Yeast cells in double layer calcium alginate–chitosan microcapsules for sparkling wine production

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ABSTRACT

This paper focuses on the use of a new type of yeast encapsulation procedure, applying the chitosan-calcium alginate double layer microcapsules, for the production of Riesling sparkling wine. Four different sparkling wines were produced by free or encapsulated yeasts. The four types of yeast used were adapted (Free EtOH-A, Encapsulated EtOH-A) and non-adapted to ethanol (Free, Encapsulated).

The different yeast-inoculating formats had a significant impact on oxygen consumption and pressure increase rate in the bottle during the *prise de mousse*. Similarly to the free form, encapsulated yeast successfully completed the secondary fermentation. After an ageing period of 6 months, volatiles and sensory profiles of sparkling wines were compared. Although, some differences in volatile profiles were found among samples, sparkling wines produced by Encapsulated EtOH-A showed sensory properties, in terms of aroma, taste and body, similar to those produced by free yeast (both adapted and non-adapted to ethanol).

1. Introduction

Sparkling wine is produced by a secondary alcoholic fermentation of base wine. In the traditional method (*méthode champenoise*) the re-fermentation takes place in the bottle after the addition of the *liquer de tirage* (composed of sugars and yeasts) (Pozo-Bayón, Martínez-Rodríguez, Pueyo, & Moreno-Arribas, 2009). It is well known that a preliminary yeast adaptation, mainly to ethanol, is essential for the success of the *prise de mousse*, because it enables yeast to acclimatize in base wine (Dinh, Nagahisa, Hirasawa, Furusawa, & Shimizu, 2008; Benucci, Liburdi, Cerreti, & Esti, 2016). The secondary fermentation is carried out to produce the desired amount of carbon dioxide (CO_2) pressure, and it is followed by an ageing period, during which yeast autolysis occurs. At the end of the ageing period the yeast lees is removed by riddling, a very labour-intensive and time consuming process which takes about 30 days. The labour involved in this process is the reason for the high final cost of sparkling wine (Kemp, Alexandre, Robillard, & Marchal, 2015).

Riddling and the subsequent disgorging procedures may be accelerated and simplified by the use of immobilized yeasts (Puig-Pujol et al., 2013). In recent decades, research has been carried out on the selection and development of suitable techniques using support materials appropriate for yeast immobilization (De Lerma et al., 2018; Wen-Tao, Wei-Ting, Yu-Bing, & Xiaojun, 2005; Liouni, Drichoutis, & Nerantzis, 2008). Capsule entrapment preserves the desired yeast metabolism by the physical localization of viable cells into the polymer matrix (Wen-Tao et al., 2005). The support materials most commonly used on an industrial level in capsule production for their properties (e.g. food-grade, low cost, abundant and suitable for ethanol–water solution) are: carrageenans, chitosan, polyvinyl alcohol and alginates (Kourkoutas, Bekatorou, Banat, Marchant, & Koutinas, 2004). Sodium alginate gels when it comes in contact with bivalent cations (e.g. Ca^{2+}), thus creating a three-dimensional network, usually in the form of a capsule or a bead. As shown by other authors, the use of calcium alginate capsules simplifies the procedure of 'remuage' (Divies, Cachon, Cavin, & Prevost, 1994). This causes the product to settle quickly into the neck of the bottle so that yeast can be easily removed. Despite the advantages of encapsulated yeasts, their application is still limited. Issues with them include: cell leakage, diffusional limitations, rupture of capsules caused by growth cell and gas production (during the *prise de mousse*), and dissolution of three-dimensional gel in an acidic media (during the sparkling wine ageing period). Research has been conducted to find a way of improving the chemical and mechanical properties of single alginate layer capsules by using chitosan (1,4-linked-2-amino-2-deoxy-b-D-glucan). This is a coating agent obtained from a natural food-grade polysaccharide called chitin (Liouni et al., 2008; Wen-Tao et al., 2005). Calcium alginate–chitosan microcapsules have been receiving considerable attention in recent years due to their

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biocompatibility, nontoxicity, and biodegradability as drug delivery systems, enzyme and cell immobilization supports and cell transplantation (Wu et al., 2018; Haque et al., 2018). Despite the efforts which have been made to improve the application of immobilized yeasts in the secondary fermentation (De Lerma et al., 2018; Puig-Pujol et al., 2013; Bozdogan & Canbas, 2011), to the best of our knowledge no study has investigated the use of encapsulated yeast in a multilayer biopolymer matrix. Thus, the aim of this study was to use a new yeast encapsulation procedure, applying the chitosan-calcium alginate double layer microcapsules, for sparkling wine production by the traditional method. Additionally, a stepwise adaptation to ethanol of yeast before encapsulation was developed for the first time. During the *prise de mousse*, the kinetics of oxygen consumption and pressure increase in the bottle were monitored. Volatiles and sensory profiles of sparkling wines produced by microencapsulated and free yeasts were measured and compared after a 6 month ageing period.

2. Materials and methods

2.1. Chemicals

The glucose, ethanol, K_2HPO_4, $MgSO_4$, peptone, yeast extract, alginic acid sodium salt and chitosan were purchased from Sigma Aldrich (Italy), whereas the quarter-strength Ringer's solution was from Oxoid (Hampshire, UK). Double distilled water was used throughout the process for the preparation of all buffers, reagents and yeast mediums. The other oenological products were kindly provided by Lallemand (Verona, Italy).

2.2. Strain and culture conditions

The commercial yeast strain *S. cerevisiae bayanus* Lalvin EC-1118® (EC1118), kindly provided by Lallemand (Verona, Italy), was routinely propagated in a yeast nitrogen base broth (YPD Broth, Sigma Aldrich, Milano, Italy) at 28 °C for 24 h in aerobic conditions. The strain was inoculated in the base wine in free or in encapsulated form, previously adapted or non-adapted to ethanol presence.

2.3. Yeast adaptation to ethanol

The yeast adaptation to ethanol was carried out in YPD broth in the presence of increasing ethanol concentrations: 4%, 7% and 10%. A standardized (final OD600 = 1.0) overnight culture of the yeast was inoculated in YPD at the lowest ethanol level. After incubation at 28 °C for 24 h in aerobic condition, cells were recovered by centrifugation (6500g × 15 min) and resuspended in YPD broth at 7% ethanol. After incubation, the cell pellet was treated in the same way, resuspended in YPD broth at 10% ethanol and incubated in the same conditions. After each ethanol adaptation phase, viable counting of the yeast cells was performed on the YPD agar at 28 °C for 24 h. Results were expressed as an average of three independent replicates.

2.4. Yeast microencapsulation

Microencapsulation of yeast cells was carried out using the Encapsulator B-395 Pro (BUCHI, Switzerland). A scheme of the Buchi Encapsulator is reported in De Prisco, Maresca, Ongeng, & Mauriello, 2015. Yeast cultures, previously adapted or non-adapted to ethanol, were centrifuged at 6500g for 15 min. The cell pellets were washed in a sterile quarter-strength Ringer's solution, harvested by centrifugation and finally suspended in an equal volume of a 17 g/L alginic acid sodium salt. The alginate-cell suspension was loaded in a syringe and then forced into the pulsation chamber and extruded through the nozzle (80 µm) (Maresca et al., 2016). The microencapsulation conditions used were: flow rate 4.5 mL/min, vibration frequency 2700 Hz, electrode voltage of 850 V. Microcapsules containing yeast cells were obtained by hardening the droplets in 100 mL of a sterile 0.5 mol/L $CaCl_2$ solution, continuously stirred at 350 rpm. After sedimentation and gentle discarding of $CaCl_2$ solution, microcapsules were stored at 4 °C for further experiments.

2.5. Efficiency of microencapsulation process

The efficiency of microencapsulation was calculated by dividing the viable count of disrupted microcapsules by the cell load of the alginate-cell suspension. Microcapsules were disrupted by serial dilution in 0.1 mol/L phosphate buffer solution pH 7.0 and a viable cell count was performed on YPD agar at 28 °C for 24 h.

2.6. Coating of microcapsules with chitosan

The chitosan-alginate microcapsules were produced according to the De Prisco et al. (2015) protocol. Briefly, 7 g/L of chitosan was dissolved in a 0.14 mol/L acetic acid solution (pH 3.2) under stirring at 8000 rpm for 20 min at 50 °C. Microcapsules (in ratio 1:10 v/v) were added to the chitosan solution (previously sterilized) and stirred at 4000 rpm for 15 min at room temperature. After sedimentation and discarding of the upper phase, alginate-chitosan microcapsules were suspended in Ringer's solution and stored at 4 °C.

