Research article

Institute of Brewing & Distilling

Received: 1 May 2014 Revised: 14 July 2014 Accepted: 14 July 2014 Published online in Wiley Online Library: 29 September 2014

(wileyonlinelibrary.com) DOI 10.1002/jib.175

Mead production: fermentative performance of yeasts entrapped in different concentrations of alginate

A. P. Pereira,[1,2] A. Mendes-Ferreira,[1] L. M. Estevinho[2]* and A. Mendes-Faia[1]

Mead is an alcoholic drink known since ancient times, produced by yeast fermenting diluted honey. However, the production of mead has suffered in recent years, partially owing to the lack of scientific progress in this field. In this study, two strains of *Saccharomyces cerevisiae*, QA23 and ICVD47, were immobilized in 2 or 4% (w/v) alginate beads to assess the most effective alginate concentration for yeast immobilization to produce mead. Neither of the alginate concentrations was able to prevent cell leakage from the beads. The fermentation length was 120 h for both yeast strains. In all cases, at the end of the fermentation, the number of cells entrapped in the beads was higher than the number of free cells, and the total 4% alginate bead wet weight was significantly higher than the 2% alginate bead wet weight. In addition, the evaluation of mead quality showed that the yeast strain had significantly more influence on the physicochemical characteristics than the alginate concentration. Although the yeasts immobilized in the two alginate concentrations were able to perform the fermentation, further research is needed in order to understand the evolution of the yeast population inside the beads throughout the fermentative process. Copyright © 2014 The Institute of Brewing & Distilling

Keywords: alginate concentration; cell leakage; mead; yeast immobilization

Introduction

Mead has been produced since ancient times, mainly in an empirical and artisanal manner. This drink has been reported to contain many of the elements required by humans and to have excellent effects on digestion and metabolism. It has also been considered to be beneficial for people who suffer from chronic anaemia and diseases of the gastrointestinal tract *(1)*. Mead, which results from the fermentation of diluted honey, can have an alcoholic content that ranges from 8 to 18% (v/v). This is accomplished by varying the proportions of honey and water and the point at which the fermentation is stopped *(2)*. The fermentative process and maturation require an extended period in which several problems may occur. For instance, the anticipated alcohol content may not be achieved, a successive addition of honey may be needed to avoid the premature end of fermentation, and there is a high likelihood of stuck fermentations *(2)*. This is related to the specific properties of the honey solution, mainly the high sugar concentration, high acidity, low protein content, low indigenous microbiota and the shortage of substances essential for yeast development *(3)*.

Indeed, this complex fermentative process depends on several factors, such as the type of honey, yeast strain, honey-must composition and pH *(4)*. In the past few years, several studies on the optimization of mead production have been carried out, mainly regarding yeast selection and honey-must formulation *(3,5–7)*. However, it is worth noting that immobilized cells were used in just two of the studies involving mead production *(4,8)*.

The application of immobilized yeast cells for the production of alcoholic beverages has been extensively studied in the past few years. Cell immobilization has some advantages over free cells, such as high cell loads, high volumetric productivities, increased substrate uptake, protection from inhibitory substances and reuse of the same biocatalyst for extended periods of time *(9–13)*. One of the most common methods of immobilization is the entrapment of cells in hydrogels, which involves entrapping living cells within a rigid network, which permits the diffusion of substrates and products, thereby making possible cell growth and the maintenance of active cells *(14)*. Calcium alginate gels have been the most widely used matrices for cell entrapment owing to their simplicity *(15)*. Alginate is a natural co-polymer that is gelled when it comes into contact with bivalent cations such as Ca^{2+}, forming beads *(16)*. Despite its numerous advantages, some problems can occur in an alcoholic fermentation using yeast cells entrapped in Ca-alginate beads, the most common being cell leakage. This phenomenon results in destabilization and rupture of beads, mainly owing to cell growth, and gas formation and accumulation within the beads, as well as the presence of chelators in the medium *(16)*. The aim of the present study was to investigate the capacity of two sodium alginate concentrations, 2 and 4%, to immobilize *Saccharomyces cerevisiae* yeast strains QA23 and ICV D47, in the context of mead production. The cells were entrapped in the gels by a drop-

