavenge DPPH free radicals at 28, 21, 14 and 7 °C.

The presence of resin confirms the antioxidant activity of the produced wines by yeast cells immobilized on resin.

### *3.6. Preservation of the Wines Produced by Immobilized and Free Cells*

The produced wines without any treatment, e.g., addition of potassium metabisulfite for preservation, were kept for at least 35 days at room temperature (22–28 °C) and 95 days at 4 °C. As shown in Table 9 (total results in Table S9), the values of the total acidity were in acceptable levels (4–6 g of tartaric acid/L). The acceptable level of the volatile acidity expressed in acetic acid must be lower than 1 g/L (Tables 10 and S10). The volatile acidity values in wines produced by yeast cells immobilized on resin are lower than 0.52 g/L at all preservation conditions. Resin terpenes, polyphenols contributed to this result as terpenes and polyphenols exhibit antioxidant and antibacterial action [32]. Some of the detected terpenes such as 4-terpineol, bornyl acetate, linalool, verbenone and L-trans-pinocarveol appearing in the wines were produced by using the immobilized yeast on *P. terebinthus* resin at each fermentation temperature contributing to their preservation [33–38]. The presence of other terpenes in wines depends on the fermentation temperature and time (Table 5). To sum up, all the detected terpenes contribute to the preservation of wine.

**Table 9.** Total acidity of wines produced from must (12.5 °Be) using immobilized and free cells at 28, 21, 14 και 7 °C and analyzed after storage at 22–28 °C and 4 °C of their production.

| Fermentation Temperature (°C) | Fermentation Using Immobilized Cells | | | | Fermentation Using Free Cells | | | |
|---|---|---|---|---|---|---|---|---|
| | | Total Acidity (g Tartaric Acid/L) | | | | Total Acidity (g Tartaric Acid/L) | | |
| | Fermentation Batch | 0 Days | >30 Days (22–28 °C) | >90 Days (4 °C) | Fermentation Batch | 0 Days | >30 Days (22–28 °C) | >90 Days (4 °C) |
| 28 | 1, 3, 5, 7, 9 | 6.0 ± 0.4 a | 5.7 ± 0.3 c | 5.4 ± 0.4 e | 1, 3, 5, 7, 8 | 5.3 ± 0.2 a | 5.1 ± 0.2 c | 4.8 ± 0.2 d |
| 21 | 10, 12, 14, 16, 17 | 5.3 ± 0.5 a | 5.1 ± 0.5 c | 4.7 ± 0.5 e | 9, 10, 11, 13, 15 | 5.3 ± 0.2 a | 5.0 ± 0.2 c | 4.6 ± 0.3 d |
| 14 | 19, 20, 21, 23 | 5.0 ± 0.7 ab | 4.9 ± 0.6 c | 4.5 ± 0.5 ef | 16, 17, 18, 19 | 5.2 ±0.6 a | 5.0 ± 0.7 c | 4.7 ± 0.5 d |
| 7 | 25, 26, 27 | 4.8 ± 0.3 ab | 4.7 ± 0.3 d | 4.6 ± 0.2 ef | 20, 21, 22 | 4.5 ± 0.1 ab | 4.4 ± 0.1 c | 4.3 ± 0.1 d |

Different letters in each column indicate statistically significant differences at the confidence level $p < 0.05$.

**Table 10.** Volatile acidity of wines produced from must (12.5 °Be) using immobilized and free cells at 28, 21, 14 and 7 °C and analyzed after storage at 22–28 °C and 4 °C of their production.

