Characterization of white and rosé sparkling wine lees surface volatiles

Alba Martín-Garcia[1,2,3], Montserrat Riu-Aumatell[1,2,3], and Elvira López-Tamames[1,2,3]

[1]Departament de Nutrició, Ciències de l'Alimentació i Gastronomia, Facultat de Farmàcia i Ciències de l'Alimentació, Campus de l'Alimentació de Torribera, Universitat de Barcelona, Av. Prat de la Riba 171, 08921, Santa Coloma de Gramenet, Spain
[2]Institut de Recerca en Nutrició i Seguretat Alimentària (INSA-UB), Av. Prat de la Riba 171, 08921 Santa Coloma de Gramenet, Spain
[3]Xarxa d'Innovació Alimentària (XIA), C/ Baldiri Reixac 4, 08028, Barcelona, Spain

Abstract. Cava is a sparkling wine that requires a second fermentation in the bottle. Its volatile fraction is conditioned by different parameters (grape, vinification process, fermentative yeast, and aging time). During the autolysis process, yeasts release compounds into the wine, but lees can adsorb certain compounds on their surface. Therefore, the aim of this work was to characterize different white and rosé Cavas, and their lees. For this, white Cavas (CGR1: 40 months; CR1: 16 months) and rosé Cavas (CRR1: multivarietal coupage; CRR2: monovarietal; both 20 months) were studied. Once disgorged, lees were freeze-dried (L-CGR1, L-CR1, L-CRR1 and L-CRR2). In addition, lees waste from the winery were collected. pH, total polyphenol index (TPI) and colour intensity (CI) of Cavas and lees were determined. The volatile fraction was analysed by Head-Space Solid Phase Microextraction followed by gas chromatography coupled to mass spectrometry. Lees showed higher values than their respective Cavas for TPI and CI, especially in the case of the L-CGR1. Most of the volatiles were identified both in Cavas and their lees, esters being the main compounds. Therefore, lees can retain phenolic and volatile compounds on their surface, which could be of interest as a new ingredient in the food industry.

1 Introduction

Cava is a Quality wine produced in specified regions (QWPSR) that requires a second fermentation in the bottle, with a minimum time of 9 months of biological ageing *sur lie* [1]. During the ageing period, fermenting yeasts undergo the autolysis process, in which they can release different compounds (lipids, carbohydrates, amino acids, peptides, and volatile compounds) to the wine [2]. Once the ageing process ends, lees are removed from the bottle (disgorgement) and become waste [3]. In fact, the Cava industry generates a great amount of organic waste, such as skins, stems and seeds from the grapes, as well as lees from the alcoholic fermentation. Indeed, lees represent a 25% of the total waste of these cellars, which is about 300 tons per year [4].

Lees consist of naturally plasmolyzed yeast cells, tartaric acid and other adsorbed compounds [5]. Actually, the cell wall of yeasts is constantly in contact with Cava during ageing [3]. It mainly consists of mannoproteins (exterior) and branched glucans (interior). It is this structure that gives the lees the properties to interact with the compounds of Cava [3, 6]. In fact, different studies have focused on the lees ability to adsorb compounds such as polyphenols and other volatile compounds that contribute to wine aroma [3, 7, 8].

Recently, since lees are rich in fiber and antioxidant compounds [5, 9], they have been used as an ingredient in several food matrices in order to re-valorize such by-product [10-12]. Since the addition of lees to food formulation may change its organoleptic properties, Cava lees should be characterized regarding volatile compounds and other physicochemical parameters such as pH and color. Therefore, the aim of this study was to characterize Cava lees regarding different parameters related to sparkling wine quality as well as to evaluate their ability to adsorb volatile compounds from Cava.

2 Materials and methods

2.1 Cava lees recovery

Four types of Cava were selected from the winery Freixenet, S.A. (Sant Sadurní d'Anoia, Spain) produced with different grapes (Macabeu, Xarel·lo and Parellada for white sparkling wines; Garnatxa and Trepat for rosé sparkling wines) as well as different ageing time (Table 1). Moreover, samples of the cellar lees waste were also obtained (L-CV1). All bottles were disgorged at the same time and wet lees were extracted. Cava samples were stored at -20°C until the analysis. Then, wet lees were frozen (-80°C, 15 min) and freeze-dried (Cryodos-50, Telstar, Terrasa, Spain). Lyophilized lees were stored in sealed tubes protected from light and humidity.

