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# Alternative Winemaking Techniques to Improve the Content of Phenolic and Aromatic Compounds in Wines

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**Abstract:** In this study, a complete physical–chemical analysis was performed for Fetească neagră wine, aged with oak staves. Red wine samples were taken from grape varieties grown in Northeast Romania and produced during 2013 vintage. At the end of the fermentation process, four oak mini staves (1 cm width × 10 cm length × 1 cm thickness) from heavy toasted French oak were added to 5 L of red wine. Samples were aged using two time periods, respectively at 1.5 and 3 months, in a room at 14–16 °C. Results showed that the initial content of total phenolic decreased during ageing, from 931.1 mg catechin/L at 1.5 months to 775.4 mg catechin/L at 3 months. In contrast, the initial content of total antioxidant activity increased after the same period of ageing to 13.3 mM Trolox as compared to the aged wines for 1.5 months, at 12.8 mM Trolox. The corregram representing the relationship between the total phenols, total antioxidant activity (TAA) and their fractions and CieLab parameters was performed. Thirty-seven minor volatile compounds were quantified by stir bars sorptive extraction and gas chromatography coupled with mass spectrometry (SBSE-GC-MS). An increase in odor activity value (OAV) with ageing time was observed, especially for fruity, fatty and woody series. The oak staves used in ageing processes can contribute positively to the aromatic profile of wines and could be considered a good choice for producing short-aged wines.

**Keywords:** red wines; oak staves; phenolic compounds; antioxidant activity; volatile compounds



**Citation:** Dumitriu (Gabur), G.-D.; Teodosiu, C.; Gabur, I.; Cotea, V.V.; Peinado, R.A.; López de Lerma, N. Alternative Winemaking Techniques to Improve the Content of Phenolic and Aromatic Compounds in Wines. *Agriculture* **2021**, *11*, 233. https://doi.org/10.3390/agriculture11030233

Academic Editor: Gianluca Caruso

Received: 30 January 2021
Accepted: 5 March 2021
Published: 11 March 2021



## 1. Introduction

Wines are appreciated by consumers due to their characteristics, aroma and health benefits, and therefore, some studies have already been undertaken to identify the wine volatiles and phenolic compounds and also to address changes during the ageing time [1]. Phenolic compounds have been widely studied because they have antibacterial [2] and antioxidant [3] properties, healthy effects on the cardiovascular [4] and nervous systems [5], and they promote benefits to consumer health associated with regular, moderate red wine consumption. Red wine antioxidant properties influenced by phenol classes are of great interest for the winemaking industry [6]. Besides, wine is a complex system where chemical changes can occur during major winemaking techniques such as ageing.

Nowadays, consumers prefer wines that are young and without a long age, due to their excellent organoleptic characteristics and plentiful fruitiness [1]. Traditionally, the ageing of red wines is performed in oak wood barrels during periods of time, dependent on the beverage to be aged and the intended final product [7]. Due to some disadvantages, such as

limited lifecycle, and the expensive cost for the storage, handling, cleaning and sanitation, oenologists are looking for alternative new products.

In the wine industry, alternative winemaking inputs are available on the market that can be used in the ageing process. These products may differ in sizes and can be found as powders, chips, staves, and other forms [8]. Oak chips (and oak barrels) contain phenolic acids, flavonols, flavanols, anthocyanins and furanic compounds which can be extracted into wine [9]. These compounds are known to express specific flavors such as vanilla, chocolate, roasted almond, spices and smoky and also influence the formation of flavano-ellagitannins, stabilize the color, or influence the bitterness and astringency levels [10]. Generally, volatile compounds are extracted from oak wood, depending on the available compound quantity, contact interval among wine-oak wood, levels of toasting and type of oak wood used. All these characteristics of oak wood directly impact the final wine aroma typicity. Additionally, a key factor in defining the final characteristics of wines is the size of the wood product. Del Alamo et al. [11] affirm that the smaller the piece of wood, the better the evolution undergone by wine. Alternative oak products are obtained from wood that are seasoned and toasted in function to its size and shape. The common seasoning used takes place under natural conditions in open air for 18–36 months [12]. In this period of time, the wood matures, changes in its chemical composition (increasing aromatic properties) and reduces the bitterness and astringency. The toasting process is the most important stage in the wood chemical composition due to the heat which provokes various chemical transformations and the degradation of wood. This process conducts a significant increase in aromatic compounds and a decrease in astringency. The consumer buying decision, overall estimation of wine quality and price are all based on the wine aroma profile [1].

Romania is an important European wine producer, with a strong history of wine-making and an increasing interest in autochthonous wines. Fetească neagră appeared through popular selection made in time, from the wild forest vine (*Vitis silvestris*) which was cultivated by the Dacian peoples, in an area between the Carpathians and the Nistru River. The Fetească neagră variety is an old Romanian–Moldavian dark-skinned grape variety developed in Uricani, Iasi, a winemaking region. Fetească neagră has been cultivated in Romania for over 2000 years but is less known in other viticultural countries [13]. Fetească neagră aged wine is considered one of the best red wines due to its smoky, plum, black currant, and chocolate characters.

The aim of this paper is to characterize one of the autochthonous red wines (Fetească neagră) aged with oak staves during two ageing periods, which includes the phenolic compounds, total antioxidant activity and their fractions (phenolic acids, flavanols, flavonols and anthocyanins), color properties and last but not least, the minor volatile compounds.

## 2. Materials and Methods

### *2.1. Winemaking and Ageing Conditions*

Red wine used for the trials was from grape varieties grown in Northeast Romania and produced during 2013 vintage. The maceration–fermentation process at 10–12 °C followed the crushing stage and lasted for 7 days. After maceration, the marc was pressed, and the wines produced were conveyed into fermentation tanks for completing the alcoholic/malolactic fermentation. Daily pumping overs were carried out to optimize the extraction of color, phenolics and aroma compounds and their respective flavors. At the end of the fermentation processes, 4 oak mini staves were added to 5 L of red wine. The dimensions in centimeters for the mini staves was 1 × 10 × 1 (width × length × thickness) and was obtained from heavy toasted French oak (*Quercus petraea*). The wines were aged for two time periods, respectively at 1.5 and 3 months, in a room at 14–16 °C. The experiment was performed in three replicates.