| Fermentation Temperature (°C) | Fermentation Using Immobilized Cells | | | | Fermentation Using Free Cells | | | |
|---|---|---|---|---|---|---|---|---|
| | | Volatile Acidity (g Acetic acid/L) | | | | Volatile Acidity (g Acetic Acid/L) | | |
| | Fermentation Batch | 0 Days | >30 Days (22–28 °C) | >90 Days (4 °C) | Fermentation Batch | 0 Days | >30 Days (22–28 °C) | >90 Days (4 °C) |
| 28 | 1, 3, 5, 7, 9 | 0.30 ± 0.04 a | 0.35 ± 0.03 b | 0.31 ± 0.04 d | 1, 3, 5, 7, 8 | 0.31 ± 0.04 a | 1.71 ± 0.16 b | 0.35 ± 0.03 d |
| 21 | 10, 12, 14, 16, 17 | 0.29 ± 0.02 a | 0.37 ± 0.03 b | 0.32 ± 0.01 d | 9, 10, 11, 13, 15 | 0.29 ± 0.02 a | 1.80 ± 0.10 b | 0.40 ± 0.04 d |
| 14 | 19, 20, 21, 23 | 0.29 ± 0.02 a | 0.41 ± 0.03 b | 0.33 ± 0.01 d | 16, 17, 18, 19 | 0.31 ± 0.03 a | 2.01 ± 0.28 b | 0.47 ± 0.02 e |
| 7 | 25, 26, 27 | 0.27 ± 0.02 a | 0.51 ± 0.01 c | 0.36 ± 0.02 d | 20, 21, 22 | 0.29 ± 0.02 a | 2.54 ± 0.40 c | 0.53 ± 0.02 f |

Different letters in each column indicate statistically significant differences at the confidence level $p < 0.05$.

### *3.7. Sensory Evaluation*

The grape variety, microbial strain, fermentation process and aging contribute to the wine aroma which is an attractive organoleptic characteristic for the consumers [39]. The selected wines for testing were those that were fermented at 14 °C as at this temperature the volatile acidity of the wines maintains low levels and the wines retain their aroma. Sensory evaluation of wine samples is presented in Figure 2. The aroma and the smell of *P. terebinthus* resin were predominant in the wines produced by: (a) immobilized cells on *P. terebinthus* resin at 14 °C and storage at 22–28 °C; and (b) immobilized cells on *P. terebinthus* resin at 14 °C and storage at 4 °C. On the other hand, the wine produced

by free cells (without resin) had a pleasant and fruity aroma. The wines with this special aroma of resin were preferred by the tasters.

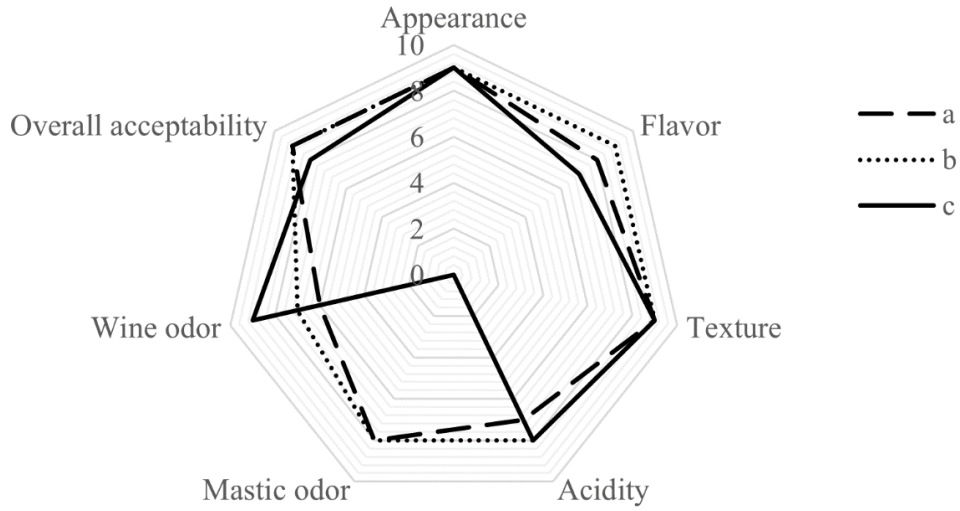


**Figure 2.** Sensory evaluation of wines produced by (**a**) immobilized cells on *P. terebinthus* resin at 14 °C and storage at 22–28 °C; (**b**) immobilized cells on *P. terebinthus* resin at 14 °C and storage at 4 °C; and (**c**) by free cells at 14 °C and storage at 4 °C at the 30th day of their storage.