* Correspondence to: L. M. Estevinho, CIMO, Centro de Investigação de Montanha, Escola Superior Agrária, Instituto Politécnico de Bragança, Campus de Santa Apolónia – Apartado 1172, 5301–855 Bragança, Portugal. E-mail: leticia@ipb.pt

[1] IBB-Institute for Biotechnology and Bioengineering, Centre of Genomics and Biotechnology, Universidade de Trás-os-Montes e Alto Douro, Apartado 1013, 5001-801 Vila Real, Portugal

[2] CIMO, Centro de Investigação de Montanha, Escola Superior Agrária, Instituto Politécnico de Bragança, Campus de Santa Apolónia – Apartado 1172, 5301-855 Bragança, Portugal

forming procedure and with the goal of evaluating the most effective cell concentration.

Material and methods

Yeast strains

Active wine dry yeasts, *S. cerevisiae* Lalvin QA23 and Lalvin ICV D47 (Lallemand, Montreal, Canada), were used in this study.

Honey

A dark honey was purchased from a local beekeeper in the northwest of Portugal. A palynological analysis of the honey was performed according to the acetolytic method *(17)* and it was determined that this multifloral honey was derived primarily from the pollen of *Castanea* spp. (45%) and *Erica* spp. (32%). The characteristics and satisfactory quality of the honey were in agreement with the requirements established by Portuguese legislation (Decreto-Lei no. 214/2003, 18 September).

Preparation of honey-must for fermentation

The honey-must for fermentation was prepared as described by Pereira *et al*. *(7)*. Honey was diluted (to 37% w/v) using natural spring-water to obtain, at the end of fermentation, an alcoholic beverage of approximately 11% ethanol, with the solution mixed to homogeneity. Insoluble materials were removed from the mixture by centrifugation (2682 ***g*** for 30 min; Eppendorf 5810 R centrifuge) to obtain a clarified honey-must. Titratable acidity was adjusted with 5 g/L of potassium tartrate (Sigma-Aldrich, St Louis, MO, USA) and pH was adjusted to 3.7 with malic acid (Merck, Darmstadt, Germany). The nitrogen content was adjusted to 267 mg/L with diammonium phosphate (DAP, BDH Prolabo, Leuven, Belgium). The parameters °Brix, pH, titatrable acidity and assimilable nitrogen concentration were determined, prior to and after the adjustments. The honey-must was pasteurized at 65 °C for 10 min and then immediately cooled. No sulphur dioxide was added to the honey-musts.

Immobilization of yeast cells

The dry yeast was hydrated by dissolving 2 g of active dry yeast in 20 mL of sterilized water at 38 °C, according to the manufacturer's instructions, to obtain 10^8 colony forming units (CFU)/mL. Sodium alginate (BDH Prolabo, Leuven, Belgium) was dissolved in distilled water at concentrations of 2 and 4% (w/v). The calcium chloride (Panreac, Barcelona, Spain) solution was prepared with distilled water at a concentration of 180 mM. Sodium alginate and calcium chloride solutions were autoclaved at 121 °C for 15 min, and then were cooled. To inoculate the honey-must with 10^6 CFU/mL, the appropriate amount of yeast suspension was added to 10 mL of a sodium alginate solution. The polymer–cell mixture was added dropwise to the $CaCl_2$ solution and left to harden in this solution for 30 min at 4 °C. The *S. cerevisiae* immobilized beads were rinsed three times with sterile distilled water, and then transferred into the honey-must.