2.7. Morphology of microcapsules

The size and surface morphology of microcapsules were analyzed using a Zeiss light microscope (200 × magnification and calibrated micrometer) before and after the chitosan coating.

2.8. Base wine and secondary fermentation

Sparkling wines were produced by the so-called traditional method using a monovarietal base wine from *Vitis vinifera* L. cv. Riesling renano white grape (accession VCR 3 obtained from VCR – Vivai Cooperativi Rauscedo collections, Rauscedo, PN, Italy). Grape berries were harvested in a vineyard located in the Umbria region (central Italy – vintage 2016), and directly pressed in a horizontal press RPF 50 – BUCHER Vaslin (Romans d'Isonzo, Italy) without crushing-stemming. The Riesling juice was treated with potassium bisulfite (5 g/hL) and pectic enzyme (2 g/hL). Fermentation took place in a stainless steel tank (Ghidi Metalli, PT, Italy) at 18 °C and the yeast used was *S. cerevisiae bayanus* Lalvin EC-1118® (EC1118). At the end of the alcoholic fermentation, the base wine was treated by adding bentonite (50 g/hL) and gelatin (1 g/hL) so as to reach a turbidity level below 2 NTU after the heat test. The cold stabilization period lasted for 4 weeks at a temperature of 2 °C. The wine was then filtered through sterile polyethersulfone membrane filter cartridges (0.45 µm). The composition of the base wine produced is reported in Table S1. Secondary fermentation was carried out in standard 750 mL sparkling wine bottles, filled with base wine containing 24 g/L of sucrose. Free cells inoculum was prepared to obtain a final concentration in the bottle of 2×10^6 cells/mL of viable yeast cells as reported by (Bozdogan & Canbas, 2011). The amount of microcapsules to be introduced into each bottle was calculated in order to achieve an equivalent inoculum (2×10^6 cells/mL of viable yeast cells). Four types of yeast were used to produce four different sparkling wines. These were: i. free yeast non adapted to ethanol (Free); ii. free yeast adapted to ethanol (Free EtOH-A); iii. encapsulated yeast non adapted to ethanol (Encapsulated); iv. encapsulated yeast adapted to ethanol (Encapsulated EtOH-A). A total of 24 bottles (6 replicates of each type) were produced. Among these, 3 replicates for each sample (total 12 bottles) were closed with bidules (an empty polyethylene cup that facilitates the disgorging process) and crown cap. The other 12 bottles were capped with aphrometers (Oenoitalia Srl, Italy) which are pressure gauges that hermetically seal the bottles. The bottles were positioned horizontally on the floor of a dedicated room

and kept in the dark at a constant temperature of 15 °C.

2.9. Analytical procedures

The OIV analytical methods (O.I.V., 2011) were applied for the determination of pH, total acidity (g/L), volatile acidity (g/L), alcoholic degree (% v/v), residual sugar (g/L), total and free sulphur dioxide (mg/L), malic and lactic acid (g/L) and glycerol (g/L). All analyses were performed in triplicate. The addition of inoculum (free or encapsulated yeast) started the *prise de mousse* phase. The oxygen consumption and the pressure increase within the bottle were then monitored. The consumption of the dissolved oxygen was measured using the NomaSenseTM O_2 Trace (PreSens GmbH, Regensburg, Germany), which is a trace oxygen meter with fiber-optic oxygen sensors based on a 2 mm polymer optical fiber. PreSens Pst6 oxygen sensors (Presens GmbH, Regensburg, Germany) were glued into the bottles before bottling and the measurements were performed in triplicate on the same 3 samples used for the pressure increase determination. The pressure increase, corresponding to the CO_2 released during the *prise de mousse*, was determined by an aphrometer.

As reported in Benucci et al. (2018), solid-phase extraction (SPE) was carried out using ENV + cartridge (IST, Ystrad Mynach, Wales) and the volatile compounds were analysed by GC/MS with a 6980 N Network GC System coupled with a 5975 XL EI/CI MSD (Agilent Technologies, Santa Clara, CA, USA), equipped with DB-Wax Bonded PEG fused silica capillary column (60 m × 320 µm i.d. × 0.25 µm film thickness; Agilent Technologies). Volatile compounds were identified by comparing the GC retention time and mass spectra with pure reference standards and by the NIST data bank. The quantitative analysis was performed using 1-heptanol as internal standard with Response factor (RF) = 1.

2.10. Estimation of the prise de mousse kinetic parameters

The oxygen consumption and the pressure increase inside the bottle resulting from the addition of inoculum were measured by means of a sigmoid or altered Gompertz decay function as previously described by other authors (D'Amato, Corbo, Nobile, and Sinigaglia, 2006; Tronchoni, Gamero, Arroyo-López, Barrio, and Querol, 2009). The following equation was applied:

$$Y = A + C * e^{-e(K*(t-M))} \quad (1)$$

where Y is the amount of oxygen consumed (ppm) or the pressure (bar) in the bottle at time t (days); A is the lower asymptote, representing the lowest amount of oxygen consumed or the lowest pressure value when t tends to infinity (t/∞); K is the oxygen consumption rate or the pressure increase rate; C is the distance between the upper and lower asymptote and M is the half-time (in day^{-1}) of oxygen consumption or pressure increase. Eq. (1) was fitted to the experimental data by a nonlinear regression procedure (GraphPad Prism 5.0, GraphPad software, Inc.) and the quality of the regression was evaluated by the coefficient of determination (R^2).

2.11. Sensory analysis

The sensory evaluation of sparkling wines was carried out at Tuscia University by a panel of 10 assessors (4 female and 6 male), ranging in age between 30 and 65, all of them experienced in wine tasting and trained in performing sensory analysis of various wine typologies (including sparkling wines). Prior to the formal evaluation, the panel underwent 12 h of training (i.e., 6 × 2 h sessions held over three weeks), during which they generated, discussed, and modified descriptive terms, using the tasting card recommended by the O.I.V. (2011) and gained familiarity in recognizing and scoring the intensity of each attribute along with practice samples.

The sensory attributes evaluated were: colour, limpidity, aroma quality and intensity, taste quality and intensity, acidity, body and overall judgment.

Testing was conducted in a sensory laboratory equipped with separate booths under daylight, and the environment was free of interference in terms of noise, visual stimulation and ambient odour. The temperature was kept at 24 ± 2 °C. Humidity values ranged from 70 to 80%. The sparkling wine samples were evaluated in duplicate and were presented in random order at 12°C in coded standard wine-tasting glasses according to ISO standard 3591 (ISO, 1977), covered with a watch-glass and marked with a three-digit code.

To reduce carryover effects, a 7 min break was taken between each sample evaluation during which judges used distilled water to rinse their mouths. The scorecard used had a 10-point structured scale to rate the intensity of each attribute (0 = attribute not perceptible, 10 = attribute strongly perceptible). The mean scores of attributes were submitted to quantitative descriptive analysis in order to generate the sensory profile of the four sparkling wines.

Moreover, attention was paid to visual evaluation of foam quality and effervescence, which is of special importance in sparkling wines. The attributes assessed were the initial quantity of foam formed, whether the foam covered the whole surface of the wine, the presence of a foam collar, the size of the bubbles, and the effervescence as reported by Hidalgo et al. (2004).

2.12. Statistical analysis

Data were analysed by one-way completely randomised Analysis of Variance (ANOVA) with the EXCEL® Add-in macro DSAASTAT (Onofri, 2006, Italy), followed by Tukey Honestly Significant Difference (Tukey HSD) post-hoc test (α = 0.05) for multiple comparisons of samples.

Principal component analysis (PCA) was applied on an autoscaled data matrix made up of the 4 sparkling wines produced (Free; Free EtOH-A; Encapsulated; Encapsulated EtOH-A), and the mean values of sparkling wine volatiles, as variables. Unscrambler for Windows (version 9.2 software package, CAMO A/S, Trondheim, Norway) was used for data processing and full cross-validation was used as validation criterion.

3. Results and discussion

To the best of our knowledge this is the first time that *Saccharomyces cerevisiae* EC1118 culture has been applied in chitosan-calcium alginate double layer microcapsules to produce sparkling wine by the traditional method. Four different types of yeast were used to produce four different types of wine. These were free yeast adapted to ethanol, free yeast non adapted to ethanol, encapsulated yeast adapted to ethanol and encapsulated yeast non adapted to ethanol (Free EtOH-A, Free, Encapsulated EtOH-A, Encapsulated).