### 2.2. Analytical Parameters

The enological parameters include alcohol content, titratable acidity (expressed as tartaric acid) and volatile acidity (as acetic acid), pH, total and free $SO_2$ for red wines. These parameters were measured according to the International Vine and Wine Organization (OIV) International Oenological Codex [14]. CieLab parameters (L*, a*, b*, C*, H* and I*) were measured in a Perkin Elmer Lambda 25 spectrophotometer. Initially, samples were filtered through a HA-0.45 µm paper (Millipore, Milford, MA, USA).

### 2.3. Fractionation of Phenolic Compounds and Total Phenoloc Content

In the first step, ethanol was removed, and distilled water was added until the sample reached the initial volume. The fractionation of phenolic compounds was analyzed according to the protocol described by Dae-Ok and Chang [15] and was realized by using tC-18 SepPak columns. In the second step, the activation of columns was carried out with 5 mL of methanol and washed with 5 mL of ultra-pure water. The columns were set up with 5 mL of water at pH 7, and 1 mL of sample adjusted to pH 7 was supplied. In the third step, phenolic acids and their respective esters (fraction 1) were eluted with 5 mL of water at pH 7. The elution of flavanols (fraction 2) was carried out with 5 mL of 16% acetonitrile at pH 2. Finally, flavonols (fraction 3) and anthocyanins, tannins and other polymeric pigments (fraction 4) were eluted with 5 mL of ethyl acetate and 5 mL of methanol, respectively.

The total phenolic content of the samples was investigated, and the phenolic fractions were obtained according to the method described by Stevanato et al. [16].

### 2.4. Total Antioxidant Activity

The chromophore $ABTS^+$ method was used to determine the antioxidant activity of samples, according the Re et al. [17]. The oxidation of 7 mM of ABTS with 2.45 mM of potassium persulphate only in state of darkness for 12–16 h produced the $ABTS^+$. The obtained $ABTS^+$ solution was diluted in 20 mM of phosphate buffer at pH 7.4 to obtain an absorbance at 734 nm of 0.7. A 100 µL wine sample, previously filtered with HA-0.45 µm papers (Millipore, Milford, MA, USA) was reacted with 900 µL of this test mixture. After 6 min, the absorbance at 734 nm of the reaction mixture was determined. The antioxidant activity was calculated and the percentage of inhibition must be in the interval of 20%–80%.

### 2.5. Minor Aroma Compounds

Minor aroma compounds extraction was performed according to the method described by Lopez de Lerma et al. [18]. Samples were diluted in a proportion 1:10 with a hydroethanolic solution (12% ethanol $v/v$) and pH was adjusted to 3.5. The stir bars were placed in a 10 mL glass container. Each container had 10 mL of the diluted sample and 0.1 mL of a solution of ethyl nonanoate (0.4464 mg/L) as internal standard. The stirring of the containers was performed at 1500 rpm, 25 °C for 100 min. After this, wine was removed from the container and the stir bar was gently dried and prepared for gas chromatography mass spectrometry (GC-MS) analysis.

The glass thermal desorption tubes were placed into a GC-MS equipped with a Gerstel TDS 2 thermodesorption system. The stir bars were heated to release and transfer the extracts into a cooled injection system/programmed temperature vaporizer (CIS 4 PTV) containing a tenax adsorption tube. The GC-MS equipment was set as the following: thermal desorption at 35 °C, ramped at 120 °C $min^{-1}$ to 280 °C for 10 min; helium flow rate was set at 3 mL/min; CIS injector at 25 °C for the total desorption time, followed by 12 °C $s^{-1}$ in splitless mode to 280 °C for 7 min. The GC was fitted with an Agilent-19091S capillary column 30 m × 0.25 mm i.d., 0.25 mm film thickness. Helium was used as the carrier gas with a column flow rate of 1 mL $min^{-1}$. Retention times, spectral libraries supplied by Wiley (version 7 N) and for the identification, confirmation and preparation of standard solutions of the volatile compounds, pure chemical compounds were used, which were

obtained from Merck, Sigma Aldrich, Riedel de Haen and Fluka. The calibration curve was obtained by using standard solutions of known concentrations previously subjected to the same treatment as the samples in conjunction with the target and qualifier ions selected for each compound by the Hewlette Packard Chemstation (Palo Alto, CA, USA).

### *2.6. Aromatic Series and the Odorant Activity Value*

The volatile compound contribution to the wine samples' aroma characteristics could be investigated both qualitativly or quantitativly, using the aroma descriptor or the odorant activity value (OAV). The OAV can be used as a measure of the influence of specific aroma compounds to the overall aroma profile of a sample. Mathematicaly, the OAV represents the ratio between the concentration of an individual aroma compound and the perception threshold of that compunds, as found in the literature [19]. Afterwords, the sum of all compounds' OAV in a given series is used to identify the total intensity of a specific aromatic series.

### *2.7. Statistical Analysis*

Statistical significance was determined using an analysis of variance (ANOVA) of the experimental data. Analyses were performed by using Statgraphics Centurion XVI of StatPoint Technologies Inc. (Warrenton, VA, USA). A correlation matrix using corrgram was conducted; therefore, Pearson's correlation was performed using phenolic compounds, antioxidant activity, and, respectively, their fractions and color parameters. The correlation analysis was performed by using the R package "corrgram".

## 3. Results

### *3.1. Enological Parameters*

After 1.5 months of ageing, all important red wine chemical characteristics were analyzed and the following values were obtained: alcohol content 14.9 % (*v*/*v*), pH 3.65, titratable acidity 5.67 g/L, volatile acidity 0.53 g/L (as acetic acid), total $SO_2$ 138 mg/L and free $SO_2$ 41 mg/L. After 3 months of ageing, the same major chemical compounds showed: alcohol content 14.9 % (*v*/*v*), pH 3.69, titratable acidity 5.40 g/L, volatile acidity 0.56 g/L, total $SO_2$ 115 mg/L and free $SO_2$ 37 mg/L.

### *3.2. Total Phenolic Content and Total Antioxidant Activity*

During the ageing process, various reactions involving co-pigmentation, condensation and polymerization take place between its phenolic compounds. Therefore, these reactions certainly influence its structure and undoubtedly, its antioxidant activity [20].