Fermentation conditions and monitoring

The immobilized beads were transferred into the honey-must for batch fermentation. All fermentations were carried out using a previously described system *(6)*, which consisted of 250 mL flasks filled to two-thirds of their volume and fitted with a side-arm port sealed with a rubber septum for anaerobic sampling. The flasks were maintained during alcoholic fermentation at 25 °C under continuous, but moderate shaking (120 rpm), mimicking an industrial environment. Aseptic sampling for monitoring the fermentation was performed using a syringe-type system as previously described *(18)*. Fermentations were monitored daily by weight loss as an estimate of CO_2 production. For determining the growth parameters of suspended cells in the medium, samples were collected and appropriately diluted for the measurement of their optical density at 640 nm in a UV–VIS spectrometer (Unicam Helios) and for counting CFU in solid Yeast Peptone Dextrose agar (YPD – 20 g/L glucose, 10 g/L peptone, 5 g/L yeast extract and 20 g/L agar) plates after incubation at 25 °C for 48 h. Determination of reducing sugars was performed using the 3,5-dinitrosalicylic acid method with glucose as the standard. At the end of the alcoholic fermentation, samples were taken from the fermented media for several analytical determinations.

Analyses performed at the end of fermentation

At the end of fermentation, the culture dry weight of the suspended cells in the medium was determined using triplicate samples of 14 mL centrifuged in pre-weighed tubes at 3890.1 ***g*** for 10 min, washed twice with sterile deionized water, dried for 24 h at 100 °C and stored in a desiccator before weighing. For determination of dry weight, determination of the concentration of viable cells immobilized in the beads and the immobilization yield at the end of fermentation, the beads were liquefied using a chemical method, according to a procedure adapted from Göksungur and Zorlu *(19)*. Fifty beads were washed with water, dissolved in 50 mL of a sterilized sodium citrate solution (50 mM), with continuous stirring for 30 min at room temperature. The dry weight of the immobilized cells was determined by the same procedure as described previously for suspended cells in a medium. For assessing the growth of immobilized cells, after appropriate dilutions of liquefied beads, these were counted as the number of CFU in solid YPD plates, after incubation at 25 °C for 48 h. The immobilization yield was calculated as the immobilized dry weight of yeasts/immobilized and free dry weight of yeasts × 100 *(15)*.

The oenological parameters, such as total sulphur dioxide (SO_2), pH, titratable acidity, volatile acidity and ethanol content, were determined according to standard methods *(20)*. Yeast assimilable nitrogen (YAN) was determined by the formaldehyde method as previously described *(21)*. After clarification, 10 mL of the sample was transferred into a 50 mL beaker and diluted with 15 mL of water. The pH was adjusted to 8.1 with 100 mM NaOH and 2.5 mL of formaldehyde at pH 8.1 was added. After 5 min, the pH was adjusted again to 8.1 by titration with 50 mM NaOH. Assimilable nitrogen was calculated using the following formula:

$$\mathrm{YAN(mg/L)} = [(\text{vol. NaOH}) \times (\text{conc. NaOH}) \times 14 \times 1000] / (\text{sample volume})$$

Statistical analysis

All of the fermentation experiments were performed in duplicate and the results are expressed as mean values and standard deviation. The data were analysed using SPSS Software, version 17.0 (SPSS Inc.). To test significant differences amongst the

physicochemical characteristics of meads and bead characteristics, a two-factor – alginate concentration (A) and strain (S) – analysis of variance (ANOVA) was applied. In order to compare the means between two unrelated groups (2 and 4% alginate) for each strain, an independent-samples *t*-test was performed. The fulfilment of the ANOVA requirements, namely the normal distribution of the residuals and the homogeneity of variance, was evaluated by means of the Shapiro–Wilks test ($n < 50$) and Levene's test, respectively. All statistical tests were performed at a 5% significance level.