3.1. Ethanol adaptation and microencapsulation

As expected, the yeast population of the strain EC1118 showed resistance to ethanol. Indeed, the results of the microbial count showed that the starting population of 5.6 × 10^8 CFU/mL was slightly reduced to 4.9 × 10^8 CFU/mL after ethanol adaptation. Adapted and non-adapted cells were microencapsulated in an alginate matrix and the results showed an microencapsulation efficiency of about 87% for both types of cultures. To the best of our knowledge this is the first study in which yeast cells are microencapsulated by using the vibrating technology. However, values of efficiency of microencapsulation in the range 80–90% are those usually obtained with different techniques combined to different types of microorganisms (Kavitakea, Kandasamya, Devib, & Shettya, 2018). Morphology of microcapsules before and after chitosan coating is shown in Fig. 1. Microcapsules showed a regular spherical shape and yeast cells seemed homogeneously distributed in the alginate matrix. Microcapsules showed an

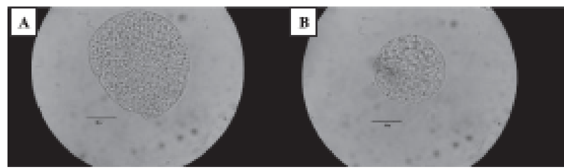

Fig. 1. Surface morphology of microcapsules before (A) and after chitosan coating (B).

average diameter, calculated by analyzing 50 microcapsules, of about 150 µm. After chitosan coating (Fig. 1A) the diameter decreased at about 110 µm (Fig. 1B). This is probably due to calcium alginate network shrinkage caused by the low pH of the coating solution (George & Abraham, 2006).

3.2. *Prise de mousse kinetic parameters*

3.2.1. *Oxygen consumption behaviour*

The dissolved oxygen consumption resulting from the yeast addition into the bottle was measured to better understand the influence of encapsulation on yeast fermentation kinetics. The initial amount was approximately 8.6 mg/L. As ascertained by other authors, yeast metabolism under anaerobic conditions normally requires oxygen in order to favour the synthesis of sterols and unsaturated fatty acids (Fornairon-Bonnefond, Demaretz, Rosenfeld, & Salmon, 2002). To the best of our knowledge, no studies have been carried out to determine the oxygen consumption by *Saccharomyces cerevisiae* in the bottle during the secondary fermentation for sparkling wine production. It is worth taking into account (Fig. 2A) that approximately 90% of the dissolved oxygen was rapidly consumed in the first few days after the inoculation of both ethanol adapted yeast forms (3 and 4 days for Free EtOH-A and Encapsulated EtOH-A, respectively), whereas the non-adapted forms needed a considerably longer time (16 and 37 days for Free and Encapsulated, respectively). The obtained data followed the sigmoid behaviour described by the Gompertz decay function, and they were adequately fitted as shown by the R^2 values (0.96–0.99) reported in Table 1. Although the entire oxygen amount initially present in the bottle was consumed during the *prise de mousse*, the kinetics of its consumption were considerably influenced by yeast-inoculating formats. The rate of oxygen consumption values (K_{Oxy}) were significantly higher when secondary fermentation was carried out by free and encapsulated yeast previously adapted to ethanol (Free EtOH-A and Encapsulated EtOH-A) (Table 1), with no significant differences between the two adapted forms. The free cells of the non-adapted yeasts showed a higher oxygen consumption rate and a lower time to consume 50% of the initial oxygen amount as compared to the encapsulated form. At the end of the prise de mousse the residual dissolved oxygen ranged from 14 µg/L (Free EtOH-A and Encapsulated EtOH-A) to 41 µg/L (Free and Encapsulated). Therefore, data revealed that the ethanol adaptation phase was crucial to enhancing yeast metabolism. Although the cells were enclosed in a double layer microcapsule, the results were still evident.

3.2.2. *Pressure increase behaviour*

Fig. 2B shows the kinetics of the *prise de mousse* phase, which is represented by the pressure increase in the bottle corresponding to the CO_2 released during the secondary fermentation. EC1118 free and encapsulated, both adapted and non-adapted to ethanol, successfully completed the secondary fermentation by reaching the expected pressure (6 bars). This result is due to the amount of sugar in the *liquer de tirage* added. The kinetic behaviour was described by a sigmoid curve, as reported by Martí-Raga, Sancho, Guillamon, Mas, and Beltran (2015) and Benucci et al. (2016) that could be fitted using a Gompertz model. The predicted curves fitted well with the experimental data and their

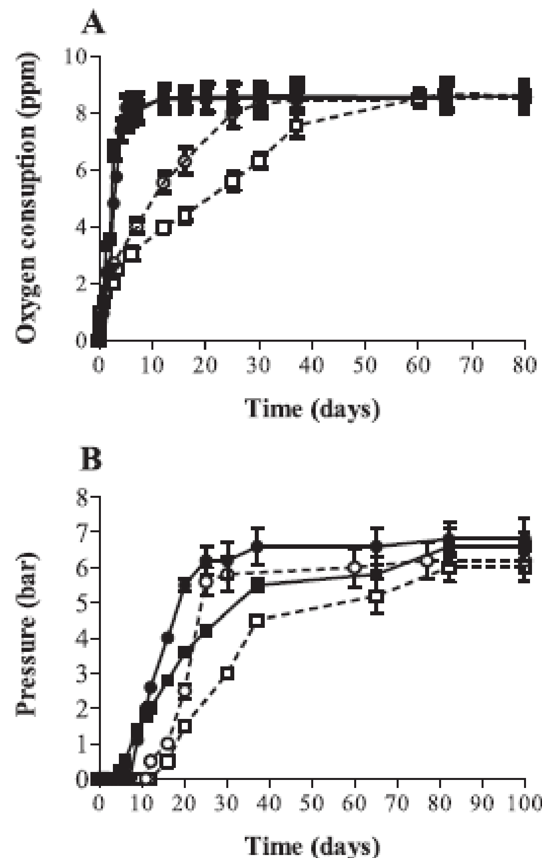

Fig. 2. Time course of oxygen consumption (A) and evolution of pressure (B) in the bottle during *prise de mousse* carried out by EC1118 yeast in Free (●, ○) or Encapsulated (■, □) form, adapted (●, ■) or non-adapted (○, □) to ethanol (EtOH). Reported values are mean ± standard deviation of 3 replicates.

Table 1
Parameters obtained by fitting the altered Gompertz equation to the experimental data of oxygen decrease and pressure increase in the bottle during *prise de mousse* carried out by EC1118 yeast in Free or Encapsulated form, adapted (EtOH-A) or non-adapted to ethanol. Different letters within a row denote significant differences among the means (one-way ANOVA, α = 0.05, Tukey multiple comparisons test).

	Free	Free EtOH-A	Encapsulated	Encapsulated EtOH-A
K_{Oxy} (mg L^{-1} day^{-1})	0.086b (0.009)	0.341 a (0.043)	0.037b (0.007)	0.388 a (0.038)
M_{Oxy} (day^{-1})	9b (1.5)	2c (0.3)	15 a (1.0)	2c (0.4)
R^2	0.99	0.96	0.98	0.98
K_P (bar day^{-1})	0.032b (0.004)	0.053 a (0.010)	0.014c (0.005)	0.038 ab (0.004)
M_P (day^{-1})	21b (3.5)	13c (2.5)	49 a (1.5)	18 bc (2.0)
R^2	0.94	0.93	0.94	0.98

K_{Oxy}, oxygen consumption rate; M_{Oxy}, half-time of oxygen consumption; K_P, pressure increase rate; M_P, half-time of pressure increase.

regression coefficients ranged from 0.93 to 0.98 (Table 1). The different yeast-inoculating formats had a significant impact on the kinetic parameters [e.g. pressure increase rate (K_P) and half-time of pressure increase (M_P)], with the highest K_P (0.053 bar day^{-1}) and the lowest M_P value (13 day^{-1}) revealed for Free EtOH-A.

As previously observed for the oxygen consumption, the fermentation kinetics appeared more vigorous when using free and encapsulated yeasts, previously adapted to ethanol (Free EtOH-A and Encapsulated EtOH-A) with significant differences compared to non-adapted forms.

Moreover, the secondary fermentation carried out by encapsulated EC1118 (non-adapted to ethanol) was the slowest, with a K_P of 0.014 bar day^{-1} and a M_P value of 49 day^{-1} to reach the 50% value of final pressure in the bottle (Table 1). As reported by Dinh et al. (2008), exposing yeast cells to a stepwise increase in the level of ethanol stress is effective for obtaining the ethanol-tolerance. This is most likely due to the different fatty acid content of cell membrane and cell morphology (You, Rosenfield, & Knipple, 2003; Stanley, Bandara, Fraser, Chambers, & Stanley, 2010). It is also important to note that the time required for starting the secondary fermentation (number of days until the start of pressure increase) confirmed the enhanced metabolic efficiency of ethanol-adapted cells, both in encapsulated and free forms (lag phase of about 6 days), as compared to the corresponding non-adapted counterparts (lag phase of about 16 days).