Table 1. Studied Cava and their respective lees.

Sample ID	Lees sample ID	Grape[1]	Biological ageing (months)	Category[2]
CGR1	L-CGR1	M-X-P	40	Gran Reserva
CR1	L-CR1	M-X-P	16	Reserva
CRR1	L-CRR1	GA-TR	20	Reserva
CRR2	L-CRR2	TR	20	Reserva

[1]M: Macabeu; X: Xarel·lo; P: Parellada; GA: Garnatxa; TR: Trepat.
[2]Categories according to PDO Cava [1].

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2.2 Determination of physicochemical parameters

The plasmolyzed cells were determined by cell counting with a Neubauer chamber (Ref.: 640110, Paul Marienfeld GmbH & Co., Germany). pH was determined in both Cava and lees using a pH meter XS PH60 Violab (XS Instruments, Carpi, MO, Italy).

The optical density (OD) for the analysis of the total phenolic index (TPI), color intensity (CI) and color hue (CH) was determined using a UV-3600 UV-Vis-NIR Spectrophotometer (Shimadzu Scientific Instruments, Inc., MD, USA). Samples were placed in quartz cuvettes with a length of 10 mm. OD was measured at a wavelength range between 280 nm and 620 nm. Ultrapure water was used as blank. For TPI it was necessary to dilute the samples with ultrapure water to obtain OD values between 0.1 and 0.9 according to OIV official analysis regulations [13].

2.3 Analysis of volatile compounds in Cava and lees

The extraction of volatile compounds in Cava and lees was performed by head-space solid phase microextraction (HS-SPME). It was carried out using a 2 cm long Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/ CAR/PDMS) fiber supplied by Supelco (Bellefonte, PA, USA). Samples of 5 mL (Cava) or 25 mg (lees) were placed in 10 mL vials. After 15 min of equilibration at 50°C under continuous agitation (250 rpm), the fiber was exposed to the headspace for 40 min.

Chromatographic analysis was carried out in a 6890N Network GC system (Agilent, Palo Alto, CA, USA) coupled to MS Agilent technologies 5973 Network selective detector (Agilent, Palo Alto, CA, USA). Helium was used as a carrier gas. Separations were accomplished in a DB Wax USN 125-7031 column (30 m × 0.25 mm × 0.25 µm) (Agilent, Palo Alto, CA, USA). A splitless injector suitable for SPME was used. After extraction, the fibre was removed from the headspace vial and inserted directly into the injection port of the GC. The SPME fibre was thermally desorbed for 2.5 min at 260°C.

The initial temperature of the column was 40°C for 10 min, and this was subsequently increased at 4°C/min up to 75°C, then temperature was increased at 2°C/min up to 260°C and hold for 5 min using splitless injection mode. GC-MS detection was performed in complete scanning mode (SCAN) in the 40-350 amu mass range with two scans per second. Electron impact mass spectra were recorded at an ionization voltage of 70 eV and ion source of 280°C. The results reported were calculated by dividing the peak area of the compounds of interest by the total area, obtaining the relative abundance of each compound. The relative response factor was considered to be 1. Identification was performed by comparison of their mass spectra with those of the mass spectra library database Wiley 6.0., and their retention times with those of pure standards when they were available.

2.4 Statistical analysis

All assays were performed in triplicate and in a randomized run order. The statistical analysis was performed using Prism 9 version 9.1.2 (225) (GraphPad Software, LLC., California, USA) statistical package. The results are reported as the means ± standard error (SE) for parametric data. A one-way ANOVA and comparison of the means were conducted using Tukey's test, with a confidence interval of 95% and significant results with a p-value of ≤ 0.05. Principal component analysis (PCA) was also performed to determine differences between Cavas and lees.

3 Results and Discussion

For this study, four different types of Cava, as well as their lees, were analyzed. In addition, wine lees from the cellar waste were also collected and analyzed, since the wineries do not separate the lees according to the type of Cava, but deposit them all together to manage them as waste.