With the exception of flavanols (F2) of total phenols and initial, phenolic acid (F1), flavanols (F2) and anthocyanins (F4) of total antioxidant activity, all the compounds exhibited significant differences when 1.5 months and 3 months were compared by analysis of variance. Phenolics acids and esters changed their concentrations significantly ($p \leq 0.01$); all the other compounds changed their concentrations with $p \leq 0.001$ (Table 1).

The initial content of total phenols decreased after 3 months of ageing (775.4 mg catechin/L) as compared to a 1.5-month ageing time (931.1 mg catechin/L). This decrease in total phenolic content is especially due to their participation in numerous condensation reactions, as well as in hydrolytic and other degradation reactions. Additionally, during ageing, proanthocyanidins suffer a spontaneous cleavage and polymerization with anthocyanins, as well as precipitation of large insoluble polymers formed [21]. In contrast, the initial content of total antioxidant activity increased after the same ageing period (13.3 mM Trolox) as compared to aged wines of 1.5 months (12.8 mM Trolox) (Table 1). The results presented in previous published articles dealing with the antioxidant activity during wine ageing are until now too antithetical. Burin et al. [22] and Alén-Ruiz et al. [23] showed a significant increase as compared to the respective wines. In a different study, Rivero-Pérez et al. [20] affirmed that antioxidant activity decreases over time during ageing.

**Table 1.** Chemical parameters of red wines aged with oak staves for 1.5 and 3 months.

| Parameters | Fractions | 1.5 Months | 3 Months | *p* Value | Sign. |
|---|---|---|---|---|---|
| Total phenolic (TP) content mg catechin/L | Initial | 931.1 ± 19.0 [b] | 775.4 ± 21.4 [a] | 0.0007 | *** |
| | Phenolic acids and esters (F1) | 120.0 ± 4.3 [a] | 147.0 ± 4.4 [b] | 0.0016 | ** |
| | Flavanols (F2) | 141.9 ± 10.5 [a] | 141.6 ± 4.3 [a] | 0.9699 | ns |
| | Flavonols (F3) | 333.1 ± 9.3 [b] | 225.9 ± 6.8 [a] | 0.0001 | *** |
| | Anthocyanins and polymeric compounds (F4) | 282.4 ± 0.5 [b] | 216.2 ± 6.5 [a] | 0.0001 | *** |
| Total antioxidant activity (TAA) mM Trolox | Initial | 12.8 ± 0.7 [a] | 13.3 ± 0.3 [a] | 0.2455 | ns |
| | Phenolic acids and esters (F1) | 1.6 ± 0.05 [b] | 1.5 ± 0.04 [a] | 0.0136 | ns |
| | Flavanols (F2) | 2.1 ± 0.06 [a] | 2.3 ± 0.07 [b] | 0.0212 | ns |
| | Flavonols (F3) | 1.1 ± 0.03 [a] | 1.9 ± 0.06 [b] | 0.0000 | *** |
| | Anthocyanins and polymeric compounds (F4) | 6.6 ± 0.2 [a] | 6.9 ± 0.2 [a] | 0.2204 | ns |
| CieLab parameters | L* | 31.95 ± 0.96 [a] | 31.48 ± 0.94 [a] | 0.5732 | ns |
| | a* | 56.52 ± 1.7 [a] | 58.10 ± 1.74 [a] | 0.3223 | ns |
| | b* | 34.40 ± 1.03 [a] | 37.24 ± 1.12 [b] | 0.0317 | ns |
| | H | 31.33 ± 0.94 [a] | 32.66 ± 0.98 [a] | 0.1640 | ns |
| | C | 66.16 ± 1.98 [a] | 69.01 ± 2.07 [a] | 0.1603 | ns |
| | I | 4.81 ± 0.14 [a] | 5.07 ± 0.15 [a] | 0.1038 | ns |

The values are mean ± standard deviation of three independent experiments. Different letters indicate significant differences at $p < 0.05$ level according to the Fisher's Least Significant Difference (LSD) test. The alphabetical order indicates an increasing content. Signs: *, **, *** and ns indicate significance at $p < 0.05$, $p < 0.01$, $p < 0.001$ and not significant, respectively. CieLab parameters: L* (lightness), a* (red-greenness component), b* (yellow-blueness component), H (Hue), C (Chroma), I (Intensity).

Oak wood used in winemaking has a complex phenolic composition that includes phenolic acids, flavanols, cumarins, gallic and ellagic tannins [24]. The high solubility of these compounds in hydroalcoholic solution makes them desirable during winemaking as they influence the overall composition of the phenolic families. In our study, the flavanols, flavonols and anthocyanins (fraction 2, 3, 4) content of total phenolic decreased with ageing time, probably due to the polymerization reactions that occurred. The results are in agreement with a previous study that used wines obtained from Cabernet Sauvignon [25]. The loss of proanthocyanidins may have been caused by the classical acid-catalyzed C–C bond-breaking [26] or by the precipitation of high molecular weight polymers [27]. On the other hand, only an increase in phenolic acid and esters (fraction 1) content of total phenolic was observed, as a consequence of the extraction of phenolic compounds from wood. Cadahía et al. [28] reported that ageing using French oak for a period of 12 months causes increased values of hydroxybenzoic acids and their derivatives in wine samples. Moreover, the accumulation of hydroxybenzoic acids during the ageing process may also be caused by hydrolysis of gallic tannins [29].

In Table 1, the result of a fraction of the total antioxidant activity can be noticed, which consist of increases in all values after 3 months of ageing. Anthocyanins have been confirmed to contribute significantly to the wine color attributes [30]. Additionally, anthocyanins and flavonols polymerization can cause deposits during the ageing process [31,32].

