

# Physico-Chemical and Microbiological Profile of Wine Lees of Red Wines from Local Grapes Varieties

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## Abstract

In the Republic of Moldova, the viticulture industry is a sector with a high economic impact, and the utilization of secondary products from winemaking represents a growing concern regarding environmental sustainability. Wine lees, one of the types of wine waste, is less studied in order to valorize it. Currently it is used in the production of ethyl alcohol, as aggregates in the soil and others. The aim of this study was to characterize from a physico-chemical and microbiological point of view the lees sediments obtained after the primary fermentation of three types of individualized red wines made from autochthonous grapes varieties. It was found that residual yeasts represent a valuable raw material containing carbohydrates (from  $14.35\% \pm 0.19\%$  to  $25.11\% \pm 1.51\%$  SU), lipids (from  $4.61\% \pm 0.21\%$  to  $9.41\% \pm 2.04\%$  SU), proteins (from  $42.62\% \pm 1.57\%$  to  $77.62\% \pm 9.14\%$  SU), anthocyanins (from  $9.18 \pm 0.15$  to  $22.78 \pm 1.60$  mg cyanid) and beta-glucans (from  $12.84\% \pm 0.01\%$  to  $17.42\% \pm 0.02\%$ ). The pH value of wine lees ranges from  $3.49 \pm 0.0$  to  $3.083 \pm 0.01$ , the dry matter from  $9.62\% \pm 0.22\%$  to  $25.06\% \pm 0.42\%$  and the ash from  $0.03\% \pm 0.42\%$  to  $0.035\% \pm 0.21\%$ . The microbiological study confirmed the presence of live yeasts of the genus *Saccharomyces cerevisiae*, which remain active due to the presence of residual sugars and oxygen. The results of the research are promising and encourage the obtaining of new products with special purpose and added value.

## Keywords

Red Wines, Wine Lees, Yeasts, By-Products, Winemaking

## 1. Introduction

The food sector produces approximately 95 - 98 million tonnes of waste and by-products every year, becoming a severe environmental and social concern facing civilization today [1]. The wine industry is one of the economic sectors that generates a significant amount of waste. This waste is often not managed properly, which raises concerns about the environmental sustainability of wine production [2] [3]. Currently, winemaking byproducts such as grape pomace, grape seeds, and residual yeast can be used to produce alcohol, serve as fertilizer or animal feed, extraction of many bioactive compounds used in the creation of functional foods, as anthocyanins from red grapes as natural colorants, phenolic compounds as antioxidants etc. [4]. The disposal of winemaking waste into the environment creates environmental problems, contributes to the pollution of underground and surface water, to the attraction of disease vectors and the excessive consumption of oxygen in the soil, and in the groundwater etc. [5]. The biodegradation of this waste is quite slow, due to the low pH level and the presence of compounds with antibacterial properties, such as polyphenols. Agricultural residue management is considered a vital strategy to achieve resource conservation and maintain environmental quality [6]. Currently, grape pomace is one of the most valuable winemaking byproducts. It is considered to be the main byproduct of winemaking [7]. It represents a material with added value and is used in various industrial processes, including as an additive in food products, beneficially influencing the physico-chemical, functional and sensory parameters of new products [8]. Another waste from winemaking are grape seeds, which contain approximately 40% fiber, 10% - 20% lipids, 10% protein, and the rest are sugars, polyphenolic compounds and minerals [8] [9]. Residual yeast is another by-product that, according to European Regulation 491/2009 of 25 May 2009, represents the sediment that settles in winemaking tanks after fermentation, during storage, or after authorized treatment, or that is obtained after filtration or centrifugation of wine.