3.1 Determination of physicochemical parameters

Cava and lees pH values and cell counts are shown in Figure 2. In general, pH values of Cava are within the requirements established in the legislation (minimum 2.8 and maximum 3.4) [1]. It should be noted that the pH of Caves with Reserva category (CR1, CRR1 and CRR2) had a pH close to 3.1, regardless of the grape variety, while Cava Gran Reserva (CGR1) obtained a lower pH (2.94 ± 0.05) ($p < 0.05$). As for the lees, the pH values showed no significant differences except for the L-CV1 (cellar residue) samples, which obtained the lowest value (3.02 ± 0.02) ($p < 0.05$). However, the pH range obtained by lees was lower than that reported by other studies, in which the values ranged from 3.6 to 7.2 [4].

Table 2. UV-Vis spectrometry results regarding total phenolic index (TPI), color intensity (CI) and color hue (CH) expressed as absorbance units.

	Sample ID	TPI[1]	$OD_{320\ nm}$	CI[2]	CH[3]
CAVA	**CGR1**	6.47 ± 0.25	3.72 ± 0.62^{ab}	0.604 ± 0.062^{a}	1.612 ± 0.162^{a}
	CR1	5.43 ± 0.57	3.11 ± 0.04^{ac}	0.370 ± 0.097^{b}	1.111 ± 0.097^{b}
	CRR1	6.96 ± 0.70	4.21 ± 0.32^{b}	1.145 ± 0.064^{c}	1.490 ± 0.064^{a}
	CRR2	5.59 ± 0.88	2.65 ± 0.06^{c}	0.540 ± 0.013^{ab}	1.247 ± 0.109^{b}

LEES	**L-CGR1**	9.68 ± 0.48[a]	4.35 ± 0.71	1.320 ± 0.035[a]	1.140 ± 0.031[a]
	L-CR1	9.64 ± 0.36[a]	4.40 ± 0.47	1.412 ± 0.038[a]	1.023 ± 0.010[b]
	L-CRR1	8.07 ± 0.64[b]	4.66 ± 0.19	0.832 ± 0.087[b]	1.025 ± 0.002[b]
	L-CRR2	7.68 ± 0.52[b]	5.01 ± 0.18	1.836 ± 0.052[c]	0.999 ± 0.016[b]
	L-CV1	6.36 ± 0.21[c]	4.24 ± 0.12	0.766 ± 0.045[d]	0.942 ± 0.002[c]

[1]TPI: Total Polyphenols Index, $OD_{280\ nm} \times 10$.
[2]CI: Color Intensity, $OD_{420\ nm} + OD_{520\ nm} + OD_{620\ nm}$.
[3]CH: Color Hue, $OD_{420\ nm} / OD_{520\ nm}$. Results are expressed as mean ± standard deviation of triplicates. Different letters denote statistically significant differences ($p < 0.05$) between the samples of Cava and between the samples of lees for each parameter.

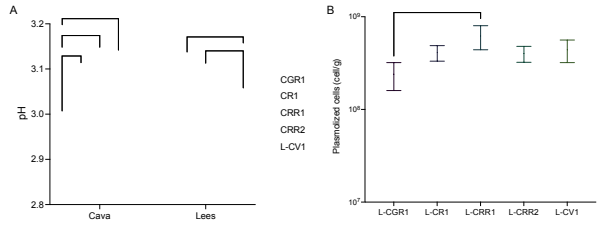

Figure 1. pH (A) and cell counts (B) of the different types of Cava and lees.

Plasmolyzed cells were then counted in lees samples using the Neubauer chamber (Fig. 1). The number of cells was found to be between $2.4 \times 10^8 \pm 8.0 \times 10^7$ cell/g (L-CGR1) and $6.2 \times 10^8 \pm 1.8 \times 10^8$ cell/g (L-CRR1). The Reserva Caves (both white and rosé) had a similar concentration of cells. Also, the residue from the winery (L-CV1) had a concentration of 4.4×10^8 cells/g.