### 3.3. Color Parameters

Wine color was investigated at pilot scale by measuring the chromatic L* (lightness), a* (red-greenness component), b*(yellow-blueness component), H (hue angle), C (chroma) and I (intensity) (Table 1). The lightness (L*) slightly decreased after 3 months of ageing, in accordance with results described by Schwarz et al. [33] in Sherry wines. Additionally,

ageing may stimulate the formation of reddish-brown polymers during the winemaking process [34]. The parameters a*, b*, H, C and I increased after 3 months of ageing. The coordinate b* and H increased slightly in red wines with ageing time due to the formation of yellow–orange pigments during wine ageing [35]. In contrast, Chaves et al. [34] noticed a decrease in coordinate b* and H in wines produced from the Pedro Ximenez variety. Additionally, higher a* values could be linked to the formation of anthocyanin-derived compounds [36]. Chroma (C*) and hue angle (H*) are the corresponding angular coordinates derived from the Cartesian coordinates a* and b*, but they are better related to the human sensory perception of color. The vividness of color (C*) shown by aged wines increased for 3-month wines ageing as compared to 1.5-month wines ageing. Moreover, these results obviously indicate that aged red wines exposed a yellow–red tonality [37]. In the ageing, substantial modification of the color of red wines takes place, from red/violet in young wine to red/orange in aged ones due to oxidation, reduction and polymerization of anthocyanins. Anthocyanins can react with catechin monomers and condensed tannins through aldehydes, and the formation of new pigments, such as anthocyanin-alkyl-catechin, can cause bathochromic shifts (bluish red hues). Thus, a formation pyrano-anthocyanin-catechin compounds takes place, that causes a hypsochromic shift (orange hues). [38].

### *3.4. Correlation between Total Phenolic Content, Total Antioxidant Activity and Color Parameters*

Polyphenol compounds are known due to their own antioxidant properties that generally are presumed to be linked to human health benefits [39]. Therefore, a correlation matrix using correlogram was conducted; Pearson's correlation was carried out using phenolic compounds and antioxidant activity, and their fractions and color parameters, respectively.

This statement is supported by the significant high positive correlations found between total phenols and F3-total phenolic (TP) ($r$ = 0.98) as well as F4-TP ($r$ = 0.97) and F1-total antioxidant activity (TAA) ($r$ = 0.91). Additionally, significant negative correlations were observed between total phenols and F1-TP ($r$ = −0.96), F2-TAA ($r$ = −0.83) and F3-TAA ($r$ = −0.97). F1-TP has a significant positive correlation with F3-TAA ($r$ = 0.97), F2-TAA ($r$ = 0.86) and a significant negative correlation with F3-TP ($r$ = −0.96), F4-TP ($r$ = −0.94) and F1-TAA ($r$ = −0.88). F3-TP presented a significant positive correlation with F4-TP ($r$ = 0.99) and F1-TAA ($r$ = 0.95) and a significant negative correlation with F2-TAA ($r$ = −0.81) and F3-TAA ($r$ = −0.98). F4-TP revealed a significant positive correlation with F1-TAA ($r$ = 0.93) and significant negative correlation with F2-TAA ($r$ = −0.84) and F3-TAA ($r$ = −0.98) (Figure 1).

No statistically significant correlation ($p < 0.05$) was observed for TP and TAA in the wines (Figure 1). F1-TAA is significantly negatively correlated with F3-TAA ($r$ = −0.86). F2-TAA presented a significant positive correlation with F3-TAA ($r$ = 0.92) and F3-TAA ($r$ = 0.90). Arnous et al. [40] demonstrated that there is a weak correlation among the principal polyphenols and the antioxidant parameters.

The L* color parameter showed a significant positive correlation with F2-TP ($r$ = 0.89). L* values were related to the involvement of F2-TP in the formation of yellow pigments, which made it seem more opaque. Anthocyanins have been related to the red color of wine, whereas flavonols and flavan-3-ols may improve the red color of young wine by the co-pigmentation process [33]. Therefore, a* was significantly positively correlated with F2-TAA ($r$ = 0.85) and F4-TAA ($r$ = 0.99), while, b* was significantly positively correlated with F1-TP ($r$ = 0.83), F2-TAA ($r$ = 1), F3-TAA ($r$ = 0.89), F4-TAA ($r$ = 0.92) and a* ($r$ = 0.88). However, a* and b* provide both quantitative and qualitative chromatic information and can be linked with the visual human color recognition [41]. Hence, changes of the color components of aged wines with oak staves led to significant modifications in C* and H*. C* is significantly positively correlated with F2-TAA ($r$ = 0.94), F4-TAA ($r$ = 1), a* ($r$ = 0.98), and b* ($r$ = 0.95), which are typical for the red color as mentioned before. H* presented a significant positive correlation with F2-TAA ($r$ = 0.93), F4-TAA ($r$ = 1), a* ($r$ = 0.98) and b*($r$ = 0.95). Moreover, C* was strongly positively correlated with H* ($r$ = 1). This aspect highlighted the decrease in the co-pigmentation effect and the presence of polymeric pigments during ageing [37]. The I* color indicator was significantly affected by the

aged wines. Thus, the justification of the significant correlations found between F2-TAA ($r$ = 0.97), F4-TAA ($r$ = 0.98), a* ($r$ = 0.96), b* ($r$ = 0.98), H* ($r$ = 0.99) and C* ($r$ = 1) with I* is presented in Figure 1.

