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Physicochemical and Nutritional Characterization of Winemaking Lees: A New Food Ingredient

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Abstract: Wine lees are defined as the sediment formed at the bottom of the tank or barrel after wine alcoholic fermentation. They have a heterogeneous composition and currently constitute 6% of the byproducts generated by each ton of wine grapes. However, it is the most under-researched of all the byproducts of the winemaking process. Therefore, with the aim of highlighting this byproduct, a physicochemical and nutritional characterization of winemaking lees from three different wine making processes (white, rosé, and red winemaking) was carried out. In addition, the technological properties of these winemaking lees were also analyzed. The lees analyzed in this research demonstrated an interesting nutritional and heterogeneous composition. Moreover, wine lees showed high values of emulsifying capacity. Thus, winemaking lees could be considered, in a preliminary way, as a new ingredient to be included in new food formulations.

Keywords: wine lees; alcoholic fermentation; winery; byproduct

1. Introduction

Lees obtained from the fermentation of grape must, or Winemaking Lees (WL), are defined according to the European regulation EEC No. 337/979 as "the sediment formed at the base of the deposit or barrel containing wine after fermentation, during storage or after performing authorized treatments to the product, as well as residues obtained from the filtration or centrifugation of said product". Their composition is variable and consists of microorganisms (mainly yeasts), tartaric acid, colloids, polyphenols, and inorganic matter [1]. Thus, after the winemaking process, the lees can be characterized as organic waste or byproducts, with a low pH value, low electrical conductivity values, and a high content of phosphorus, potassium, and organic matter, as well as a low content of micronutrients and heavy metals [2]. Once alcoholic fermentation is complete, the lees still play an important role in the ageing of wines, as the yeasts' autolysis processes cause the rupture of cellular membranes and the consequent release of intracellular components and hydrolytic enzymes, the latter causing the hydrolysis of intracellular biopolymers [3]. In addition, the release of compounds such as polysaccharides, proteins, amino acids, and lipids, nutritionally enrich the wine [4]. This great variability of compounds present in WL has allowed them to be used as fermentative activators after an enzymatic or thermal treatment [5], or as a source to obtain purified mannoprotein extracts, whose use improves wine stabilization and sensory properties [6]. For this reason, wine aging in contact with lees can modify the sensorial profile of final wines [7].

WL represent 6% of each ton of grapes destined for vinification [8]. Worldwide, 49.4 million tons of wine grapes are produced annually [9], generating 2.96 million tons of lees during winemaking. Currently, most wineries use lees to obtain alcohol through distillation [10] and tartaric acid by

crystallization [11]. However, other wineries accumulate lees along with other winemaking products for composting, in order to obtain microbiologically stable organic amendments for the vineyard [12].

WL have been analyzed for different purposes given their heterogeneous composition. The presence of relevant compounds in winemaking lees has appealed to the pharmaceutical and cosmetic industries. In this way, several authors report the possibility of extracting polyphenols by microwave [13], ultrasonic [14], its combination [15], or supercritical fluid techniques [16]. Moreover, the high nutritional load of winemaking lees allows for creating a culture medium based on lees for the development of lactic acid bacteria [17]. In addition to the presence of these kinds of compounds, its inorganic matter content has turned WL into a test substrate for biorefining. The chemical industry has managed to implement a pilot scale system that allows the production of ethanol, tartrates, and polyphenols, as well as byproducts that are suitable as nutritional supplements for microorganisms [18]. Despite all of the above, unlike the different uses given to other byproducts from the agrifood industry, the use of winemaking lees in the field of human feeding is not currently developed. Only Cechini et al. [19] and Hwang et al. [20] have reported that winemaking lees have the potential for its incorporation into food processing.

Therefore, in this study, a physicochemical, nutritional, and technological characterization of WL from different vinification processes was performed with the aim of studying, in a preliminary way, the possibility of applying this byproduct as a new ingredient in the food industry.