The different types of polyphenols found in a wine have absorbance depending on the wavelength: at 280 nm the absorption is related to the benzene ring common to all phenolic compounds; at 320 nm are flavones and non-flavonoid compounds (hydrocinnamic acids, stilbenes, and hydrobenzoic acids); and finally, 520 nm is related to the presence of anthocyanins, which provide a reddish or purple pigment [15, 16]. Therefore, the white Cavas will mainly show absorbance in the 280 nm and 320 nm region, while in the pink cava samples there will be an extra absorption region at 520 nm. On the other hand, the color intensity (CI) represents the amount of color, varies depending on the wine and the grape variety used during vinification, and is in the range of 0.3 to 1.8 units. In addition, the color tone (TC) shows the development of orange tones with the aging of the wine, obtaining values between 0.5 and 0.7 for young wines and increasing up to 1.2-1.3 for aged wines [14]. The values obtained by UV-Vis spectrometry are found in Table 2.

Recent studies indicate a first increase and a subsequent decrease in the values of TPI, CI and CH as a result of the absorption of polyphenols by lees, as well as their polymerization and precipitation during the aging of Cava [15]. In fact, a higher TPI was observed in the samples of white lees compared to their respective Cava, obtaining the highest values in L-CGR1 and L-CR1 (white Cava). In rosé samples the same tendency of increase of the TPI in the lees was observed, obtaining a difference of 1.11 (CRR1 and L-CRR1) and 2.09 (CRR2 and L-CRR2) between the Cava and its lees. The sample of the cellar waste (L-CV1) showed the lowest TPI values (6.36 ± 0.21).

Regarding the CI, among Cavas of white varieties CGR1 had a higher intensity than CR1 ($p < 0.05$). However, the CI of L-CR1 was slightly higher than that of L-CGR1. Therefore, color intensity of Cava increases with the biological ageing. In contrast, for rosé samples, CRR1 had a higher CI than CRR2 ($p < 0.05$). In fact, single-variety rosé Cava (CRR2) had a very pale coloration, so adding the Garnatxa variety to Trepat (CRR1) significantly increased the CI. With respect to the lees obtained from these Cavas, L-CRR1 had a lower CI, as opposed to L-CRR2, that significantly increased its CI ($p < 0.05$). Similarly to TPI, the CI of L-CV1 was the lowest.

Finally, CH results were as expected for Cava with biological ageing, obtaining values between 1.111 ± 0.097 (CR1) and 1.612 ± 0.162 (CGR1); and 0.942 ± 0.002 (L-CV1) and 1.140 ± 0.031 (L-CGR1) for lees. In fact, the highest CH values were those of Cava with the highest aging.

3.2 Analysis of the volatile compounds of Cava and lees

The volatile fraction, or aroma, of Cava is one of the most relevant quality factors of such product. Aroma is influenced by different parameters, such as grape variety, the vinification process, the fermenting yeasts, and biological ageing in contact with lees [7, 15, 16]. In fact, the second fermentation and biological ageing have a great impact on the volatile compounds of Cava. For instance, during autolysis, yeasts release compounds to the wine [6]. Nevertheless, yeast lees are able to adsorb certain compounds in their surface [3]. Therefore, ageing time can determine the volatile profile of a product such Cava [15, 17].

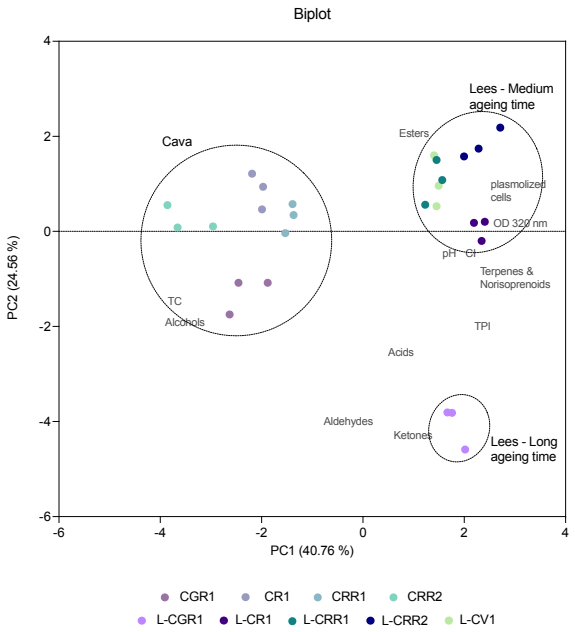

Figure 2. Principal component analysis (PCA) biplot of Cava and lees.