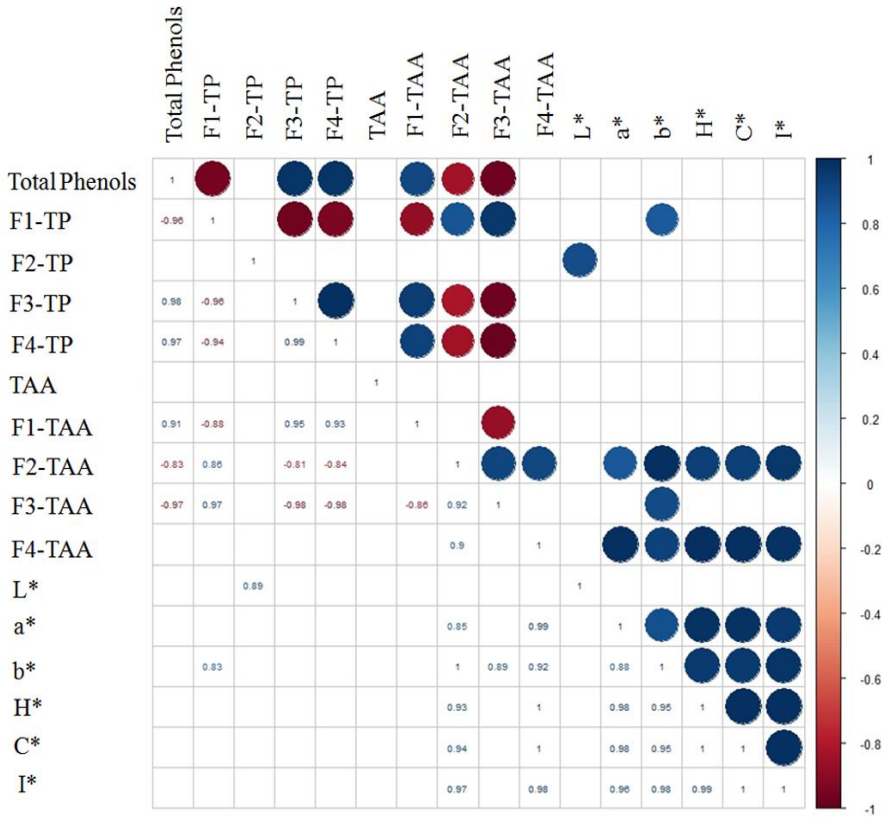


**Figure 1.** Correlations between total phenols, total antioxidant activity (TAA), their fractions (F1, F2, F3 and F4) and color parameters. Blue circles represent significant positive correlations and red circles represent significant negative correlations.

Blue circles correspond to significant positive correlations and red circles to significant negative correlations. The size of the circle reflects the magnitude of the Pearson correlation coefficient. These correlation coefficients range between −1 and +1 and measure the strength of the association between the variables.

### *3.5. Volatile Compounds*

#### 3.5.1. Chemical Families

Thirty-seven minor volatile compounds were identified and quantified, and then were grouped into alcohols (five), carbonyls (five), carboxylic acids (four), esters (14), lactones (four), terpenes (one), volatile phenols (2two) and oak compounds (two).

In Figure 2, the concentration of the chemical families for aged wines with oak staves at 1.5 and 3 months is represented. Wines aged for 1.5 months with oak staves present the highest concentration of carboxylic acids, esters, followed by alcohols and the lowest amount of terpenes. After 3 months of ageing, wines present similar behaviors, but with concentrations much higher than at 1.5 months. Additionally, all chemical families were characterized by the increases of concentrations at 3 months. Esters are generally produced during the alcoholic fermentation by the activity of yeasts and are known to influence on fruity and floral aromas in wines [42]. One more chemical family with a positive impact on the red wines were the oak compounds, due to these compounds being directly extracted from wood. Coconut, vanilla, and woody-like are the main characteristics for whiskey lactone [43].

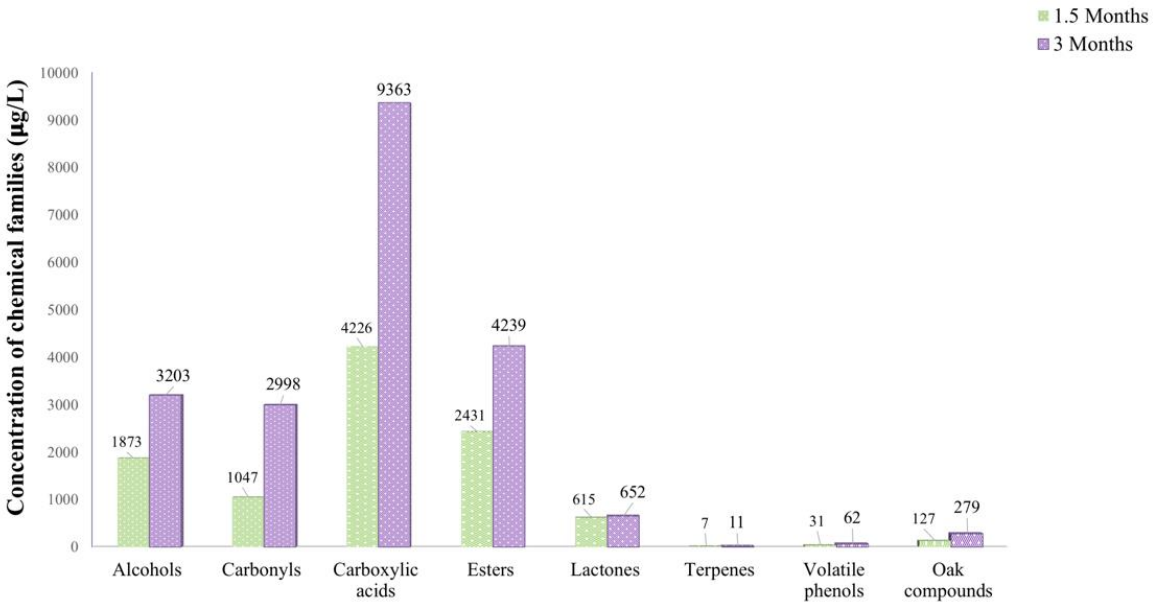


**Figure 2.** Concentration of chemical families (µg/L) in wines aged with oak staves at 1.5 and 3 months.

#### 3.5.2. Volatile Compounds Identified and Quantified in Wines Aged with Oak Staves

With the exception of furfuryl alcohol, heptanal, octanal, butanoic acid, hexanoic acid, ethyl vanillate, decalactone, all the compounds exhibited significant differences when 1.5 months and 3 months ageing time were compared by analysis of variance. E-2-hexenol, phenylethyl acetate, ethyl decanoate, ethyl hexadecanoate, butyrolactone, limonene modified their concentrations significantly ($p \leq 0.01$); all the others compounds changed their concentrations significantly ($p \leq 0.001$).

The concentration of minor alcohols for samples with oak staves increased with the ageing time. These results are comparable to those obtained by Petropulos et al. [44]. Hexanol was the main minor alcohol, with a higher concentration in wines aged for 3 months (2387.37 µg/L) than in wines aged for 1.5 months (1349.47 µg/L) (Table 2). This is associated with the grape variety [45] and should be considered as positive for the wines, as the presence of high values of this compound can give herbaceous odor nuances [46].