Results and discussion

In this work the immobilization of *S. cerevisiae* yeast strains QA23 and ICV D47, using two alginate concentrations (2 and 4%), with a population corresponding to approximately 10^6 CFU/mL, was studied. The effectiveness of the immobilization was determined by counting the yeast cells released from the beads into the medium and by analysing the reducing sugar consumption profile (Fig. 1). Minor differences were detected in the number of CFU in the medium and in reducing sugars of the fermentations carried out with the cells immobilized in 2 or 4% Ca-alginate, using both strains. The strain ICV D47 immobilized in 4% of Ca-alginate showed a slightly higher sugar consumption until 72 h of fermentation. Nevertheless, all fermentations ended after 120 h with similar concentrations of residual sugars, ranging from 15.13 ± 0.49 to 19.89 ± 2.57 g/L, in meads fermented by the strains ICV D47 and QA23, respectively, and entrapped in 2% alginate beads. Similar concentrations of residual sugars and times of fermentation were obtained for mead production using free yeast cells *(6,7)*. These residual sugars include disaccharides such as sucrose, maltose, isomaltose, trisaccharides and tetrasaccharides *(7)*. The growth profile shows that, at the beginning of fermentation, the number of free cells in the medium was higher when yeast cells were entrapped in 2% than in 4% alginate. This difference was seen more clearly for strain QA23. For this strain, at the end of fermentation, the number of free cells in medium was 6.8×10^6 and 4.8×10^6 CFU/mL when immobilized in 2 and 4% Ca-alginate, respectively. For the strain ICV D47, minor differences were observed at the end of fermentation in the number of free cells in the medium for both alginate concentrations (1.3×10^7 CFU/mL). The strain, ICV D47, presented a higher number of free cells in medium. However for both strains, independent of the alginate concentration used, the number of cells in medium had increased to 10^6–10^7 CFU/mL in the first 48 h and then remained constant until the end of the fermentation. The evolution of CO_2, particularly in the first 48 h of fermentation, may cause an internal mechanical loading of the beads, leading to the disintegration of the majority of beads *(19)*. The increase in the cell population in the medium was exponential, resulting from the combined effects of cell leakage from the beads and the proliferation of free cells in the medium. Other authors have obtained similar results 30 h after cultivation, when a different entrapment agent (LentiKat® carrier) was applied *(12)*.

Yeast cell growth was confirmed by cellular dry weight values and it was verified that the dry weight of cells in the beads was higher than that of the free cells in medium, irrespective of the concentration of Ca-alginate and the yeast strain used (Fig. 2). This observation corroborates previous results obtained with *S. cerevisiae* encapsulated in polyvinyl alcohol particles for a beer fermentation *(12)*, in which a higher final cell concentration in the LentiKats® carrier than in suspended cells (4×10^8 vs 3×10^7 cells/mL of carrier) was observed.

At the end of the fermentation, several growth parameters were determined after dissolution of the beads in a sodium citrate solution and these are presented in Table 1. The total wet weight of the 4% Ca-alginate beads was significantly higher