According to the report of the International Organization of Vine and Wine, 258 million hectoliters of wine were produced globally in 2022 [10]. Wine lees represents approximately 2% - 6% of the wine volume and is composed mainly of ethanol, tartaric acid, yeast cells, polysaccharide complexes, polyphenols, and inorganic matter [11] [12]. If we calculate the volume of wine lees, then globally in 2022 approximately 10.32 mhl of wine lees were produced. For countries where winemaking is a dominant branch of the economy, the recovery of wine lees (waste from winemaking) represents a major challenge [13]. Republic of Moldova is an agricultural country and the image vector is wine produced in the 4 IGP wine regions (**Figure 1**), recognized (registered) at the EU level, such as: the region IGP “Codru”, located in the center of the country, recognized for the production of white wines, the region IGP “Ștefan Vodă”, located in the south-east of the country, it is known for its red wines—varietal and blend, the region IGP “Valul lui Traian”, located in the south-west of the country, known for the

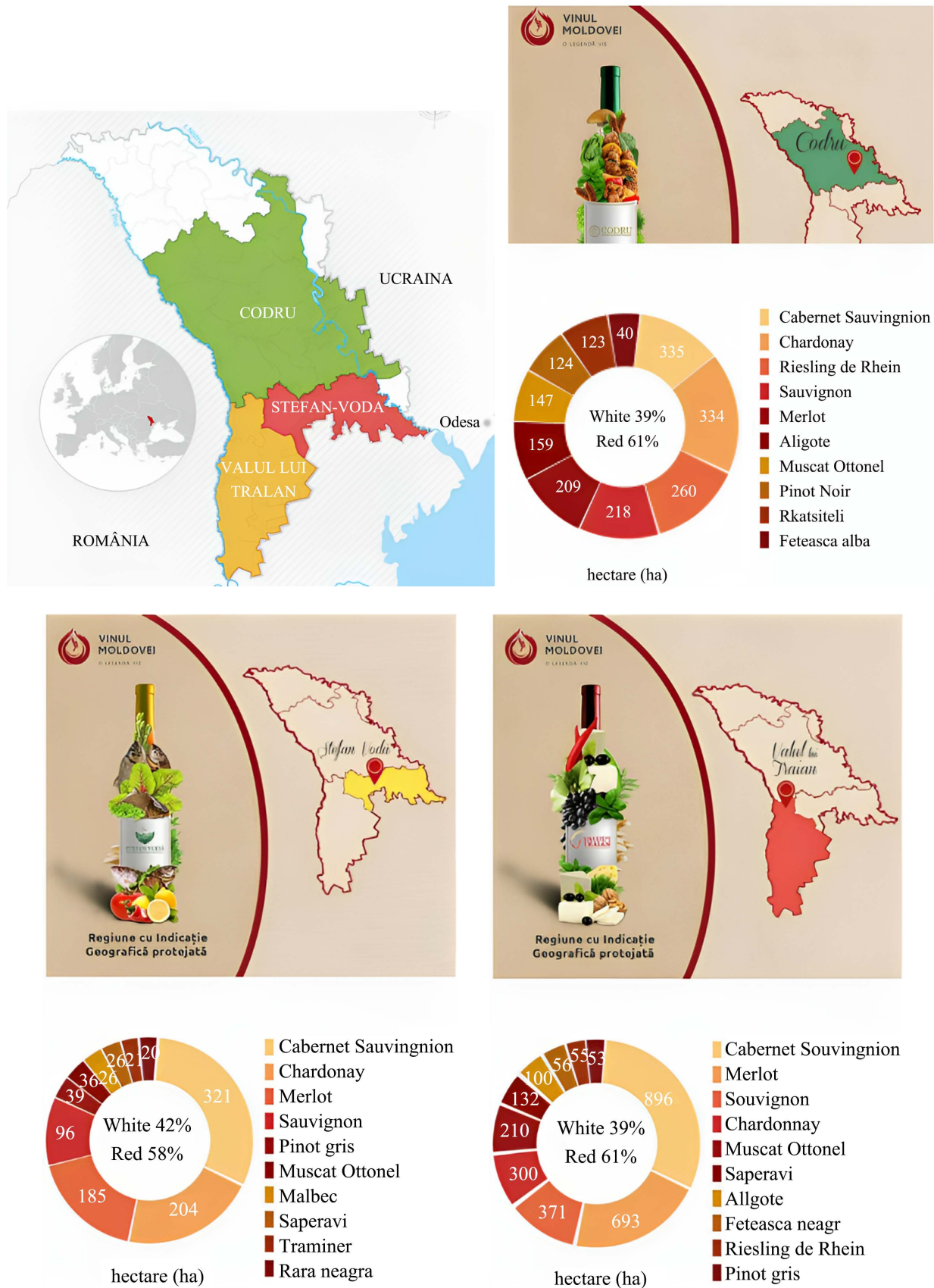


Figure 1. Wine production in the wine regions of the Republic of Moldova (year 2021) [14] [15].

production of high-quality liqueur wines and red wines and the region IGP “Di-vin”, known for the production of distilled wine brandies, matured in oak barrels.