Furfural and 5-methylfurfural increased considerably with ageing time. These compounds are obtained by the degradation of carbohydrates during oak staves toasting steps. Furfural is formed when pentoses are exposed to high heat levels and 5-methyl furfural is obtained from rhamnose [47]. The concentration of furfural was higher in wines aged for 3 months (1952.61 µg/L) than 1.5 months (696.07 µg/L) and the 5-methylfurfural show similar behavior, being higher at 3 months (892.98 µg/L) than at 1.5 months (229.67 µg/L). These changes were similar to those previously reported by Fernández de Simón et al. [48] in a Spanish artificially aged wine, using chips and staves from oak wood at three toasting levels, varying from light to heavy, and by Del Álamo et al. [49] in a micro-oxygenation strategy that investigated oak chips or staves of different sizes and origins during the ageing process.

Furfural and 5-methylfurfural (sweet caramel-like, nutty, almond odor and flavor), heptanal (rancid and herbal aroma), octanal (citrus, fresh) and benzaldehyde (bitter almond, smoked aroma) are described as having the aroma presented by Peinado et al. [50].

A slight decrease was observed in butanoic and hexanoic acid content, and in contrast the octanoic and decanoic acid highly increased their contents during wine ageing. These results are in good agreement with Rodríguez-Bencomo et al. [51] and Petropulos et al. [44] who reported increased concentrations of octanoic acid when oak chips were added to the fermentation process of red wines. Even if a direct negative impact of oak wood compounds on the wines flavor was not reported, sometimes small concentrations

of carboxylic acids could generate unpleasant odors. Their aroma goes to rancid, cheese, sweat, fat and sour [52]. These compounds (butanoic, hexanoic, octanoic and decanoic acids) are the product of a biosynthesis of long-chain fatty acids performed by yeasts [53].

Ethyl propionate, ethyl butanoate, ethyl octanoate, ethyl 2-methyloctanoate, phenylethyl acetate, isoamyl acetate, ethyl decanoate, ethyl vanillate, ethyl tetradecanoate, ethyl hexadecanoate concentrations increased with the ageing time. Isoamyl acetate presents the highest concentration among other esters and increases from 1177.86 to 2030.80 μg/L at 3 months (Table 2). Rodrıguez-Bencomo et al. [51] and Ruiz-Moreno et al. [54] also observed higher concentrations of these compounds in a wine fermented with oak wood chips. In contrast, decreased values of ethyl isobutanoate, ethyl furoate, ethyl dodecanoate and hexyl hexanoate may be caused by chemical hydrolysis at low pH values due to the esterification processes [55].

In general, esters concentration found in wine aged is above the sensory detection threshold levels of humans. In red wines, ethyl propionate (apple, pineapple, strawberry), ethyl isobutanoate (apple, strawberry), ethyl butanoate (pineapple aroma), ethyl 2-methyloctanoate (fruity), isoamyl acetate (banana-like aroma) ethyl octanoate (pineapple, cognac, and apricot aroma), ethyl decanoate (pear aroma), ethyl tetradecanoate (tropical fruit) and hexyl hexanoate (fruity) made a very important contribution to the fruity character of samples [56] (Table 2).

Guaiacol (from 7.77 to 19.00 μg/L) and 4-vinylguaiacol (from 22.99 to 43.16 μg/L) increased with time of storage, in a similar way to that observed by Sanchez-Palomo et al. [57].

*Trans* and *cis*-whiskey lactone increased during ageing time and play an important part in the overall sensory character of wines. The *cis*-isomer threshold limit is around 74 μg/L and positively influences the specific aroma compounds as woody, vanilla and chocolate. The concentration of *trans*-whiskey lactone was higher in wines aged for 3 months (34.77 μg/L) than for 1.5 months (17.43 μg/L), and the *cis*-whiskey lactone showed the same behavior, being higher at 3 months (244.75 μg/L) than at 1.5 months (110.05 μg/L). Bautista-Ortín et al. [58] showed that higher concentrations were found when cubes or shavings were used, rather than powder. According to this author, *trans*-oak lactone was found in lower concentrations than *cis*-oak lactone, and similarly to the *cis* isomer, after 3 months of wood contact time, the maximum concentration had already been reached.

#### 3.5.3. Aromatic Series

A method of assessing the contribution of a compound to wine aroma is by calculating its odor activity value (OAV). The mathematical formula is the ratio analytical concentration to odor perception threshold. The compounds with a high OAV can be assumed to contribute noticeably to the wine aroma. Furthermore, OAVs can be used to set up the fingerprint of a wine.

The overall wine aroma was estimated using the odor descriptors grouped according to their aromatic series. In particular cases, a low number of aroma compounds were included in more aromatic series, as previously described in the literature [50,53].

The aroma fingerprint for the ageing wine can be defined, from a quantitative point of view, as fruity, fatty, woody, buttery, green, chemistry, toasty, spicy, floral and citric fruits. A comparison of the OAVs for these series allows for a better understanding of the contribution levels of specific aroma compounds extracted during wines' ageing with oak staves. Additionally, to compare the aroma specificity of wines aged for 1.5 months or 3 months, the OAVs were classified into the ten-aromatic series, describing the representing aroma of a particular wine. Figure 3 shows the aroma characteristics of the aged wine for 1.5 and 3 months, by arranging the mean values for the first six aromatic series from high to low. The results exhibit higher OAVs obtained during ageing, and especially for the fruity, fatty and woody series.