3.3. Sparkling wine characterization after a 6 month ageing period

It is well known that the kinetics of secondary fermentation in the bottle affects the qualitative characteristics of the final outcome of sparkling wine produced, including CO_2 integration in the product, physical-chemical characteristics, and volatile aroma compounds (Martínez-García, García-Martínez, Puig-Pujol, Mauricio, & Moreno, 2017; Pozo-Bayón et al., 2009). Thus, the main oenological parameters, volatiles and sensory properties of the 4 different sparkling wines produced were determined after an ageing period of 6 months.

3.3.1. Oenological parameters

The oenological parameters are summarized in Table S1. No significant differences were observed among the 4 sparkling wines in alcohol content, titratable and volatile acidity, pH, free and total sulphite content, and residual sugar. In addition, data showed a significant increase (about 1.5 g/L) in glycerol content in all samples as compared to the base wine (3.2 g/L). This increase could be due to the glycerol-pyruvic fermentation pathway, during the early phase of *prise de mousse*, when glycerol synthesis makes it possible to reoxidize surplus NADH produced by anabolism. No significant differences related to yeast-inoculating formats were determined, thus proving that the ethanol adaptation process, before secondary fermentation, did not affect this yeast metabolism.

3.3.2. Volatile aroma compounds

The 101 volatile compounds (e.g. terpenes and norisoprenoids, aldehydes and ketones, esters, alcohols, acids, phenols and lactones) identified and quantified by GC–MS in the Riesling base wine and in all sparkling wines produced using EC1118 yeast, in free or encapsulated form adapted or non-adapted to ethanol, are shown in Table 2. When one-way ANOVA was performed significant differences on the volatile composition was observed among the samples.

3.3.2.1. Terpenes and norisoprenoids. Terpenoids and C13-norisoprenoids, which are important secondary plant metabolites, contribute to the varietal character of many wines. These compounds, mainly characterized by floral aroma, are transferred from the grape to the must during solid–liquid extraction phases, either in a free volatile form or bound to sugars.

Observing data in Table 2, the secondary fermentation and the following 6 month ageing period lead to a decrease of total terpenes compared to the initial content in the base wine, although significant differences did not appear among the four sparkling wines. Recently, Soares et al. (2015) found that the quantity of some monoterpenes was reduced in the early stages of Moscatel sparkling wine production. This is thought to be caused by yeast membrane incorporation and acetylation. There were significant differences between Riesling base wine and sparkling wines concerning the occurrence of linalool, cis and *trans*-furan linalool oxide, endiol and *cis*-8-hydroxy–linalool. However, ho-trienol only decreased when encapsulated yeasts were used, whereas an increase was observed in sparkling wines produced by yeast in free forms (Free and Free EtOH-A), regardless of the ethanol adaptation.

Although the total amount of norisoprenoids remained almost unchanged after the secondary fermentation and the 6 month ageing period, 1,1,6-Trimethyl-1,2-dihydronaphthalene (TDN, well-known as petroleum-like aroma of aged Riesling wines) slightly increased in the aged sparkling wines and did not come near the odor perception threshold (20 µg/L, Sacks et al., 2012).

3.3.2.2. Aldehydes and ketones. An increase in the amount of total aldehydes and ketones was revealed in the four sparkling wines (Free, Free EtOH-A, Encapsulated, Encapsulated EtOH-A) compared to the base wine, after the secondary fermentation and the 6 month ageing period. A significantly higher level was found in Free and Free EtOH-A sparkling wines compared to the encapsulated ones. Data in Table 2, show that the rise in total aldehydes and ketones is mainly due to the increase of furan compounds (deriving from sugar degradation), responsible for the yeasty and toasty notes, and in particular for furfural and norfuraneol volatiles, which remains far from the odor perception threshold [14100 and 2000 µg/L, respectively (Sarrazin, Dubourdieu, & Darriet, 2007; Ferreira, Lopez, & Cacho, 2000)].

3.3.2.3. Esters. Esters are the major family of volatile compounds released during yeast autolysis (Alexandre and Guillox-Benatier, 2006), and characterized by low perception thresholds, thus strongly contributing to the fruity and floral-like aroma of sparkling wine.

After the secondary fermentation and the 6 month ageing period, the four sparkling wine profiles showed a higher content of total esters compared to the base wine, with an increase ranging from 12% to 24% in free and encapsulated samples, respectively (Table 2). Comparing the four sparkling wines, the encapsulated sample was the richest in total esters, due to the greatest increase in the amount of diethyl malate, ethyl lactate and diethyl succinate. Ethyl lactate and diethyl succinate were described as volatiles that increase in long aged sparkling wines (Torrens, Riu-Aumatell, Vichi, López-Tamames, & Buxaderas, 2010; Riu-Aumatell, Bosch-Fusté, López-Tamames, & Buxaderas, 2006). Their formation could be enhanced by using lees during the ageing period (Bautista, Fernández, & Falqué, 2007).

Moreover, all sparkling wines showed a decrease in the concentration of some esters (e.g. hexyl acetate and ethylphenyl acetate) after the secondary fermentation and the 6 month ageing period as compared to the base wine, with no significant differences among the four samples. According to the literature, these compounds decrease during cava production (Spanish sparkling wine), thus causing the loss of the fresh and fruity aroma (Torrens et al., 2010; Riu-Aumatell et al., 2006).

3.3.2.4. Alcohols. A slight decrease in total alcohols was observed in the four sparkling wines after the secondary fermentation and the 6 month ageing period. The amount of total alcohols appeared significantly lower in sparkling wines produced by ethanol-adapted yeasts, both in free and encapsulated forms (−16% and −8% for Free EtOH-A and Encapsulated EtOH-A, respectively) (Table 2). Therefore, data reveal that the ethanol adaptation phase has a significant effect on the concentration of these volatiles, both for yeast in free and entrapped forms. Although in all sparkling wines, 2-phenylethanol (the most abundant alcohol, having a rose and honey-like aroma) exceeds the corresponding thresholds (14000 µg/L, Ferreira et al., 2000), its concentration is lower in Free EtOH-A and Encapsulated EtOH-A compared to non-adapted counterparts. In contrast, the concentration of its corresponding acetate ester (2-phenylethyl acetate) increased, remaining far from the odor perception threshold (250 µg/L, Francis & Newton, 2005). However, 3-(methylthio)-propanol or methionol (off-

Table 2
Concentrations of the quantified volatile compounds (μg/L of 1-heptanol) of Riesling base wine and sparkling wines obtained by EC1118 yeast in Free o Encapsulated form, adapted (EtOH-A) or non-adapted to ethanol. Different letters within a row denote significant differences among the means (one-way ANOVA α = 0.05, Tukey multiple comparisons test).