In this study, a total of 68 different compounds were identified in the samples of Cava and lees. The results obtained were subjected to a PCA (Fig. 2). Generally, Cava with more time of biological ageing (CGR1) and their lees (L-CGR1) were the samples with a greater variety of identified compounds. On the other hand, Cavas with the same biological ageing time (CR1, CRR1 and CRR2) showed differences regarding the grapes variety used for vinification, being significant between CR1 and rosé Cavas, but not between CRR1 and CRR2 for most of the compounds.

Both Cava and lees presented a similar volatile profile (Fig. 3). In both matrices esters were the major volatile compounds (45%–79%), highlighting ethyl hexanoate and ethyl octanoate in Cava, and ethyl octanoate and ethyl decanoate in lees. It can be observed that Cava lees presented more variability regarding the relative abundance of each family compound, although their general profile was very similar to Cava.

Acids are a product of long chain fatty acids catabolism and, depending on their concentration, they are related to a decrease in wine quality [18, 19]. It has been reported that when acids between C6 and C10 are above 20 mg/L have a negative impact on wine organoleptic quality, while below that concentration, acids contribute with pleasant aromas [18]. In the present study, acids accounted for 12%–17% of the total volatile compounds identified, increasing with biological ageing. Octanoic acid was the major acid, in accordance with other studies [15, 18, 20]. Moreover, Mendes de Souza Nascimento et al. (2018) [18] reported that higher values of chromatographic areas of acids can be related to the double fermentation that take place in sparkling wine vinification following the traditional method, as it is the case of Cava production. Regarding lees, acids showed a greater area for L-CV1 (34.37 ± 10.91), L-CGR1 (31.95 ± 9.34) and L-CR1 (16.85 ± 2.10), being a 26%, 22%, and 17%, respectively. Lees from rosé Cava presented lower values with a relative abundance of 10.27 ± 0.61 (9%, L-CRR1) and 7.01 ± 1.00 (7%, L-CRR2).

Figure 3. Relative abundance of the volatile compounds identified in Cava and lees A) White Cava and lees; B) Rosé Cava and lees.

Aldehydes are the result of carbohydrate and lignin degradation and are responsible for toasty notes in wine [18, 21]. Even though aldehydes can be reduced during ageing to form their respective alcohols [21], in this study, both Cava and lees increased the total abundance of aldehydes with the ageing time. Furthermore, it has been reported that aldehydes are easily adsorbed by lees [3, 21]. For instance, a few aldehydes were identified in CR1 (furfural, benzaldehyde and 2-methylbenzaldehyde), CRR1 (furfural and benzaldehyde) and CRR2 (furfural and 3-methylbenzaldehyde), but the aldehydes found in their lees presented a higher diversity.

During the fermentation process, higher alcohols are produced from sugars and amino acids. They are an important fraction of the sparkling wine volatile profile, even though alcohols may have a positive or a negative impact on wine aroma [18]. In the present study, isoamyl alcohol and 2-phenylethanol, both major products of alcoholic fermentation, where the dominant alcohols in Cava, being in accordance with other studies [15, 18, 22]. Although 2-phenylethanol was found in all lees samples, isoamyl alcohol was only identified in L-CGR1 (Cava Gran Reserva) and L-CV1 (cellar waste), with a low relative abundance (1.11 ± 0.12 and 2.03 ± 0.62, respectively). 2-butanol, 1-hexanol, (Z)-3-hexen-1-ol and

2-methylpropanol were only found in Cava. Moreover, 2-butanol and 2-methylpropanol were exclusive of rosé Cava, while 1-octanol and (Z)-3-hexen-1-ol were only identified in white Cava. On the other hand, 1,3-butanediol, 1-nonanol, 2-hexanol and 2-ethilhexanol were only obtained in lees. In fact, Gallardo et al. (2009) [3] studied the volatile profile of lees and concluded that lees have a scarce capacity of retaining higher alcohols on their surface.