**Table 2.** Minor volatile compounds (μg/L) in wines aged with oak staves for 1.5 and 3 months. Variance analysis.

| | Families/Compounds | 1.5 Months | 3 Months | Sign. | Odor Descriptors | Odor Threshold | Aroma Series |
|---|---|---|---|---|---|---|---|
| | **Alcohols** | 1872.82± 93.57 $^{a}$ | 3203.34 ± 150.88 $^{b}$ | *** | | | |
| 1 | Hexanol | 1349.47 ± 74.90 $^{a}$ | 2387.37 ± 128.23 $^{b}$ | *** | Grass, oily, herb, resin | 2500 [59] | Green |
| 2 | E-3-hexenol | 134.00 ± 7.21 $^{a}$ | 233.00 ± 10.00 $^{b}$ | *** | Cut grass | 1000 [59] | Green |
| 3 | E-2-hexenol | 308.87 ± 17.71 $^{a}$ | 489.42 ± 32.09 $^{b}$ | ** | Green tomato | 400 [59] | Green |
| 4 | Furfuryl alcohol | 67.48 ± 5.76 $^{a}$ | 65.88 ± 5.17 $^{b}$ | ns | Burnt, coffee | 8000 [60] | Toasty |
| 5 | Benzyl alcohol | 13.00 ± 0.89 $^{a}$ | 27.67 ± 1.58 $^{a}$ | *** | Floral, rose, phenolic, balsamic, sweet, fruity | 200,000 [61] | Floral |
| | **Carbonyls** | 1047.38 ± 51.51 $^{a}$ | 2997.72 ± 146.74 $^{b}$ | *** | | | |
| 6 | Heptanal | 65.08 ± 2.67 $^{a}$ | 67.33 ± 2.75 $^{a}$ | ns | Herbal, ozone, rancid, nut | 3 [62] | Chemistry, Green |
| 7 | Octanal | 25.23 ± 2.66 $^{a}$ | 19.98 ± 1.00 $^{b}$ | ns | Citrus, green, fresh | 2.5 [63] | Chemistry, Citric fruit |
| 8 | Furfural | 696.07 ± 43.05 $^{a}$ | 1952.61 ± 102.03 $^{b}$ | *** | Burned almonds, fusel alcohol | 770 [64] | Chemistry, Toasty |
| 9 | Benzaldehyde | 31.33 ± 2.52 $^{a}$ | 64.82 ± 5.36 $^{b}$ | *** | Bitter almond, smoked, cherry | 350 [62] | Toasty |
| 10 | 5-methylfurfural | 229.67 ± 20.01 $^{a}$ | 892.98 ± 44.92 $^{b}$ | *** | Caramel | 1100 [64] | Toasty |
| | **Carboxylic acids** | 4225.96 ± 335.98 $^{a}$ | 9363.44 ± 147.38 $^{b}$ | *** | | | |
| 11 | Butanoic acid | 35.24 ± 2.29 $^{a}$ | 31.33 ± 1.53 $^{a}$ | ns | Rancid, cheese, sweat, sour | 173 [52] | Fatty |
| 12 | Octanoic acid | 3605.48 ± 295.05 $^{a}$ | 8009.06 ± 159.37 $^{b}$ | *** | Cheese, fat, grass, oil, sweat | 500 [52] | Fatty |
| 13 | Decanoic acid | 550.00 ± 40.00 $^{a}$ | 1291.71 ± 53.02 $^{b}$ | *** | Rancid fat, dust, grass | 1000 [52] | Fatty |
| 14 | Hexanoic acid | 35.24 ± 2.29 $^{a}$ | 31.33 ± 1.53 $^{a}$ | ns | Rancid, fatty, soapy | 420 [52] | Fatty |
| | **Esters** | 2430.93 ± 124.96 $^{a}$ | 4238.81 ± 112.21 $^{b}$ | *** | | | |
| 15 | Ethyl propionate | 271.99 ± 23.65 $^{a}$ | 466.70 ± 23.19 $^{b}$ | *** | Apple, pineapple, rum, strawberry | 45 [65] | Fruity |
| 16 | Ethyl isobutanoate | 15.12 ± 1.07 $^{a}$ | 5.67 ± 0.14 $^{b}$ | *** | Apple, strawberry | 15 [60] | Fruity |
| 17 | Ethyl butanoate | 307.74 ± 11.67 $^{a}$ | 446.69 ± 20.81 $^{b}$ | *** | Fruity, floral, apple, pineapple | 20 [66] | Fruity, Floral |
| 18 | Isoamyl acetate | 1177.86 ± 76.01 $^{a}$ | 2030.80 ± 62.62 $^{b}$ | *** | Banana | 30 [52] | Floral |
| 19 | Ethyl furoate | 5.43 ± 0.46 $^{a}$ | 0.71 ± 0.03 $^{b}$ | *** | Glue, paint | 1000 [61] | Floral |
| 20 | Ethyl octanoate | 346.94 ± 16.48 $^{a}$ | 912.57 ± 13.49 $^{b}$ | *** | Pineapple, floral, apricot, fat | 5 [52] | Fruity, Floral |
| 21 | Ethyl 2-methyloctanoate | 12.50 ± 0.90 $^{a}$ | 20.50 ± 1.07 $^{b}$ | *** | Fruity | 20 [63] | Fruity |
| 22 | Phenylethyl acetate | 83.42 ± 5.93 $^{a}$ | 110.99 ± 6.60 $^{b}$ | ** | Fruity, floral, tobacco | 250 [66] | Floral |
| 23 | Ethyl decanoate | 92.53 ± 9.25 $^{a}$ | 134.62 ± 5.01 $^{b}$ | ** | Sweet, fruity, pear | 200 [52] | Fruity |
| 24 | Ethyl vanillate | 8.24 ± 0.17 $^{a}$ | 9.19 ± 0.46 $^{b}$ | ns | Smoky, burnt | 990 [61] | Toasty, Spice |
| 25 | Ethyl dodecanoate | 64.53 ± 4.84 $^{a}$ | 48.43 ± 2.23 $^{b}$ | ** | Creamy, floral, fruit, leaf | 500 [60] | Buttery, Floral |
| 26 | Ethyl tetradecanoate | 24.09 ± 0.41 $^{a}$ | 28.36 ± 0.50 $^{b}$ | *** | Tropical fruit | 4000 [60] | Fruity |
| 27 | Ethyl hexadecanoate | 19.12 ± 0.77 $^{a}$ | 23.08 ± 0.87 $^{b}$ | ** | Caramel | 2000 [60] | Buttery |
| 28 | Hexyl hexanoate | 1.42 ± 0.11 $^{a}$ | 0.49 ± 0.03 $^{b}$ | *** | Fruity, green | 700 [60] | Citric fruit |