It should be mentioned that in the Republic of Moldova, local varieties such as Răra Neagră, Fetească Neagră, Fetească Albă, Viorica are becoming more and more important to make a difference and give the wines original, authentic and local characteristics. Currently, these varieties represent 5% of the vineyards, but the areas are continuously increasing, thanks to the increased international interest and thanks to the support provided by government programs [13].

In the Republic of Moldova in 2020, the volume of wines produced was 9.1 million dal, respectively the volume of wine lees represented approximately 360 thousand dal. The largest volume of yeast sediments are obtained before and after alcoholic and malolactic fermentation [16] [17]. At the same time, the composition of wine lees depends on numerous parameters largely related to the types of yeast and grapes used and the winemaking method, resulting in a wide compositional heterogeneity [18] [19]. Winemaking yeasts synthesize bioactive compounds of different molecular classes, such as peptides [20] [21], polyphenols [22] and terpenoids [23]. At the same time, tartaric acid is extracted from wine lees, but the use of wine lees for this purpose was limited in the Republic of Moldova. Tartaric acid is currently used in considerable quantities in winemaking and the food industry, being an import product with a rather high price for the Republic of Moldova [23]. Thus, yeast biomass—the main component of wine lees, is used only occasionally but with quite promising perspectives [24]. *Saccharomyces cerevisiae* yeast extract is rich in amino acids, proteins, peptides, polysaccharides, vitamins B6 and B12, minerals (enzyme cofactors) such as zinc, copper and manganese, phytosterols and phenols, including catechins and transveratrol with antioxidant activity, as they are produced of *Saccharomyces cerevisiae* as the adaptive response to oxidative stress [25].

The potential of the wine lees from the winemaking resulting from the production of wines from local varieties of the vine seems not to be studied. In this context, the purpose of the study, the results of which are presented in this work, was to evaluate the physico-chemical and microbiological parameters of the wine lees collected after the manufacture of red wines from native grape varieties: Rara Neagra and Feteasca Neagra, but also from a homemade wine, manufactured under artisanal conditions.

## 2. Materials and Methods

### 2.1. Raw Material

Wine lees were collected from 3 types of red wines obtained from local varieties Rara Neagră and Feteasca Neagră—offered by Purcari winery and antizan wine (blend of 70% 1001 variety and 30% Izabela) from the central part of Moldova, Calarasi district.

## 2.2. Reagents

Reagent Folin-Ciocalteu, Anhydrous sodium sulfate, Chloroform, Gentian violet, Lugol's solution, Fuchsin, Sodium hydroxide 0.1 N, Ethanol 96%, Anthrone, Sulfuric acid 66%, D-glucose, Muller Hinton Agar culture medium.

## 2.3. Physico-Chemical Methods of Analysis

The pH value of the wine lees samples were directly determined using the inoLab pH 7110 pH meter. Total ash was determined in wine lees samples according to the method described by the International Organization of Vine and Wine OIV-MA-VI-07: R2000 [26], and the dry substance was determined by the simple method of removing water by heating to a temperature of 100°C - 110°C and determining the mass difference between the initial and final sample. Lipids were determined according to the Bligh and Dyer method using the mixture of ethanol, chloroform and acetic acid [27]. The content of amino acids was determined spectrophotometrically, at a wavelength of 535 nm and anthocyanins were extracted using a polar solvent (ethanol) [28]. Carbohydrates in wine lees were determined using the Antron method [29], using the Antron reagent that colors the solution in blue, and the total carbohydrate content was measured with spectrophotometer "SHIMADZU UV1800 SPECTROPHOTOMETER", measuring the color intensity at 620 nm wavelength and comparing the absorbance with a standard curve using known concentrations. The total protein content of the sample was determined using the Lowry method, which is a spectrophotometric assay that detects the presence of peptide bonds. The assay uses a combination of copper ions and the Folin-Ciocalteu reagent to produce a blue color, which can be quantified to determine the amount of protein present in the sample [30]. The enzymatic activity of catalase and superoxide dismutase enzymes were determined spectrophotometrically. In the case of catalase (CAT), the method is based on the ability of hydrogen peroxide to inhibit the catalase enzyme by changing its active structure [31]. For superoxide-dismutase (SOD), the method consists in the inhibition of the salt of tetrazolium nitroblue NBT, in the system containing phenazine metasulfate and NADH under the action of SOD. Following the reduction of NBT, nitroformazan is formed, which has a blue color, the intensity of which is proportional to the amount of reduced NBT. The degree of inhibition of this process depends on the activity of the enzyme [32] [33].