2. Materials and Methods

2.1. Materials

Three types of WL from three different winemaking processes were used for this study: white, rosé, and red WL. Sauvignon Blanc was employed for white winemaking, while Tempranillo was used for rosé and red winemaking. In all cases, the same strain of *Saccharomyces cerevisiae* was used (Lallemand, Barcelona, Spain). Diamin phosphate (Agrovin, Ciudad Real, Spain) was used as a fermentative activator at a 10 g/hL dose. For white and rosé winemaking, Alcoholic Fermentation (AF) was performed at 16–17 °C, while for red winemaking, it was performed at 22–23 °C. Skin contact time was between 6 and 8 h for rosé, while grape skins were in contact with red wine throughout the AF process. All the winemaking took place in the same winery. One kilogram of WL from each wine was collected just after alcoholic fermentation and was divided into two batches. The first batch, for physicochemical analysis, was stored in dark conditions and at −14 °C until analysis. At the time of analysis, WL were thawed and centrifuged in order to remove as much wine as possible. In the second batch, for nutritional and technological analysis, ultra-freezing was used and the batch was freeze-dried on a Virtis Benchtop K^{TM} model (SP Industries, Warminster, PA, USA) for a 72 h period. Once freeze-dried, each sample was homogenized in a Vowerk's Thermomix TM31 (Wuppertal, Germany) for 1 min and stored in zip bags in an automated desiccator (SP Industries, Warminster, PA, USA). In this regard, all analyses performed from the second batch of lees onwards were carried out with freeze-dried lees, and therefore the results are expressed per gram of dry WL.

2.2. Methods

For pH determination, a direct measure was carried out in a pH-meter Basic20 (Crison, Loveland, OH, USA). In order to determine Total Anthocyanins (TA) content, the methodology proposed by the Australian Wine Research Institute (AWRI) was followed [21], expressing the results in mg of malvidin-3-glucoside (M3G) per gram of WL. For the determination of antioxidant capacity, the protocol proposed by Hwang et al. [20] with slight modifications was followed. A mixture of fresh lees with bidistilled water in a 1:3 ratio was extracted with methanol for 12 h. After extraction, it was centrifuged and 0.3 mL of supernatant was placed in 1 cm plastic cuvettes alongside with 1.2 mL of 50% methanol and 0.5 mM DPPH (in methanol). The solution was kept at room temperature for 90 min and absorbance

at 517 nm was measured [22]. Antioxidant activity was quantified from a TROLOX calibration line (R^2 = 0.9963), obtaining the results in mg TROLOX/g WL.

For ash and mineral content determination, one gram of sample was incinerated in porcelain crucibles in a Carbolite ELF 11/148 muffle oven (Sigma-Aldrich, Saint Louis, MO, USA). The ash content was determined by weight difference. To determine the mineral content (Ca, Mg, K, Na, Fe, Cu, P, Mn, Zn, Cr, Co, Ni, Cd, and Pb), the protocol proposed by AFNOR [23] was followed. Fat content was determined using the Soxhlet method [24] using an automated Soxhlet extractor Soxtec^{TM} ST255 (Foss Industries, Barcelona, Spain) and n-hexane as solvent. Total fat was calculated as the difference between dry sample weight (0.5 g) before and after extraction. Hexane–fat mix was evaporated using a rotary evaporator HEI-VAP G1 (Heidolph, Germany) at 60 °C until hexane evaporation, and the fat was collected and stored in the dark and at 4 °C until methylation for fatty acid determination was performed. Fatty Acids (FA) were determined by gas chromatography after derivatization to methyl esters (FAMEs), following the methodology proposed by Rodríguez-Alcantara et al. [25]. WL total nitrogen was determined following the Kheldahl method [24].

In order to determine the suitability of WL as a new food ingredient, Emilsufying Activity (EA) and Foaming Capacity (FC) were assessed. EA was determined according to the adapted Yasumatsu et al. [26], and FC according to Patel et al. [27].

2.3. Statistical Analysis

All the analyses were performed in triplicate, in order to ensure statistical significance. For all the results, mean values and Standard Deviations (SD) were calculated.

3. Results

Table 1 shows the results of the physicochemical and nutritional characterization of the three WL studied. The pH value ranged between 3.38 for red WL and 3.45 for white and rosé WL, without showing great differences in any case. The acidity in WL is due to the presence of tartaric salts (mainly K and Ca tartrate) precipitated after the alcoholic fermentation along with yeasts [1]. Therefore, acidity in WL depends on wine alcoholic degree and its K and Ca levels, among others. This acid component, very noticeable from a sensory point of view, should be highlighted and can be decisive in regard to the potential use of WL as a food ingredient.

Table 1. Winemaking Lees (WL) physicochemical and nutritional characterization.

	White WL	Rosé WL	Red WL
pH	3.450 ± 0.010	3.450 ± 0.040	3.380 ± 0.050
TA (mg M3G/L)	n.d	1.147 ± 0.004	2.149 ± 0.059
Antioxidant capacity (g Trolox/ L WL)	0.190 ± 0.065	0.646 ± 0.041	2.919 ± 0.031
Total Nitrogen * (%)	4.106 ± 0.037	3.135 ± 0.125	0.855 ± 0.025
Total fat * (%)	0.783 ± 0.063	1.802 ± 0.009	0.132 ± 0.047
Ashes * (%)	32.753 ± 0.218	10.733 ± 0.265	33.283 ± 0.171

* Corresponds to dry-weight. Results show the means ± SD of three repetitions. N.d; not detected.