**Table 2.** *Cont.*

| | Families/Compounds | 1.5 Months | 3 Months | Sign. | Odor Descriptors | Odor Threshold | Aroma Series |
|---|---|---|---|---|---|---|---|
| | **Lactones** | 614.56 ± 39.23 [a] | 651.98 ± 19.28 [a] | ns | | | |
| 29 | γ-Crotonolactone | 146.17 ± 7.76 [a] | 317.08 ± 18.48 [b] | *** | Toasty, buttery | 1000 [64] | Buttery, Green |
| 30 | γ-Butyrolactone | 437.00 ± 38.59 [a] | 286.27 ± 11.51 [b] | ** | Sweet, caramel, roasted nut | 1000 [64] | Buttery |
| 31 | γ-Nonalactone | 27.13 ± 2.09 [a] | 44.47 ± 2.37 [b] | *** | Coconut, creamy, apricot, peach, sweet | 30 [56] | Fruity, Buttery |
| 32 | γ-Decalactone | 4.26 ± 0.21 [a] | 4.17 ± 0.04 [a] | ns | Peach, milky, sweet, fat | 47 [65] | Fruity, Buttery |
| | **Terpenes** | 6.87 ± 0.44 [a] | 10.62 ± 0.86 [b] | ** | | | |
| 33 | Limonene | 6.87 ± 0.44 [a] | 10.62 ± 0.86 [b] | ** | Flowery, green, citrus | 200 [67] | Citric fruit |
| | **Volatile phenols** | 30.76 ± 0.83 [a] | 62.16 ± 2.02 [b] | *** | | | |
| 34 | Guaiacol | 7.77 ± 0.39 [a] | 19.00 ± 1.00 [b] | *** | Medicine, smoke | 75 [68] | Chemistry, Toasty |
| 35 | 4-vinylguaiacol | 22.99 ± 1.11 [a] | 43.16 ± 1.61 [b] | *** | Clove, woody, smoke, phenol | 40 [69] | Spice, Woody |
| | **Oak compounds** | 127.48 ± 12.01 [a] | 279.53 ± 20.53 [b] | *** | | | |
| 36 | *trans*-whiskey lactone | 17.43 ± 1.36 [a] | 34.77 ± 2.84 [b] | *** | Woody, vanilla | 32 [70] | Woody |
| 37 | *cis*-whiskey lactone | 110.05 ± 12.07 [a] | 244.75 ± 18.00 [b] | *** | Woody, vanilla | 74 [70] | Woody |

The values are mean ± standard deviation of three independent experiments. Different letters indicate significant differences at $p < 0.05$ level according to the LSD test. The alphabetical order indicates an increasing content. Signs: *, **, *** and ns indicate significance at $p < 0.05$, $p < 0.01$, $p < 0.001$ and not significant, respectively.

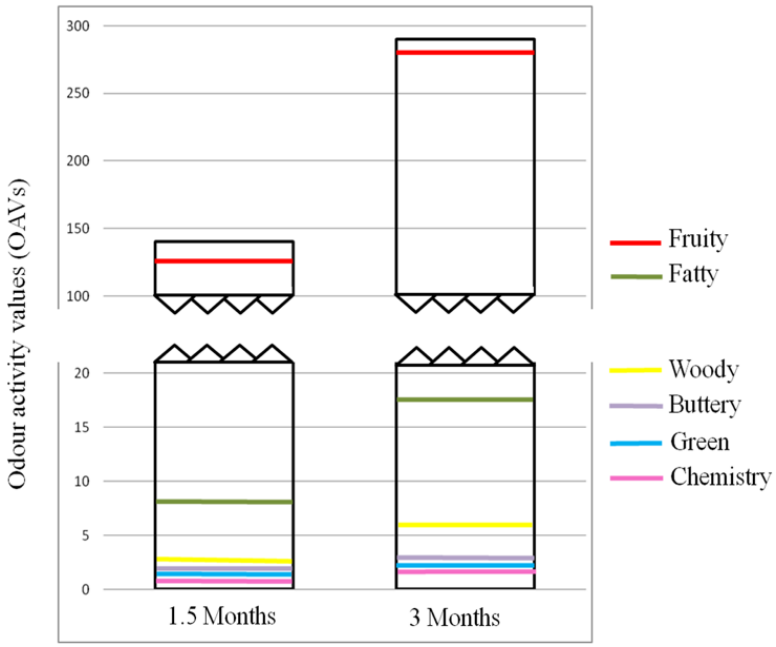


**Figure 3.** The fingerprints of red wines aged with oak staves for 1.5 months and 3 months using aromatic series.

Fruity was an aromatic series that showed very high overall values and mostly contains seven esters and two lactones, quantified by stir bars sorptive extraction and gas chromatography coupled with mass spectrometry (SBSE-GC-MS). Additionally, fatty and woody series showed increases during ageing (Figure 3), while other aromatic series, such as buttery, green, and chemistry showed a minor contribution to the wine aroma profiles.

## 4. Conclusions

Oak staves used in the Fetească neagră wine ageing processes led to an increase in volatile compounds amounts with time. Additionally, changes appear in the phenolic content, total antioxidant activity, their fractions and in the color. Wines aged for 3 months had higher antioxidant activity levels and higher color properties. The antioxidant activity of red wines aged with oak staves afforded the higher accumulation of bioactive compounds, thus increasing the overall red wine quality. Ageing carried with oak staves is characterized by an increased accumulation of the oak flavor, and wines aged for 3 months have more complex aroma and flavor, than wines aged for 1.5 months.

OAV results indicate that the use of oak staves during ageing can improve aroma series such as fruity, fatty and woody notes in wines. Therefore, oak staves can be used effectively in the ageing process to improve the aroma fingerprint of young red wines, which may result in a positive impact on the wine mouthfeel and appearance.

Overall, the use of oak staves in winemaking may be an important factor in the production of specific wine types with a distinctive touch and improved sensory characteristics. The alternative technology is a favorable choice for the wine industry, together the encouragement of faster, cheaper and sustainable ageing processes.

**Author Contributions:** Conceptualization, G.-D.D., R.A.P., C.T., N.L.d.L.; methodology, G.-D.D., R.A.P.; writing—original draft preparation, G.-D.D.; writing—review and editing, G.-D.D., C.T., R.A.P., N.L.d.L., V.V.C., I.G. All authors have read and agreed to the published version of the manuscript.

**Funding:** This study was supported by a grant of the Romanian Ministry of Education and Research, CNCS—UEFISCDI, project number PN-III-P1-1.1-PD-2019-0652, and also by the project PN-III-P1-1.1-PD-2016-0325, both projects being funded within PNCDI III.

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

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Data is contained within the article.

**Acknowledgments:** We are also grateful to the University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania and the University of Córdoba, Córdoba, Spain.

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

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