## 2.4. Extraction Method of $\beta$ -Glucans

For the extraction of  $\beta$ -glucans from wine lees, the enzymatic method assisted with ultrasound was used [34]. 100 g of the sample was adjusted to a pH of 10 - 11 with the help of 1 N NaOH. After, the sample was placed in an ultrasonic bath 30 kHz to 70°C for 30 min. Then the pH of the sample was lowered to 6 using sulfuric acid (H<sub>2</sub>SO<sub>4</sub>). The sample was then treated with the enzyme, 0.2 g of Mannanase enzymes was added to the wine lees. The sample was placed in a

water bath for 90 min. at 48°C, centrifuged at 10,000 rpm for 10 min and the precipitate was washed until pH 6.5 - 7 of the water or neutral pH. Finally dried for 3 days at 50°C.

## 2.5. Microbiological Analysis Methods

To microbiologically analyze the wine lees of wines, they were cultivated on a culture medium. Each type of wine lees was incubated on 2 media, so a total of 6 samples were analyzed. The samples were cultured on the Mueller Hinton Agar and Sabouraud medium, the samples were thermostated at a temperature of 30°C ± 1°C for 72 hours. After which the total number of microorganisms was determined according to ISO 4833-1:2013 [35], Gram staining [36] and morphological analysis of micro-organism colonies were performed.

## 2.6. Statistical Analysis

Statistical processing of the results was performed using the MO Excel and Statistics 9.0 software suite. The obtained results of the 3 repetitions were expressed by calculating the mean, standard deviation, and confidence interval for a mean. All differences were considered statistically significant for  $P \leq 0.05$ .

## 3. Results and Discussion

### 3.1. Physico-Chemical and Nutritional Parameters

In this study, the biomass of wine lees collected from 3 types of wines presented in **Table 1** was analyzed. Analyzing the physico-chemical and nutritional parameters of red wine lees, we can understand if they are of interest for further valorization and finding out from their characteristics the most important domains of possible use.

**Table 1.** Physico-chemical parameters of red wine lees.

No.	Parameters	Red wine less		
		Rara Neagra	Feteasca Neagra	Craft Wine
1	pH	3.15 ± 0.12	3.49 ± 0.02	3.08 ± 0.01
2	Ash, %	0.03 ± 0.42	0.04 ± 0.21	0.04 ± 0.12
3	Dry matter, %	25.06 ± 0.42	24.69 ± 0.09	9.62 ± 0.22
4	Carbohydrates, % SU	19.26 ± 0.95	14.35 ± 0.19	25.11 ± 1.51
5	Lipids, % SU	9.41 ± 2.04	4.61 ± 0.21	6.23 ± 1.56
6	Proteins, % SU	42.62 ± 1.57	77.62 ± 9.14	52.24 ± 3.23
7	Antociani, mg cyanidin/100g %	9.18 ± 0.15	22.78 ± 1.60	11.23 ± 1.80
8	SOD activity, U/mg	18.83 ± 2.37	19.11 ± 2.24	54.11 ± 1.88
9	CAT activity, mU/mg	2.95 ± 0.02	1.49 ± 0.09	8.90 ± 0.26
10	β-glucans, %	17.42 ± 0.02	16.88 ± 0.04	12.84 ± 0.01

The pH value falls within the values characteristic of red wines, 2.74 - 4.01 if compared with the average statistical data for red wines [37] [38]. At the same time, the acidity of the wine lees is due to the presence of tartaric salts (mostly K and Ca tartrate) precipitated together with the yeasts after alcoholic fermentation [12]. The ash content is approximately the same in the analyzed wines, but is much lower compared to other similar studies for red wines [12] [39]. While the dry substance is approximately the same for commercial wines, 25.06% and 24.69%, and for home wine it is only 9.62%. This fact is due to the different production method, applied in the case of commercial and home wines. The content of dry matter is close to that reflected in other studies [40].