Anthocyanin content differed between the three WL analyzed. White WL did not show presence of anthocyanin, while, as expected, red WL exhibited the greatest value (2.149 mg/g WL). The differences observed between rosé and red WL are mainly the result of the winemaking process. Maceration time of grape skins and must in rosé winemaking was substantially shorter than in red, thus the release of polyphenolic compounds such as anthocyanins was 53.37% lower (Table 1). However, the anthocyanin content in WL has been shown to be higher when applying assisted extraction techniques [14–16]. Winemaking lees consumption as a food ingredient could therefore contribute to the dietary intake of bioactive compounds that are beneficial to health [28]. Related to TA, an exponential correlation (R^2 = 0.9902) was observed with the antioxidant capacity of WL. This capacity ranged between 0.190 to 2.919 g TROLOX/L WL for white and red WL, respectively, showing differences in all samples.

However, white WL has shown antioxidant capacity despite the absence of anthocyanin. This fact could be due to the presence of β-glucans in WL, which have antioxidant capacity [19].

In regard to the proximal analyses carried out to evaluate the nutritional potential of WL, the nitrogen content ranges from 4.106% for white WL to 0.855% for red WL (Table 1). These results show how WL nitrogen content is affected by the winemaking style, considering that the presence of alcohol, temperature, and the presence of exocellular proteases can affect the protein content, and therefore, the nitrogen content of the lees [29]. Furthermore, these differences could be due to the use of different strains of *Saccharomyces cerevisiae* yeasts during alcoholic fermentation. This nitrogen content is substantially lower than in foods considered rich in nitrogen such as eggs (12%), lamb (15.6%), and walnuts (14.4%) [30]. However, the presence of nitrogen compounds may be of interest and benefit for the use of WL as a food ingredient. As regards the fat content of the lees, values ranging from 0.132% (red WL) to 1.802% (rosé WL) were observed with differences in all cases. The high variability observed between the different samples may be due, as in the nitrogen content case, to the fact that the fat content in the samples could be attributed to the yeast strain used. Although in all cases *Saccharomyces cerevisiae* yeasts strains were used, lipid content can increase in the absence of growth factors for yeast [31]. A deficiency of a particular nutrient and carbonate substrates excesses are the main requirements for the accumulation of yeasts lipids [32]. Cell proliferation stops when the limiting nutrient is lacking, while the excess carbonate compounds continue to be assimilated by yeast cells and targeted into lipid synthesis [32]. In this sense, the presence of phospholipids in this wine byproduct could be beneficial to use as emulsifiers, as has already been demonstrated with those from fruits such as avocado [33]. Finally, differences in ash content were observed for all samples analyzed. Values ranged from 10.733 to 33.283% for rosé and red WL, respectively. Wine lees have a higher ash concentration than spent grain, unlike other agrifood residues from the production of fermented beverages, such as beer [34]. Related to WL ash content, the mineral fraction of WL is shown in Table 2.

Table 2. Mineral content in Winemaking Lees (WL).

(mg/L)	White WL	Rosé WL	Red WL
Ca	105.500 ± 0.707	74.350 ± 2.758	18.850 ± 3.748
K	756.500 ± 0.707	1392.500 ± 26.163	2405.050 ± 319.612
Mg	7.740 ± 0.255	10.000 ± 0.141	6.490 ± 0.983
Na	3.700 ± 0.240	4.415 ± 3.260	3.010 ± 1.047
Fe	0.746 ± 0.105	2.605 ± 0.064	1.195 ± 0.177
Cu	1.480 ± 0.014	0.473 ± 0.016	4.115 ± 0.700
P	42.000 ± 2.830	62.300 ± 1.980	6.250 ± 0.100
(µg/L)			
Mn	121.500 ± 28.991	133.000 ± 0.828	296.000 ± 42.430
Zn	151.000 ± 12.782	102.900 ± 11.455	815.500 ± 13.345
Cr	8.605 ± 2.397	1.400 ± 13.081	51.150 ± 5.303
Co	0.581 ± 0.127	1.080 ± 0.170	4.350 ± 0.325
Ni	7.670 ± 0.594	18.280 ± 4.667	23.650 ± 2.192
Cd	0.168 ± 0.115	0.175 ± 0.012	0.279 ± 0.016
Pb	4.660 ± 3.705	3.565 ± 0.021	11.950 ± 0.212

Results show the means ± SD of three repetitions.