As previously stated, esters were the major volatile compounds of both Cava and lees, contributing to aroma with fruity notes. Regarding Cava, they represented between 65% (CGR1) and 75% (CR1); while in lees there was a greater variability, ranging between 64% (L-CGR1) and 82% (L-CRR2). Because of their great hydrophobic capacity, esters can easily be retained on lees surface [3]. Among the detected esters, most of them were ethyl esters. Those are produced by yeasts during alcoholic fermentation, contributing with floral and fruity aromas [22]. In accordance with other studies, ethyl hexanoate, octanoate and decanoate and diethyl succinate, were the major esters found in Cava [18, 22]. Similarly, they were also the most outstanding esters, in agreement with the results reported by Gallardo-Cachón et al. (2009) [3].

Finally, vitispirane A and 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) were the norisoprenoids identified in Cava and lees. They are both considered ageing markers due to their concentration increase with time [3, 17]. In fact, the obtained results showed a greater area percentage in lees and Cava Gran Reserva when compared to younger Cavas, being in agreement with other studies [3, 15, 17, 19]. Similarly to esters, those compounds are hydrophobic, therefore, they have a great capacity of being retained in lees surface [3].

In general, volatile compounds of Cava and lees differ from each other (Fig. 4). Regarding white Cavas, CGR1 (Gran Reserva) there is a coincidence of 53% of the compounds with respect to their lees (L-CGR1); while CR1 (Reserva) there is a 43% similarity between Cava and lees. As for rosé Cavas, between CRR1 and L-CRR1 there was a 31% coincidence, and a 35% similarity between CRR2 and L-CRR2. Thus, longer times of ageing in contact with lees may result in greater adsorption or release of compounds and, consequently, more similarity between the wine and the lees.

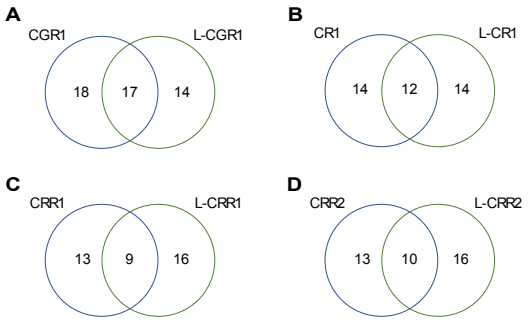

Figure 4. Venn diagram of the volatile compounds Shared by each Cava and their lees: A) Gran Reserva White Cava (CGR1); B) Reserva White Cava (CR1); C) Reserva Rosé Cava coupage Garnatxa-Trepat (CRR1); D) Reserva Rosé Cava Trepat (CRR2).

4 Conclusions

During biological ageing of Cava (sparkling wine) compounds are both released and retained by lees, therefore modifying the volatile and phenolic profiles of such wine product.

Different physicochemical parameters of both Cava and lees were studied. Generally, it was observed that lees presented higher values of TPI and CI, pointing towards the adsorption of phenolic compounds in the lees surface. It was found that pH values were lower for Cava and lees with a longer ageing period. Regarding the number of plasmolyzed cells in Cava lees, values were around 10^8 cells/g of lees.

On the other hand, a total of 68 volatile compounds were identified in Cava and lees, of which 19 were only found in lees samples. Most of these differences were found in L-CV1 (cellar lees waste) in which lees from different origins are mixed.

In conclusion, lees could be a potential source of flavor as a new ingredient for the food industry. That might be a new strategy for the valorization of such by-product. Therefore, future research should focus on the use of different lees in the formulation of foodstuff.

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References

1. Resolución de 24 de enero de 2022, de la Dirección General de la Industria Alimentaria, por la que se publica la resolución favorable de modificación normal del pliego de condiciones de la Denominación de Origen Protegida Cava. Boletin Oficial del Estado (BOE) núm. 28, de 2 de febrero de 2022, p. 14734-14735
2. H. Alexandre, M. Guilloux-Benatier, Aust. J. Grape Wine Res. **12**, 119 (2006) doi:10.1111/j.1755-0238.2006.tb00051.x
3. J. Gallardo-Chacón, S. Vichi, E. López-Tamames, S. Buxaderas, J. Agric. Food Chem. **57**, 3279 (2009) doi:10.1021/jf803493s
4. De Iseppi, A.; Lomolino, G.; Marangon, M.; Curioni, A. Current and Future Strategies for Wine Yeast Lees Valorization. Food Res. Int. **137**, 109352 (2020) doi:10.1016/j.foodres.2020.109352
5. J. Gallardo-Chacón, S. Vichi, P. Urpi, E. López-Tamames, S. Buxaderas, Int. J. Food Microbiol. **143**, 48 (2010)
6. H. Alexandre, Yeasts and Sparkling Wine Production. In *Yeasts in the Production of Wine* (Springer, New York, NY, 2019)
7. S. Pérez-Magariño, M. Ortega-Heras, M. Bueno-Herrera, L. Martínez-Lapuente, Z. Guadalupe, B.