In the case of the carbohydrate content, we can also observe a difference between the sediments of commercial and homemade wines. We assume that this fact is due to the different yeast strains used, in the case of commercial wines these are predominantly *Saccharomyces cerevisiae*, while in homemade wines the indigenous strains predominate. Soluble carbohydrates in the cell wall of wine yeasts, which come from grapes, are of great interest due to their organoleptic properties [41].

Depending on the type of wine, less on the winemaking technology, we also observe a variation in the lipid content. We observe the highest lipid content in the wine lees of Rara Neagra  $9.41\% \pm 2.04\%$ , followed by the homemade wine  $6.23\% \pm 1.56\%$ , and Feteasca Neagra is poorer in lipids and contains only  $4.61\% \pm 0.21\%$  lipids. These values are close to those described in the study carried out by Barreto de An-drade Bulos on Tempranillo and *Vitis coignetiae* red wine lees, 4.85% - 9.20% [37], but they are higher compared to another study conducted by Pau Sancho-Galán  $0.132\% \pm 0.047\%$  [12], the same on the Tempranillo red wine lees.

The proportion of proteins found in the wine lees biomass is  $42.62\% \pm 1.57\%$ ,  $77.62\% \pm 9.14\%$  and  $52.24\% \pm 3.23\%$ . The obtained values are higher compared to the data from the study published by De Iseppi [17] [42] carried out also on the red wine lees 14.5% - 15.7%. The protein content extracted from yeasts *Saccharomyces cerevisiae* is 33% - 54% [43]. We note that the values obtained for Rara Neagra and the homemade wine fall within these limits, while the sediment from Feteasca Neagra has a higher protein content and constitutes  $77.62\% \pm 9.14\%$ . This fact is also applicable if we compare the protein content with that of autolyzed dry yeast *Saccharomyces cerevisiae*, which is on average 50.10% [44]. It is known that the cell wall of yeasts is rich in mannoproteins, the content of which is approximately 40% [45].

The polyphenols extracted from the winemaking yeast contribute to the antioxidant effect of the wine lees [46]. Anthocyanins are components of wine lees with well-known antioxidant properties. According to the study carried out by Antonio Costa-Pérez, 15 types of anthocyanins are present in the wine lees [47] and namely they differ from those present in the rest of the secondary products of winemaking. In this study, the anthocyanin content was between 22.78 mg cyanidin/100g %  $\pm 1.60$  mg cyanidin/100g % and 9.18 mg cyanidin/100g %  $\pm$

0.15 mg cyanidin/100g %. At the same time, the vinification method can affect the total content of polyphenols and, consequently, their biological activities [48].

Enzyme activity is directly proportional to the winemaking method and applied technologies. SOD activity is the highest for homemade wine and is  $54.11 \pm 1.88$  U/mg, compared to industrially produced wine,  $18.83 \pm 2.37$  U/mg in the wine lees of Rara Neagra and  $19.11 \pm 2.24$  U/mg in the lees of Feteasca Neagra. The highest catalase values are recorded for homemade wine and is  $8.90 \pm 0.26$  mU/mg. Comparing the results obtained with those from the study conducted by Landeka Jurčević, we can see that the activity of the SOD enzyme on average is close to that obtained by Landeka Jurčević, which is 35%. Instead, the CAT activity we obtained is much lower compared to the mentioned study [49].