Compounds	Base wine	Sparkling wine			
		Free	Free EtOH-A	Encapsulated	Encapsulated EtOH-A
Terpenes					
Linalool	5.8 ± 0.3 a	4.2 ± 0.1b	4.1 ± 0.1b	3.2 ± 0.2c	4.2 ± 0.2b
Ho-trienol	8.7 ± 0.3b	10.8 ± 0.3 a	10.4 ± 0.1 a	5.7 ± 0.1 d	7.0 ± 0.3c
α-terpineol	11.0 ± 0.4 ab	11.1 ± 0.2 ab	11.3 ± 0.3 a	10.5 ± 0.2b	10.6 ± 0.1 ab
Citronellol	1.0 ± 0.2 a	1.4 ± 0.4 a	1.0 ± 0.0 a	1.0 ± 0.1 a	1.0 ± 0.1 a
Nerol	< 1 a	< 1 a	< 1 a	< 1 a	< 1 a
Geraniol	1.7 ± 0.1 a	1.0 ± 0.1b	1.0 ± 0.2b	1.0 ± 0.0b	1.0 ± 0.3b
trans-furan linalool oxide	53.7 ± 1.8 a	5.9 ± 0.5 bc	3.9 ± 0.9c	7.4 ± 0.3b	5.4 ± 0.1 bc
cis-furan linalool oxide	5.4 ± 0.1 a	3.9 ± 0.4 d	4.3 ± 0.1 cd	4.6 ± 0.3 bc	5.0 ± 0.3 ab
trans-pyran linalool oxide	4.2 ± 0.2 a	4.8 ± 0.5 a	4.5 ± 0.3 a	4.7 ± 0.4 a	4.1 ± 0.4 a
cis- pyran linalool oxide	3.0 ± 0.20 a	2.5 ± 0.5 a	2.7 ± 0.3 a	2.9 ± 0.4 a	2.9 ± 0.3 a
Diendiol 1	56.6 ± 2.3 a	53.7 ± 1.2 a	54.7 ± 0.9 a	53.2 ± 2.0 a	53.9 ± 1.4 a
Diendiol 2	1.9 ± 0.1 a	1.7 ± 0.1 a	1.9 ± 0.1 a	1.8 ± 0.2 a	1.4 ± 0.1b
Endiol	30.7 ± 1.1 a	20.6 ± 1.7 b	22.3 ± 0.8b	22.1 ± 1.0b	22.9 ± 1.5b
Cis-rose oxide	< 1 a	< 1 a	< 1 a	< 1 a	< 1 a
Trans-rose oxide	< 1 a	< 1 a	< 1 a	< 1 a	< 1 a
trans-8-hydroxy-linalool	< 1 a	< 1 a	< 1 a	< 1 a	< 1 a
cis-8-hydroxy-linalool	18.2 ± 0.8 a	1 ± 0.1c	4.3 ± 0.3b	3.8 ± 0.1b	1 ± 0.1c
Terpinen-4-ol	< 1 a	< 1 a	< 1 a	< 1 a	< 1 a
p-Cymene	< 1 a	< 1 a	< 1 a	< 1 a	< 1 a
Terpinolene	< 1 a	< 1 a	< 1 a	< 1 a	< 1 a
Total	**209.2 ± 8.0 a**	**129.9 ± 2.4b**	**133.8 ± 2.4b**	**128.9 ± 0.6b**	**127.6 ± 0.7b**
C_{13}- Norisoprenoids					
β-damascenone	2.4 ± 0.1 a	2.2 ± 0.0b	2.2 ± 0.0b	1.8 ± 0.1c	2.5 ± 0.0 a
a-ionone	< 1 a	< 1 a	< 1 a	< 1 a	< 1 a
β-ionone	< 1 a	< 1 a	< 1 a	< 1 a	< 1 a
TDN	0.9 ± 0.0b	1.2 ± 0.0 a	1.2 ± 0.0 a	1.2 ± 0.0 a	1.1 ± 0.1b
Etossi TDN	< 1 a	< 1 a	< 1 a	< 1 a	< 1 a
Vitispiran 1	2.7 ± 0.1 a	2.8 ± 0.1 a	2.6 ± 0.1 a	1.9 ± 0.2c	2.3 ± 0.1b
Vitispiran 2	1.6 ± 0.0 a	1.5 ± 0.1 a	1.2 ± 0.1b	1.0 ± 0.0c	1.0 ± 0.1c
Etossiactinidolo 1	< 1 a	< 1 a	< 1 a	< 1 a	< 1 a
Etossiactinidolo 2	< 1 a	< 1 a	< 1 a	< 1 a	< 1 a
Actinidol 1 (*cis*)	5.1 ± 0.1b	5.0 ± 0.1b	5.5 ± 0.2 a	5.0 ± 0.1b	4.8 ± 0.1b
Actinidol 2 (trans)	9.1 ± 0.4 a	8.9 ± 0.1 a	8.6 ± 0.1 a	9.0 ± 0.3 a	8.9 ± 0.3 a
3-oxo-α-ionol	13.5 ± 0.4b	15.0 ± 0.4 a	14.5 ± 0.4 a	14.5 ± 0.1 a	15.0 ± 0.4 a
Total	**40.4 ± 1.4 a**	**41.9 ± 1.1 a**	**41.2 ± 0.6 a**	**39.4 ± 1.4 a**	**40.5 ± 1.5 a**
Aldehydes and ketones					
Furfural	3.1 ± 0.1 d	22.1 ± 0.8b	24.5 ± 1.0 a	16.3 ± 0.3c	16.4 ± 0.5c
Syringaldehyde	2.0 ± 0.1c	2.3 ± 0.0b	1.5 ± 0.0 d	2.0 ± 0.1c	3.8 ± 0.1 a
5-Methylfurfural	0.9 ± 0.1a	1.0 ± 0.1 a	1.1 ± 0.1 a	1.0 ± 0.1 a	0.9 ± 0.1 a
Furaneol	1.6 ± 0.0c	2.4 ± 0.1 a	2.3 ± 0.1 ab	1.5 ± 0.1c	2.0 ± 0.1b
Homofuraneol	5.9 ± 0.2c	6.9 ± 0.2 ab	7.2 ± 0.2 a	3.6 ± 0.1 d	6.5 ± 0.1b
Norfuraneol	11.0 ± 0.4 e	58.3 ± 1.4b	70.5 ± 1.5 a	36.4 ± 1.4 d	49.1 ± 0.9c
Vanillin	4.8 ± 0.1 a	3.1 ± 0.2 d	3.5 ± 0.1c	5.0 ± 0.2 a	4.1 ± 0.1b
Phenylacetaldehyde	1.6 ± 0.1 a	1.0 ± 0.0b	1.1 ± 0.1b	1.6 ± 0.1 a	1.4 ± 0.1 a
Benzaldehyde	2.7 ± 0.1 d	3.0 ± 0.1 d	3.5 ± 0.1c	4.4 ± 0.1b	7.8 ± 0.3 a
Total	**33.7 ± 1.2 e**	**100.2 ± 2.8b**	**115 ± 2.7 a**	**71.9 ± 2.2 d**	**92.2 ± 0.4c**
Esters					
Hexyl acetate	142.2 ± 4.0a	15.9 ± 0.4b	16.9 ± 0.6b	16.8 ± 0.2b	16.2 ± 0.5b
Isoamyl acetate	232.1 ± 4.7c	320.5 ± 8.8b	355.5 ± 9.3a	311.8 ± 5.8b	315.5 ± 5.0b
2-phenylethyl acetate	38.9 ± 2.3c	68.3 ± 1.2ab	69.4 ± 2.5a	62.7 ± 1.6b	63.0 ± 2.6ab
Ethylphenyl acetate	7.9 ± 0.5a	2.6 ± 0.0b	2.4 ± 0.1b	2.6 ± 0.0b	2.3 ± 0.1b
Ethyl butanoate	163.6 ± 4.9b	196.8 ± 6.2a	194.3 ± 3.3a	164 ± 3.0b	193.1 ± 7.6a
Ethyl hexanoate	809.8 ± 12.8b	895.2 ± 11.2a	890.6 ± 13.0a	881.9 ± 7.8a	830.3 ± 14.6b
Ethyl octanoate	909.3 ± 30.2ab	986.8 ± 17.8a	916.5 ± 31.5ab	881.3 ± 16.0b	843 ± 38.5b
Ethyl decanoate	258.4 ± 11.1a	136.6 ± 5.0b	126.3 ± 4.5bc	114.3 ± 4.0c	121.8 ± 4.3bc
Ethyl 9-decenoate	3.9 ± 0.2c	6.4 ± 0.1a	4.6 ± 0.1b	4.5 ± 0.1b	3.4 ± 0.1d
Ethyl 3-hydroxybutanoate	163.8 ± 6.2a	159.3 ± 3.0a	160.6 ± 3.8a	170.8 ± 5.1a	155.9 ± 5.9a
Ethyl 4-hydroxybutanoate	674.6 ± 23.0a	509.0 ± 9.9bc	459.0 ± 11.3c	541.7 ± 14.6b	471.3 ± 14.2cd
Ethyl 2-hydroxyvalerate	5.7 ± 0.1a	4.3 ± 0.2b	3.9 ± 0.1bc	3.4 ± 0.1d	3.5 ± 0.1 cd
Ethyl 2-hydroxy-4-methylpentanoate	42.5 ± 1.5a	29.2 ± 0.6b	28.9 ± 1.2b	29.0 ± 1.2b	26.7 ± 1.6b
Diethyl succinate	2116 ± 64.0c	2519.6 ± 59.7ab	2647.5 ± 47.7ab	2699.6 ± 42.7a	2487 ± 95.3b
Ethyl lactate	2803.6 ± 97.0e	3073.3 ± 51.8d	3322.3 ± 70.9c	3963.1 ± 53.1a	3655.3 ± 105.0b
Isoamyl lactate	12.7 ± 0.7c	16.2 ± 0.6a	15.1 ± 0.4ab	15.2 ± 0.7ab	13.3 ± 0.9bc
Ethyl 3-methylbutyl succinate	< 1 a	< 1 a	< 1 a	< 1 a	< 1 a
Diethyl malate	5130.2 ± 125.0c	5431.3 ± 50.4c	5767.1 ± 47.4b	6306 ± 103.9a	5889.8 ± 155.0b
Diethyl 2-hydroxyglutarate	272.3 ± 7.7b	382.8 ± 9.1a	412.3 ± 15.8a	411.3 ± 12.2a	392.0 ± 8.5a
Methyl vanillate	13.0 ± 0.6a	12.9 ± 0.4a	13.1 ± 0.6a	13.0 ± 0.5a	11.5 ± 0.6a
Ethyl vanillate	1.2 ± 0.1'	< 1b	1.2 ± 0.0a	1.3 ± 0.0a	< 1b