There are great differences in mineral content in most cases for the different WL analyzed. In all cases, the cation content observed in lees was lower than what is usually observed for bottled wines [35]. However, the concentration of the different minerals in the lees is determined by the cultural practices applied at the vineyard and in the winemaking process, such as tartaric precipitation in wine [1]. Ca and K have been found to be the cations with the highest concentration on WL compared to the other cations. However, the concentration of each cation in the wine could vary depending on different parameters and conditions, such as the grape variety used in winemaking, climate conditions, and vegetative development of the vine, together with technological parameters such as time, temperature, and pH

of the alcoholic fermentation process [36,37]. As for the Mg content, no large amounts have been observed on the lees. Its origin may be mainly the result of the presence of this element in the vineyard soils, the absorption of which is carried out by the plant during its development [38]. An explanation for the Na, Cu, and P content in lees might be their presence in various plant protection products applied to the vine [35]. Finally, the iron content observed in WL comes mostly from the grape must, since it is an element absorbed by the vine during its vegetative development and the development of its fruit. Moreover, this low content could be due to the fact that this element is not present in the facilities and equipment where the wine was made or the different lees were preserved. As for trace elements, observed in µg/L concentrations, their presence is mainly due to cultural practices and/or the use of pesticides in the field [38]. Thus, the differences observed between the different WL samples studied could be the result of differences between grape varieties planted in different plots. In addition, climatic and ecological factors have also influenced the variability in the concentration of these elements in wines [39], and therefore, in their winemaking lees.

Regarding fatty acids composition (Figure 1, Table 3), the different lees samples studied could be distributed in two different groups. On the one hand, red WL, where a higher content of saturated FA is observed (>80%), and on the other hand, the group formed by rosé and white WL, where saturated FA represents less than 40% of the total fatty acids. In relation to red WL, undecanoic and hexadecanoic (palmitic) acids must be highlighted, representing almost the totality of saturated fatty acids, while octadecatrienoic (omega-3) and eicosadienoic (omega-6) represent more than 90% of the total fatty acids with one or more unsaturations. The mono/polyunsaturated fatty acid content, considered beneficial to the health, exceeded 60% in the rosé and white WL samples. Among this group of fatty acids, octadecatrienoic acid is again the main one, although high concentrations of eicosadienoic acid (omega-6) were also observed (Table 3). Moreover, significant concentrations of other fatty acids that are considered important in the diet, such as eicosapentanoic acid (EPA), were also observed in WL.

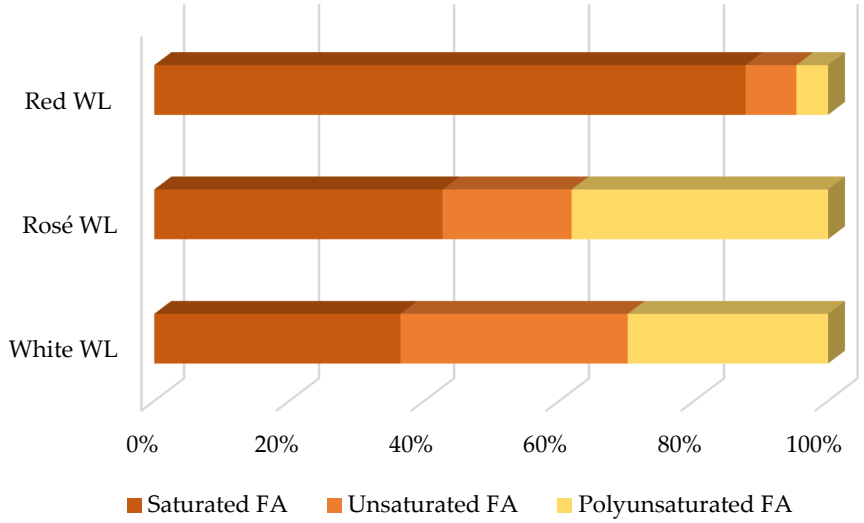

Figure 1. Distribution of fatty acid categories for three Winemaking Lees (WL), expressed as percent total fatty acids.

Table 3. Fatty acid composition of Winemaking Lees.