$\beta$ -glucan has become an extremely popular research topic both in the world of infectious diseases [50] [51], as well as in tumor immunology [52] [53]. At the same time, the content of  $\beta$ -glucans is diverse in different natural sources, such as cereals, mushrooms, etc. For example, the  $\beta$ -glucans content of oats grown in the USA is a maximum of 6.6%, oats grown in Sweden contain about 2.2% [54]. The content of  $\beta$ -glucans in oats grown in Turkey is from 3.9% to 5.7% [55]. It should be mentioned that the examination of yeasts as a potential source of  $\beta$ -glucans has gained momentum in recent years, proposing *Saccharomyces cerevisiae* as a potential source for obtaining  $\beta$ -glucans, based on advantageous procedures of oriented synthesis that would ensure a synthesis up to 25% and which contributes to improving the quality and cost of the final product [56]. In wine lees sediments from winemaking, we obtained a  $\beta$ -glucan content of  $12.84\% \pm 0.01\%$  in homemade wine,  $16.88\% \pm 0.04\%$  in Feteasca Neagră wine and  $17.42\% \pm 0.02\%$  in Rara Neagra wine.

### 3.2. Fractional Composition of Lipids

Lipids are an important group of molecules that contribute directly to the development of wine aroma [57]. The fractional composition of the lipids from the yeast sediment from the vinification of the three types of red wines under study is presented in the Table 2. We observe that in the analyzed red wines esters predominate in amounts of  $34.91\% \pm 0.01\%$  (for Feteasca Neagră),  $24.22\% \pm 0.03\%$  (for Rara Neagră) and  $23.22\% \pm 0.12\%$  (for Homemade wine). Triglycerides are an important part of the lipids in the sediments in quantities of  $22.60\% \pm 0.09\%$  (for Homemade wine),  $18.82\% \pm 0.04\%$  (in Feteasca Neagră) and  $15.36\% \pm 0.10\%$  (in Rara Neagră). High triglyceride content was also detected in

**Table 2.** Fractional composition of lipids in wine lees, %  $\Sigma$  lipids.

No.	Wine lees from red wines	Phospholipids	Sterols	Monoglycerides	Diglycerides	Triglycerides	Esters
1.	<b>Feteasca Neagră</b>	$11.37 \pm 0.17$	$9.80 \pm 0.03$	$12.94 \pm 0.10$	$12.16 \pm 0.14$	$18.82 \pm 0.04$	$34.91 \pm 0.01$
2.	<b>Rara Neagră</b>	$21.35 \pm 0.07$	$8.85 \pm 0.13$	$11.98 \pm 0.06$	$18.23 \pm 0.16$	$15.36 \pm 0.10$	$24.22 \pm 0.03$
3.	<b>Craft Wine</b>	$17.03 \pm 0.12$	$7.74 \pm 0.04$	$12.10 \pm 0.12$	$17.34 \pm 0.05$	$22.60 \pm 0.09$	$23.22 \pm 0.12$



the study conducted by José C. del R o [58] in the yeast biomass from beer which constituted about 67% of the total lipids. We observe the same trend in the study conducted by Piritta Niemi [59], where a high content of triglycerides (55%) is reported in the yeast sediment, but the percentage of phospholipids and diglycerides is lower (9.1% and 5.7% respectively). The obtained results show that the wine lees is very rich in esters that are formed during the alcoholic fermentation as well as during the storage or maturation of the wine [60]. Grapes, in general, are rich in sterols and fatty acids the yeasts in phospholipids. The Lipids are mainly found in seeds of grapes including fatty acids, tocopherols, tocotrienols, and phytosterols. Different grape varieties have a different concentration and profile of fatty acids and tocopherol in the seed oils, where the oil concentration varies between 7.3% and 22.4%. As a rule, linoleic acid is in the amount of 51.6% - 67.8% [61].


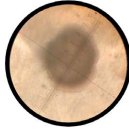

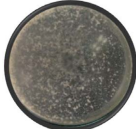
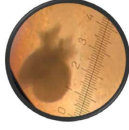

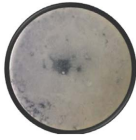
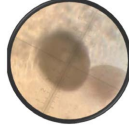

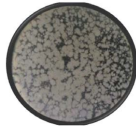
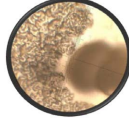
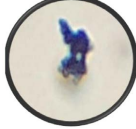

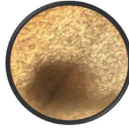


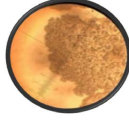
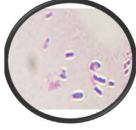
Phospholipids are the predominant structural components of the yeast cell membrane and are essential for yeast cell viability. They have the role of maintaining the stability of the cell membrane, regulation of permeability, transport of molecules through the cell membrane [62]. At the same time, sterols are a component of the yeast cell. They are responsible for maintaining the structure of the plasma membrane and essential for yeast development [63].