(continued on next page

Table 2 (*continued*)

Compounds	Base wine	Sparkling wine			
		Free	Free EtOH-A	Encapsulated	Encapsulated EtOH-A
Ethyl cinnamate	12.7 ± 0.7*	< 1b	< 1b	< 1b	< 1b
Ethyl dihydrocinnamate	< 1 a	< 1 a	< 1 a	< 1 a	< 1 a
Ethyl salicylate	< 1 a	< 1 a	< 1 a	< 1 a	< 1 a
Ethyl pyroglutamate	1463.9 ± 45.3c	2374.4 ± 52.6b	2755.0 ± 70.7 a	2456.3 ± 110.1b	2480 ± 80.0b
Total	**15282 ± 442.3 d**	**17147 ± 65c**	**18167 ± 64b**	**19055 ± 268 a**	**17981 ± 14b**
Alcohols					
1-Hexanol	203.6 ± 9.3a	209.4 ± 6.6a	209.7 ± 6.2a	220 ± 10.6a	207.9 ± 12.8a
trans-3-Hexen-1-ol	9.9 ± 0.4c	10.9 ± 0.2ab	10.8 ± 0.1abc	11.4 ± 0.3a	10.4 ± 0.5bc
cis-3-Hexen-1-ol	15.9 ± 0.5a	14.8 ± 0.4ab	15.9 ± 0.6a	13.1 ± 0.8b	15.1 ± 0.8ab
2-Hexen-1-ol	1.9 ± 0.0b	2.4 ± 0.1a	2.3 ± 0.1a	1.4 ± 0.1c	2.4 ± 0.1a
Benzyl alcohol	22.5 ± 0.5b	26.6 ± 0.6a	25.1 ± 0.6ab	25.7 ± 0.6a	22.4 ± 1.5a
2-phenylethanol	19200 ± 700.0a	17900 ± 636.4ab	14990 ± 495.0c	18300 ± 353.6ab	16900 ± 600.0b
3-(methylthio)- propanol or Methionol	105.5 ± 4.6c	163.8 ± 4.1ab	172.9 ± 5.0a	162.4 ± 1.8ab	153.7 ± 6.2b
Furfuryl alcohol	25.1 ± 1.0a	16.1 ± 0.4d	15 ± 0.4d	21.8 ± 0.5b	19 ± 1.0c
Homovanillyl alcohol	13.1 ± 0.4a	11.9 ± 0.6ab	10.4 ± 0.4c	11.2 ± 0.1bc	11.2 ± 0.3bc
Vanillic alcohol	2.8 ± 0.1a	2.3 ± 0.1b	2.1 ± 0.0c	2.9 ± 0.1a	2.4 ± 0.0b
1-Octen-3-ol	11.8 ± 0.6a	< 1b	< 1b	< 1b	< 1b
Total	**19612 ± 692 a**	**18358 ± 862 ab**	**15455 ± 698c**	**18771 ± 480 ab**	**17346 ± 604b**
Acids					
Butyric acid	886.6 ± 35.0a	714.6 ± 25.0b	782.7 ± 30.2b	772.3 ± 19.6b	790.8 ± 40.0ab
3-Methylbutanoic acid (Isovaleric acid)	132.3 ± 4.0a	130.5 ± 3.2a	135.7 ± 3.0a	131.1 ± 4.9a	129.2 ± 5.5a
Hexanoic acid	3966.5 ± 180.0a	3299.4 ± 92.3c	3516.5 ± 82.4bc	3814.9 ± 60.2ab	3686.6 ± 108.5ab
Octanoic acid	6423.3 ± 194.5a	5456.7 ± 101.3b	5824.2 ± 95.3b	6296.2 ± 89.2a	6249.9 ± 129.4a
Decanoic acid	1087.7 ± 13.0b	998.4 ± 8.2c	1108.5 ± 15.2b	1231.1 ± 13.5a	1249.9 ± 10.5a
Homovanillic acid	5.1 ± 0.3a	5.1 ± 0.2a	4.9 ± 0.1a	5.4 ± 0.1a	5.5 ± 0.3a
Total	**12502 ± 348 a**	**10605 ± 325c**	**11373 ± 17b**	**12251 ± 26 a**	**12112 ± 66 a**
Phenols					
4-ethylphenol	2.4 ± 0.1e	5.8 ± 0.1b	3.3 ± 0.1d	4.9 ± 0.1c	7.7 ± 0.3a
4-ethylguaiacol	< 1 a	< 1a	< 1 a	< 1 a	< 1 a
4-vinylphenol	11.2 ± 0.4d	31.3 ± 0.8a	24 ± 0.7b	11.8 ± 0.6 cd	13.8 ± 0.3c
4-vinylguaiacol	41.2 ± 1.8c	65.3 ± 2.5a	62.6 ± 1.8a	43.2 ± 1.6c	50.1 ± 2.1b
Eugenol	1.3 ± 0.1a	1.1 ± 0.1b	1.1 ± 0.0b	1.3 ± 0.1a	< 1b
Guaiacol	< 1 a	< 1 a	< 1 a	< 1 a	< 1 a
o-cresol	< 1 a	< 1 a	< 1 a	< 1 a	< 1 a
p-cresol	< 1 a	< 1 a	< 1 a	< 1 a	< 1 a
Vanillin	4.8 ± 0.1a	3.1 ± 0.1d	3.5 ± 0.0c	5 ± 0.1a	4.1 ± 0.1b
Acetovanillone	11.3 ± 0.3b	12.1 ± 0.3a	12 ± 0.1a	12.2 ± 0.0a	11.2 ± 0.1b
Phenol	1.6 ± 0.1c	2.7 ± 0.1b	3 ± 0.1ab	2.7 ± 0.0b	3.3 ± 0.2a
Total	**78 ± 2e**	**126 ± 1a**	**114 ± 1b**	**85 ± 1d**	**96 ± 3c**
Lactones					
cis-Wisky lactone	2.2 ± 0.0c	2.4 ± 0.0bc	2.6 ± 0.1ab	2.2 ± 0.0c	2.7 ± 0.1a
trans-Wisky lactone	3.7 ± 0.1b	3.9 ± 0.1b	5 ± 0.2a	5.2 ± 0.1a	4.9 ± 0.1a
γ-nonalactone	2.7 ± 0.2a	1.3 ± 0.0c	1.5 ± 0.0c	1.8 ± 0.1b	1.3 ± 0.0c
γ-decalactone	623.9 ± 23.0a	< 1b	< 1b	< 1b	1 ± 0.1b
γ-Butyrolactone	1096.9 ± 47.0b	1226.9 ± 38.5ab	1204.2 ± 45.0ab	1258.5 ± 42.0a	1176.4 ± 53.5ab
4-carboxyethoxy-butyrolactone	440 ± 9.5c	559.5 ± 14.1a	577.5 ± 12.9a	565.5 ± 8.0a	522.5 ± 5.3b
Total	**2170 ± 14 a**	**1797 ± 24 bc**	**1793 ± 32 bc**	**1834 ± 34b**	**1708 ± 84c**
Others					
N-(3-Methylbutyl)acetamide	16.6 ± 0.4c	17.5 ± 0.4 bc	18.8 ± 0.4 a	17.9 ± 0.3 ab	18.9 ± 0.3 a

flavour associated with the perception of cauliflower and cooked cabbage) was the only alcohol revealed at a higher amount in all sparkling wines compared to the base wine, remaining far from the odor perception threshold (1000 μg/L; Guth, 1997). The lowest methionol concentration was found in the encapsulated EtOH-A sample.

3.3.2.5. Acids. These volatiles, predominantly fermentation products, are generally recognized as unpleasant odors (being described as ranging from sweaty and cheesy to goaty and rancid), nevertheless, some volatile fatty acids could improve the complexity of wine aroma (Swiegers & Pretorius, 2005).

After the secondary fermentation and the 6 month ageing period, the total amount of acids was significantly lower in free and in free EtOH-A samples as compared to sparkling wines produced by encapsulated forms (Encapsulated and Encapsulated EtOH-A), thus suggesting that total acid concentration was considerably influenced by yeast-inoculating formats. In particular, data in Table 2 show that the amount of hexanoic, octanoic and decanoic acid (having rancid fat and cheese-like aroma) is significantly lower in free and in free EtOH-A samples.