Fatty Acid (mg/L)			White WL	Rosé WL	Red WL
IUPAC Name	***Common Name***	***Saturation***			
Butanoic acid	Butiric acid	S	59.663 ± 3.594	23.352 ± 1.598	20.280 ± 0.331
Decanoic acid	Capric acid	S	56.183 ± 0.32	25.391 ± 1.918	24.010 ± 0.894
Undecanoic acid	Undecylic acid	S	1683.143 ± 11.670	627.158 ± 10.033	134,081.000 ± 1.193
Dodecanoic acid	Lauric acid	S	1919.208 ± 60.615	477.789 ± 27.899	455.876 ± 15.804
Tetradecanoic acid	Miristic acid	S	36.207 ± 3.265	4.668 ± 0.215	0.000 ± 0.000
Tetradecenoic acid	Miristoleic acid	U	44.419 ± 0.314	29.132 ± 1.475	10.530 ± 1.194
Pentadecanoic acid	Pentadecilic acid	S	6418.080 ± 83.427	1172.515 ± 34.407	463.881 ± 9.713
Hexadecenoic acid	Palmitoleic acid	U	1850.926 ± 67.714	440.892 ± 16.064	57.923 ± 0.442
Hexadecanoic acid	Palmitic acid	S	3082.708 ± 31.075	8380.932 ± 115.950	7433.159 ± 499.714
Heptadecanoic acid	Margaric acid	IP			
Octadecanoic acid	Estearic acid	S	1851.276 ± 67.219	440.892 ± 16.064	57.923 ± 0.442
Octadecadienoic acid	Linoleic acid	P	24.970 ± 0.641	15.715 ± 0.453	6.212 ± 0.043
Octadecatrienoic acid	Linolenic acid	P	12,037.146 ± 95.509	9176.035 ± 263.204	7452.159 ± 49.714
Eicosanoic acid	Arachidic acid	S	2.200 ± 0.223	5.757 ± 0.139	2.990 ± 0.200
Eicosadienoic acid		U	12,050.210 ± 96.056	4522.236 ± 33.005	12,322.756 ± 297.580
Eicosatrienoic acid		P	133.162 ± 5.327	598.605 ± 16.205	184.081 ± 71.904
Eicosapentanoic acid	EPA	P	174.640 ± 11.186	187.845 ± 7.470	138.690 ± 28.922
Docosanoic acid	Behenic acid	S	186.020 ± 4.646	189.846 ± 2.182	205.515 ± 7.096
Docosadienoic acid		U	6.730 ± 0.413	7.232 ± 0.357	5.391 ± 1.124
Docosapentaenoic acid		P	8.096 ± 0.248	3.960 ± 0.184	5.874 ± 3.927
Docosenoic acid	Erucic acid	S	2.000 ± 0.223	5.757 ± 0.139	2.023 ± 0.055

Results show the means ± SD of three repetitions. S: Saturated, U: Unsaturated, P: Polyunsaturated, IS: Internal Standard.

The presence of significant concentrations of fatty acids in WL makes this byproduct a possible new ingredient to incorporate in food processing. Its application has been previously tested with success in processes such as ice-cream elaboration [20]. One of the benefits of the inclusion of fatty acids from WL is that they facilitate the absorption of fat-soluble components in the diet, such as vitamins, and therefore enhance the taste and acceptability of foods [40]. The Food and Agriculture Organization (FAO) and the World Health Organization (WHO) recommend keeping the intake of saturated fatty acids as low as possible, given the adverse effects they may have on the health of the consumer [41]. However, it has also been observed that the intake of lauric, myristic, or palmitic acids may be beneficial to plasma cholesterol levels [40]. With regard to mono/polyunsaturated fatty acids, the FAO and WHO have not stipulated a dietary reference intake. However, consumption of unsaturated fatty acids has been shown to have potential benefits in term of blood lipid profile and cardiovascular risk factors [42]. Finally, the presence of polyunsaturated fatty acids has been observed in the form of linoleic acid, which cannot be synthetized and must be supplied through diet [43], as well as docosahexaenoic acid (DHA), which has physiological benefits on blood pressure [44].

Lastly, it should be noted that the three WL analyzed showed a good emulsifying activity. The results obtained were 86.36%, 79.67%, and 75.00% for white, rosé, and red WL, respectively. However, none of them showed foaming capacity. In this sense, the use of WL in the development of novel foods or their inclusion in existing foods is expected to be possible, because of their high emulsifying activity (e.g., Oil-in-Water (O/W) emulsions).

4. Conclusions

The results of this preliminary research led to the conclusion that wine lees have an interesting nutritional and heterogeneous composition. Furthermore, we observed how the type of winemaking affects their physicochemical characteristics, and consequently their properties as a possible new food ingredient. Regarding their mineral composition, it was observed that the three WL studied have high contents of K, Ca, and P, which can be very beneficial from a nutritional point of view. Concerning their technological characterization, winemaking lees have a high emulsifying activity, thus lees could be considered of interest in a preliminary way as a new ingredient that could be included in new food formulations. Further research should be carried out in order to study how the inclusion of lees in food formulation affects the nutritional, technological, and sensory aspects of food.

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