Ayestarán, LWT - Food Sci. Technol. **61**, 47 (2015) doi:10.1016/j.lwt.2014.11.011

8. R. Nieto-Rojo, C. Ancín-Azpilicueta, J.J. Garrido, Food Chem. **152**, 399 (2014) doi:10.1016/j.foodchem.2013.11.157
9. M.Á. Rivas, R. Casquete, M. de G. Córdoba, S. Ruíz-Moyano, M.J. Benito, F. Pérez-Nevado, A. Martín, Foods **10**, 1510 (2021) doi:10.3390/foods10071510
10. S. Hernández-Macias, N. Ferrer-Bustins, O. Comas-Basté, A. Jofré, M. Latorre-Moratalla, S. Bover-Cid, M. del C. Vidal-Carou, Foods **10**, 1916 (2021) doi:10.3390/foods10081916
11. M. Felix, I. Martínez, A. Sayago, M.Á.F. Recamales, Wine Lees: From Waste to O/W Emulsion Stabilizer. Innov. Food Sci. Emerg. Technol. **74**, 102810 (2021) doi:10.1016/j.ifset.2021.102810
12. J.-Y. Hwang, Y.-S. Shyu, C.-K. Hsu, LWT - Food Sci. Technol. **42**, 312 (2009) doi:10.1016/j.lwt.2008.03.008
13. International Organisation of Vine and Wine *COMPENDIUM OF INTERNATIONAL METHODS OF ANALYSIS–OIV Chromatic Characteristics*
14. P. Ribereau-Gayon, Y. Glories, A. Maujean, D. Dubourdieu, *Handbook of Enology Vol. 2: The Chemistry of Wine Stabilization and Treatments*; 2nd ed.; (John Wiley & Sons Ltd, Chichester, UK)
15. C. Ubeda, I. Kania-Zelada, R. del Barrio-Galán, M. Medel-Marabolí, M. Gil, A. Peña-Neira, Food Res. Int. **119**, 554 (2019), doi:10.1016/j.foodres.2018.10.032
16. L. Martínez-Lapuente, Z. Guadalupe, B. Ayestarán, M. Ortega-Heras, S. Pérez-Magariño, Am. J. Enol. Vitic. **64**, 39 (2013) doi:10.5344/ajev.2012.12013
17. M. Riu-Aumatell, J. Bosch-Fusté, E. López-Tamames, S. Buxaderas, Food Chem. **95**, 237 (2006) doi:10.1016/j.foodchem.2005.01.029
18. A. Mendes de Souza Nascimento, J. Fagundes de Souza, M. dos Santos Lima, G.E. Pereira, Beverages **4**, 103 (2018) doi:https://doi.org/10.3390/beverages4040103
19. J. Torrens, M. Rlu-Aumatell, S. Vichi, E. López-Tamames, S. Buxaderas, J. Agric. Food Chem. **58**, 2455 (2010) doi:10.1021/jf9035518
20. V. Caliari, C.P. Panceri, J.P. Rosier, M.T. Bordignon-Luiz, LWT - Food Sci. Technol. **61**, 393 (2015) doi:10.1016/j.lwt.2014.11.039
21. J.M. Del Fresno, A. Morata, J.M. Ricardo-da-Silva, C. Escott, I. Loira, J.A.S. Lepe, Int. J. Food Sci. Technol. **54**, 2690 (2019) doi:10.1111/ijfs.14179
22. C. Ubeda, R.M. Callejón, A.M. Troncoso, A. Peña-Neira, M.L. Morales, Food Chem. **207**, 261 (2016) doi:10.1016/j.foodchem.2016.03.117