3.3.2.6. Phenols and lactones. The total level of volatile phenols, recognized as off-flavours, was greater in the four sparkling wines compared to the base wine, with the lowest content in encapsulated and encapsulated EtOH-A samples. In particular, 4-vinylphenol and 4-vinylguaicol were significantly less abundant in these two latter sparkling wines.

Moreover, total lactones, which are desirable compounds in wines (floral and fruity-like odor), significantly decreased in all sparkling wines due to the relevant reduction of γ-decalactone after the secondary fermentation and the following 6 month ageing period.

Fig. 3. Biplot of the principal components analysis (PC 1 vs. PC 2) of volatile compounds in Riesling sparkling wines obtained by EC1118 yeast in Free or Encapsulated form, adapted (EtOH-A) or non-adapted to ethanol.

3.3.3. Principal component analysis

Aiming to classify the sparkling wines produced with free or encapsulated yeast forms, adapted or non-adapted to ethanol, a principal component analysis (PCA) was performed considering as classifying variables all volatile aroma compounds with a concentration above 1 μg/L. The results of the PCA are shown as bi-plot score loading (Fig. 3). The first two principal components accounted for 76% of the total variance of samples, PC1 explains 42% of the variance, whereas the PC2 explains 34%. PCA showed the potential to discriminate between Riesling sparkling wines produced. The first component, mainly influenced by acids, fatty acid ethyl esters and phenols, differentiates among the sparkling wine produced with free or encapsulated yeast forms. The second principal component differentiates among Encapsulated and Encapsulated EtOH-A. Instead, no differences can be observed among the sparkling wine produced with free yeasts adapted or non-adapted to ethanol, where the aromatic profile is relatively close.

While Free and Free EtOH-A are both characterized by terpenols (Ho-trienol and α-terpineolo), aldehydes and ketones (furaneol and homofuranol), phenols (4-vinylphenol and 4-vinylguaicol), the aromatic profile changed when the same EC1118 yeast is used in both encapsulated forms.

Instead, these latter samples grouped with high levels of some acids (hexanoic, octanoic, decanoic acid) and esters (e.g. ethyl lactate, diethyl malate).

3.3.4. Sensory profile

Sensory analysis of four sparkling wines, produced by free or encapsulated yeast, both adapted and non-adapted to ethanol was performed by evaluating the global organoleptic quality through quantitative descriptive analysis, as reported in the radar plot (Fig. 4). The analysis of variance of the sensory data highlighted no differences among the sparkling wines concerning the visual appearance with the only exception of the encapsulated sample, which was characterized by a higher yellow colour intensity score, a lower limpidity, and an overall bad judgment. The delayed and slow secondary fermentation, carried out by the encapsulated EC1118 non adapted to ethanol. In contrast, the application of Encapsulated EtOH-A allowed us to produce a sparkling wine having the same scores in terms of aroma, taste and body, as compared to those produced by free yeasts (both adapted and non-adapted to ethanol). Moreover, during the visual analysis of the foam characteristic of each sparkling wine (Table S2), the judges described the foam as abundant and covering the full surface in every sample with the only exception of the sparkling wine produced with yeast non adapted to ethanol where foam was little and only partly covering the surface of the wine. A total foam collar was observed in all samples except for the one produced with the encapsulated form. Nevertheless, the bubbles were small and effervescence was normal in all sparkling wines.

4. Conclusion

The yeast strain *S. cerevisiae bayanus* Lalvin EC-1118 entrapped into chitosan-calcium alginate double layer microcapsules was successfully used for the production of Riesling sparkling wine by the traditional method. A stepwise adaptation to ethanol of yeast before encapsulation was developed for the first time and this phase resulted crucial to enhance yeast metabolism. EC1118 free and encapsulated, both adapted and non-adapted to ethanol, successfully completed the secondary fermentation which reached about 6 bars in the bottle, but followed different kinetics of oxygen consumption and pressure increase during the *prise de mousse*.

Comparing volatile compounds of the produced sparkling wines after a 6 month ageing period, a significantly higher level of total aldehydes, phenols, ketones (due to the increase of furan compounds), as well as a significantly lower amount of acids was found in samples inoculated with free yeast (adapted or non-adapted to ethanol) compared to the encapsulated ones. Moreover, it was evident that the ethanol adaptation phase has a significant effect on the concentration of total alcohols, which was significantly lower in sparkling wines produced by ethanol-adapted yeasts, both in free and entrapped forms.

Finally, sensory analysis revealed that the application of encapsulated yeast adapted to ethanol allowed us to produce a sparkling wine having sensory properties similar to those produced by free yeasts (both adapted and non-adapted to ethanol) in terms of aroma, taste and body.

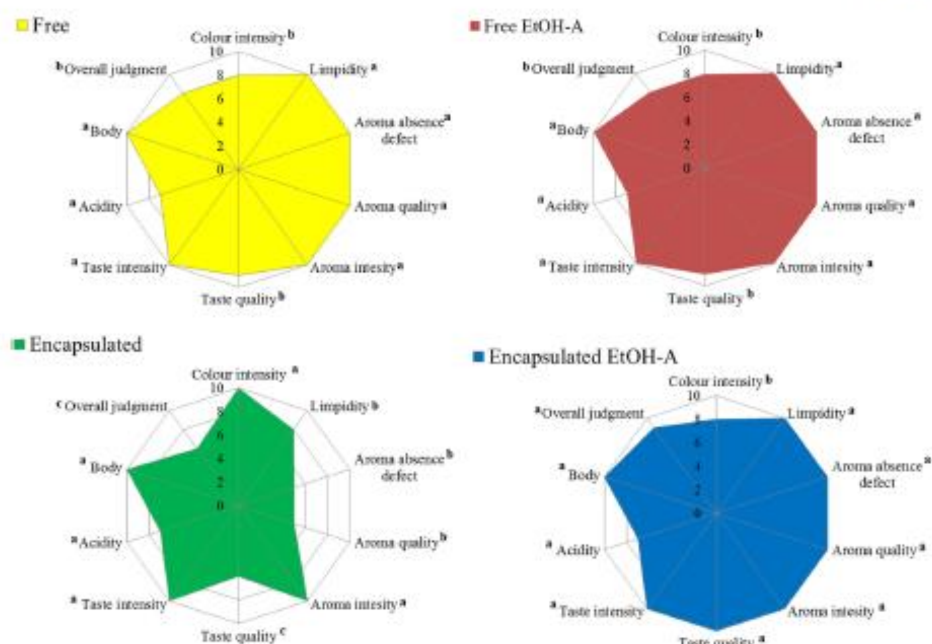

Fig. 4. Radar plot of sensory descriptors, obtained by the mean of the scores given by the panellists, of the different sparkling wines made with *S. cerevisiae* strain EC1118 [Yellow: Free form non-adapted to ethanol (Free); Red: Free form adapted to ethanol (Free EtOH-A); Green: Encapsulated form non-adapted to ethanol (Encapsulated); Blue: encapsulated form adapted to ethanol (Encapsulated EtOH-A)]. For each descriptor, different letters indicate significant differences (one-way ANOVA, $\alpha = 0.05$, Tukey multiple comparisons test). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2019.125174.

References

Alexandre, H., & Guilloux-Benatier, M. (2006). Yeast autolysis in sparkling wine–a review. *Australian Journal of Grape and Wine Research, 12*, 119–127.

Bautista, R., Fernández, E., & Falqué, E. (2007). Effect of the contact with fermentation-lees or commercial-lees on the volatile composition of white wines. *European Food Research and Technology, 224*, 405–413.

Benucci, I., Liburdi, K., Cerreti, M., & Esti, M. (2016). Characterization of active dry wine yeast during starter culture (pied de cuve) preparation for sparkling wine production. *Journal of Food Science, 81*, M2015–M2020.

Benucci, I., Luziatelli, F., Cerreti, M., Liburdi, K., Nardi, T., Vagnoli, P., ... Esti, M. (2018). Pre-fermentative cold maceration in the presence of non-Saccharomyces strains: Effect on fermentation behaviour and volatile composition of a red wine. *Australian Journal of Grape and Wine Research, 24*, 267–274.

Bozdogan, A., & Canbas, A. (2011). Influence of yeast strain, immobilization and ageing time on the changes of free amino acids and amino acids in peptides in bottle-fermented sparkling wines obtained from Vitis vinifera cv. Emir. *International Journal of Food Science & Technology, 46*, 1113–1121.