## 4. Conclusions

The use of *Pistacia terebinthus* resin as carrier of *Saccharomyces cerevisiae* AXAZ-1 had beneficial effects in the winemaking process and the white wine produced. The immobilized AXAZ-1 strain gave a higher fermentation rate than the free one and so the system resin/*Saccharomyces cerevisiae* AXAZ-1 strain acted as a promoter of the alcoholic fermentation.

The resin has many phytochemicals which extracted in the wine, giving a functional beverage with antioxidant potential due to extracted terpenes, terpenoids and polyphenols. The extracted phytochemicals contributed to a particularly pleasant aroma profile and long preservation time compared to the winemaking with free cells.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app12189097/s1, Figure S1: Classification of fermentation temperature (batches 1–22) based on fermentation time (h), ethanol (%, *v*/*v*), ethanol productivity (g/L/d), residual sugar (g/L), sugar conversion (%), and LDA; Figure S2: Classification of fermentation temperature (batches 1–27) based on fermentation time (h), ethanol productivity (g/L/d), residual sugar (g/L), sugar conversion (%), and LDA; Figure S3: Classification of fermentation temperature (batches 1–27) based on volatile compounds and LDA; Figure S4: Volatile compounds correlated with the fermentation temperature in batches 1–27 as principal components during factor analysis; Figure S5: Classification of fermentation temperature (batches 1–22) based on volatile compounds and LDA; Figure S6: Volatile compounds correlated with the fermentation temperature in batches 1-22 as principal components during factor analysis; Table S1: Kinetic parameters of must repeated fermentation batches (12.5 °Be) using free cells at 28, 21, 14 and 7 °C; Table S2: Kinetic parameters of must repeated fermentation batches (12.5 °Be) using immobilized biocatalyst at 28, 21, 14 and 7 °C; Table S3: Major volatile by-products of must repeated fermentation batches (12.5 °Be) using immobilized biocatalyst at 28, 21, 14 and 7 °C.; Table S4: Major volatile by-products of must repeated fermentation batches (12.5 °Be) using free cells at 28, 21, 14 and 7 °C; Table S7: Polyphenolic content of wines produced from must (12.5 °Be) using immobilized and free cells at 28, 21, 14 and 7 °C; Table S8: Antioxidant activity of wines produced from must (12.5 °Be) using immobilized and free cells at 28, 21, 14 and 7 °C; Table S9: Total acidity of wines produced from must (12.5 °Be) using immobilized and free cells at 28, 21, 14 and 7 °C and analyzed after storage at 22–28 °C and 4 °C of their production; Table S10: Volatile acidity of wines produced from must (12.5 °Be) using immobilized and free cells at 28, 21, 14 και and 7 °C and analyzed after storage at 22–28 °C and storage at 4 °C of their production; Table S11: Volatile

compounds extracted from *Pistacia terebinthus* resin in methanolic solutions (20, 15, 10, 5 and 0%); Table S12: Number of volatile compounds extracted from *Pistacia terebinthus* resin in methanolic solutions (20, 15, 10, 5 and 0%). References [23,24,40] are cited in the Supplementary Materials.

**Author Contributions:** Conceptualization, M.K. (Michalis Kallis), M.K. (Maria Kanellaki) and A.A.K.; methodology, M.K. (Michalis Kallis), M.K. (Maria Kanellaki) and A.A.K.; software, I.K.K.; validation, M.K. (Maria Kanellaki), K.B. and A.A.K.; statistical analysis, I.K.K.; investigation, M.K. (Michalis Kallis); resources, M.K. (Maria Kanellaki), K.B.; writing—original draft preparation, M.K. (Michalis Kallis), M.K. (Maria Kanellaki) and K.B.; writing—review and editing, M.K. (Michalis Kallis), M.K. (Maria Kanellaki), K.B. and I.K.K.; supervision, M.K. (Maria Kanellaki) and A.A.K.; project administration, M.K. (Maria Kanellaki) and A.A.K. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Data available in a publicly accessible repository.

**Conflicts of Interest:** The authors declare no conflict of interest.

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