### 3.3. Microbiological Analysis

For a more complete characteristic of the analyzed wine lees, the microbiological analysis was also performed. Following the microbiological analysis, live microorganisms were detected and in almost all samples the presence of *Saccharomyces cerevisiae* yeasts, which remain viable due to the presence of residual sugars and oxygen. We can also observe that most of the microorganisms identified in the yeast lees from winemaking are Gram negative (Table 3). At the same time, there are some microbiological studies of wine lees that show the absence of living microorganisms [36].

Thus, the wine lees of red wines is a secondary product rich in proteins, lipids and phenolic compounds. Due to this fact, the wine lees can be used in a multitude of food and non-food products. It is of particular interest from a nutritional point of view, due to the presence of significant amounts of proteins and antioxidant compounds. At the same time, from a technological point of view, the presence of proteins and fats gives it emulsifying properties. Wine lees can also be used as a fat substitute, for example in mayonnaise [64] [65] in bakery [66] or meat products [67]. Some studies have already demonstrated the advantages of using wine sediment incorporated in certain products, like texture agent in yogurts and yogurt drinks, fermented and non-fermented sports drinks, white-brined cheeses, ice cream [68], functional ingredient in bread [69] [70]. At the same time, the yeast biomass resulting from winemaking is known as a source of mannoproteins and  $\beta$ -glucans, both cell wall polysaccharides proposed as emulsifiers and thickening agents [71] [72].

**Table 3.** Microbiological analysis of wine lees.

Source/ volume, ml	Surface appearance	CFU/mL	Colonies		Microorganisms		Coloring Gram
			Properties	Edge aspect 10 × 40	Properties	Edge aspect 10 × 100	
Wine lees Feteasca Neagra Feteasca Neagră 0.1 ml		50 × 10	The profile is flat White color Edges—wavy Shape—irregular Luster—less Size—6 mm Transparency—trans-lucid		Yeast <i>Saccharomyces cerevisiae</i>		+
		66 × 10 <sup>4</sup>	The profile is flat White color Edges—wavy Point shape Luster—less Size—3 - 4 mm Transparency—trans-lucid		Sporeless sticks		-
Wine lees Rara Neagra Rara Neagra 0.1 ml		90% coverage	The profile is flat White color Edges—wavy Form—circular axis Luster—less Size—2 - 3 mm Transparency—trans-lucid		Sporeless sticks		-
		47 × 10 <sup>4</sup>	The profile is flat White color Margins—lobed Shape—irregular Luster—less Size—3 - 4 mm Transparency—trans-lucid		Yeast <i>Saccharomyces cerevisiae</i>		+
Wine lees homemade red wine Homemade red wine 0.1 ml		100% coverage	The profile is flat White color Edges—wavy Shape—irregular Luster—less Size—4 mm Transparency—translucent		Sticks with spores		-
		90 × 10 <sup>4</sup>	The profile is flat White color Edges—wavy Shape—irregular Luster—less Size—4 mm Transparency—trans-lucid		Sporeless sticks		+

## 4. Conclusion

The results of this study show that the wine lees of red wines have a heterogeneous composition that is influenced by the way the wine is produced. From a nutritional point of view, these by-products of winemaking are rich in proteins and lipids, due to the majority presence of yeasts. At the same time, red wine lees have a high content of phenolic compounds (antocyanins) with antioxidant properties. The content of the lipid fractions shows us which type of lipids dominates in the wine lees. Thus, we can observe the presence of esters, mono-, di- and triglycerides, phospholipids and sterols. These lipids are characteristic of yeast cells. Thus, the yeast biomass from the wine lees is of interest as an aroma enhancer, due to the abundance of esters, and at the same time, as an emulsifier due to the presence of triglycerides. The given study highlighted interesting and promising properties of red wine lees, but additional studies are needed to understand and argue for the effective use of these secondary products of winemaking.

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## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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