D'Amato, D., Corbo, M. R., Nobile, M. A. D., & Sinigaglia, M. (2006). Effects of temperature, ammonium and glucose concentrations on yeast growth in a model wine system. *International Journal of Food Science & Technology, 41*, 1152–1157.

De Lerma, N. L., Peinado, R. A., Puig-Pujol, A., Mauricio, J. C., Moreno, J., & García-Martínez, T. (2018). Influence of two yeast strains in free, bioimmobilized or immobilized with alginate forms on the aromatic profile of long aged sparkling wines. *Food Chemistry, 250*, 22–29.

De Prisco, A., Maresca, D., Ongeng, D., & Mauriello, G. (2015). Microencapsulation by vibrating technology of the probiotic strain *Lactobacillus reuteri* DSM 17938 to enhance its survival in foods and in gastrointestinal environment. *LWT-Food Science and Technology, 61*, 452–462.

Dinh, T. N., Nagahisa, K., Hirasawa, T., Furusawa, C., & Shimizu, H. (2008). Adaptation of *Saccharomyces cerevisiae* cells to high ethanol concentration and changes in fatty acid composition of membrane and cell size. *PLoS ONE, 3*, e2623.

Divies, C., Cachon, R., Cavin, J. F., & Prevost, H. (1994). Theme-4- Immobilized cell technology in wine production. *Critical Reviews in Biotechnology, 14*, 135–153.

Ferreira, V., Lopez, R., & Cacho, J. F. (2000). Quantitative determination of the odorants of young red wines from different grape varieties. *Journal of the Science of Food and Agriculture, 80*, 1659–1667.

Fornairon-Bonnefond, C., Demaretz, V., Rosenfeld, E., & Salmon, J. M. (2002). Oxygen addition and sterol synthesis in *Saccharomyces cerevisiae* during enological fermentation. *Journal of Bioscience and Bioengineering, 93*, 176–182.

Francis, I. L., & Newton, J. L. (2005). Determining wine aroma from compositional data. *Australian Journal of Grape and Wine Research, 11*, 114–126.

George, M., & Abraham, T. M. (2006). Polyionic hydrocolloids for the intestinal delivery of protein drugs: Alginate and chitosan — a review. *Journal of Controlled Release, 114*, 1–14.

Guth, H. (1997). Quantification and sensory studies of character impact odorants of different white wines varieties. *Journal of Agricultural and Food Chemistry, 45*, 3027–3032.

Haque, T., Chen, H., Ouyang, W., Metz, T., Lawuyi, B., & Prakash, S. (2018). Effect of integrating polyethylene glycol to alginate-poly-l-lysine and alginate chitosan microcapsules for oral delivery of live cells and cell transplant for therapy. *CMBES Proceedings, 28*.

Hidalgo, P., Pueyo, E., Pozo-Bayón, M. A., Martínez-Rodríguez, A. J., Martín-Álvarez, P., & Polo, M. C. (2004). Sensory and analytical study of rosé sparkling wines manufactured by second fermentation in the bottle. *Journal of Agricultural and Food Chemistry, 52*, 6640–6645.

ISO (1977). Sensory analysis. Apparatus. Wine-tasting glass, ISO 3591: 1977, Group B, p. 3.

Kavitakea, D., Kandasamya, D., Devib, P. B., & Shettya, P. H. (2018). Recent developments on encapsulation of lactic acid bacteria as potential starter culture in fermented foods – A review. *Food Bioscience, 21*, 34–44.

Kemp, B., Alexandre, H., Robillard, B., & Marchal, R. (2015). Effect of production phase on bottle-fermented sparkling wine quality. *Journal of Agricultural and Food Chemistry, 63*, 19–38.

Kourkoutas, Y., Bekatorou, A., Banat, I. M., Marchant, R., & Koutinas, A. A. (2004). Immobilization technologies and support materials suitable in alcohol beverages production: A review. *Food microbiology, 21*, 377–397.

Liouni, M., Drichoutis, P., & Nerantzis, E. T. (2008). Studies of the mechanical properties and the fermentation behavior of double layer alginate-chitosan beads, using *Saccharomyces cerevisiae* entrapped cells. *World Journal of Microbiology and*

Biotechnology, 24, 281–288.

Maresca, D., De Prisco, A., La Storia, A., Cirillo, T., Esposito, F., & Mauriello, G. (2016). Microencapsulation of nisin in alginate beads by vibrating technology: Preliminary investigation. *LWT-Food Science and Technology, 66*, 436–443.

Martínez-García, R., García-Martínez, T., Puig-Pujol, A., Mauricio, J. C., & Moreno, J. (2017). Changes in sparkling wine aroma during the second fermentation under CO_2 pressure in sealed bottle. *Food Chemistry, 237*, 1030–1040.

Martí-Raga, M., Sancho, M., Guillamon, J. M., Mas, A., & Beltran, G. (2015). The effect of nitrogen' addition on the fermentative performance during sparkling wine production. *Food Research International, 67*, 126–135.

Onofri, A. (2006). Enhancing Excel capability to perform statistical analyses in agriculture applied research. *Computational Statistics and Data Analysis & Statistical Software Newsletters*.

O.I.V. Organisation Internationale de la Vigne et du Vin (2011). Compendium of international methods of wine and must analysis. Paris, France.

Pozo-Bayón, M.Á., Martinez-Rodriguez, A., Pueyo, E., & Moreno-Arribas, M. V. (2009). Chemical and biochemical features involved in sparkling wine production: From a traditional to an improved winemaking technology. *Trends In Food Science & Technology, 20*, 289–299.

Puig-Pujol, A., Bertran, E., García-Martínez, T., Capdevila, F., Mínguez, S., & Mauricio, J. C. (2013). Application of a new organic yeast immobilization method for sparkling wine production. *American Journal of Enology and Viticulture, 64*, 386–394.

Riu-Aumatell, M., Bosch-Fusté, J., López-Tamames, E., & Buxaderas, S. (2006). Development of volatile compounds of cava (Spanish sparkling wine) during long ageing time in contact with lees. *Food Chemistry, 95*, 237–242.

Sacks, G. L., Gates, M. J., Ferry, F. X., Lavin, E. H., Kurtz, A. J., & Acree, T. E. (2012). Sensory threshold of 1, 1, 6-trimethyl-1, 2-dihydronaphthalene (TDN) and concentrations in young Riesling and non-Riesling wines. *Journal of Agricultural and Food Chemistry, 60*, 2998–3004.

Sarrazin, E., Dubourdieu, D., & Darriet, P. (2007). Characterization of key-aroma compounds of botrytized wines, influence of grape botrytization. *Food Chemistry, 103*, 536–545.

Soares, R. D., Welke, J. E., Nicolli, K. P., Zanus, M., Caramão, E. B., Manfroi, V., & Zini, C. A. (2015). Monitoring the evolution of volatile compounds using gas chromatography during the stages of production of Moscatel sparkling wine. *Food Chemistry, 183*, 291–304.

Stanley, D., Bandara, A., Fraser, S., Chambers, P. J., & Stanley, G. A. (2010). The ethanol stress response and ethanol tolerance of Saccharomyces cerevisiae. *Journal of Applied Microbiology, 109*, 13–24.

Swiegers, J. H., & Pretorius, I. S. (2005). Yeast modulation of wine flavor. *Advances in Applied Microbiology, 57*, 131–175.

Torrens, J., Riu-Aumatell, M., Vichi, S., López-Tamames, E., & Buxaderas, S. (2010). Assessment of volatile and sensory profiles between base and sparkling wines. *Journal of Agricultural and Food Chemistry, 58*, 2455–2461.

Tronchoni, J., Gamero, A., Arroyo-López, F. N., Barrio, E., & Querol, A. (2009). Differences in the glucose and fructose consumption profiles in diverse *Saccharomyces* wine species and their hybrids during grape juice fermentation. *International Journal of Food Microbiology, 134*, 237–243.

Wen-Tao, Q., Wei-Ting, Y., Yu-Bing, X., & Xiaojun, M. (2005). Optimization of *Saccharomyces cerevisiae* culture in alginate–chitosan–alginate microcapsule. *Biochemical Engineering Journal, 25*, 151–157.

Wu, T., Huang, J., Jiang, Y., Hu, Y., Ye, X., Liu, D., & Chen, J. (2018). Formation of hydrogels based on chitosan/alginate for the delivery of lysozyme and their antibacterial activity. *Food Chemistry, 240*, 361–369.

You, K. M., Rosenfield, C. L., & Knipple, D. C. (2003). Ethanol tolerance in the yeast *Saccharomyces cerevisiae* is dependent on cellular oleic acid content. *Applied and Environmental Microbiology, 69*, 1499–1503.