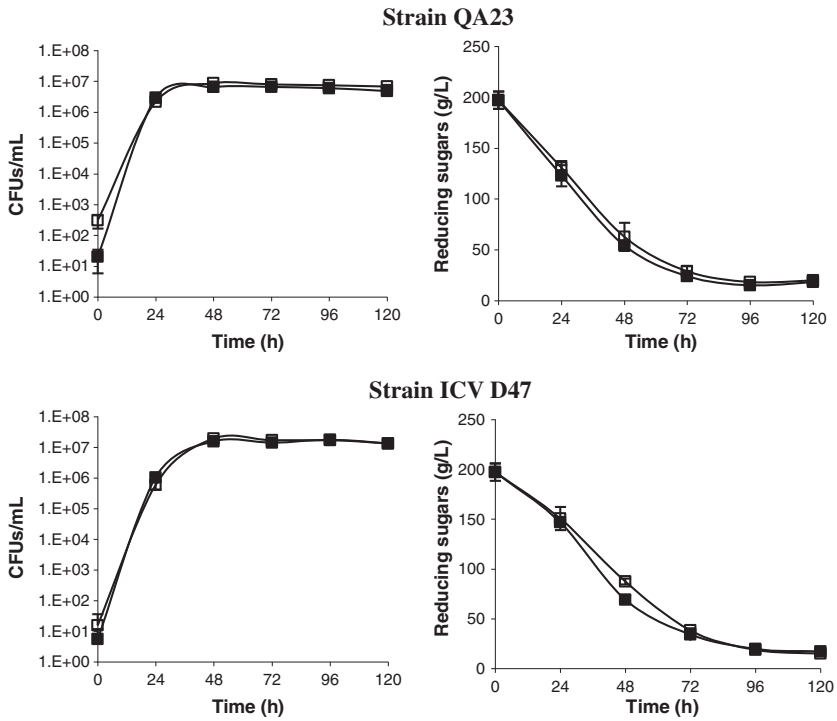

Figure 1. Growth of the free cells in medium and reduced sugar consumption by *Saccharomyces cerevisiae* QA23 and ICV D47 immobilized cells in 2% (□) and 4% (■) alginate.

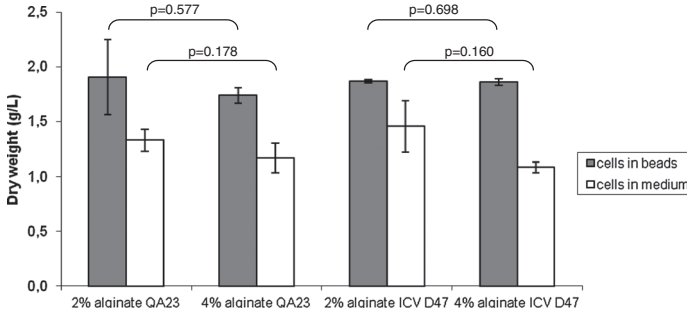

Figure 2. Cell dry weight of *S. cerevisiae* QA23 and ICV D47, at the end of fermentation, suspended in the medium (□) and inside the beads (■).

Table 1. Total beads wet weight, colony forming units (CFU) and immobilization yield of *Saccharomyces cerevisiae* QA23 and ICV D47 immobilized cells in 2 or 4% alginate

Meads	Strain QA23		Strain ICV D47		Significance		
	2% Alginate	4% Alginate	2% Alginate	4% Alginate	Alginate (A)	Strain (S)	A × S
Total bead wet weight (g)	8.36 ± 0.25*	10.15 ± 0.09*	7.65 ± 0.33*	11.10 ± 0.52*	<0.001	n.s.	0.025
CFU/mL of alginate	$1.40 \pm 0.61 \times 10^8$	$9.89 \pm 1.64 \times 10^7$	$1.82 \pm 0.43 \times 10^8$	$1.88 \pm 0.07 \times 10^8$	n.s.	n.s.	n.s.
Immobilization yield (%)	58.73 ± 6.15	59.82 ± 3.79	56.43 ± 4.12	63.24 ± 1.45	n.s.	n.s.	n.s.

* Significant difference between the alginate concentrations for each strain ($p < 0.05$); lack of superscript indicates no significant difference. n.s., No significant difference at $p < 0.05$.

when compared with the 2% Ca-alginate beads ($p = 0.011$ for QA23 and $p = 0.015$ for ICV D47). Significant differences were found in the total bead wet weight between the alginate concentrations ($p < 0.001$). No significant differences were detected in the number of CFU/mL between the strains or the alginate concentrations, with the lower value of $9.89 \pm 1.64 \times 10^7$ for mead produced using 2% alginate QA23 beads and the higher value of $1.88 \pm 0.07 \times 10^8$, corresponding to the mead produced using 4% alginate ICV D47 beads. Regarding the immobilization yield, although the values obtained with 4% alginate were higher for both strains, the differences between the two concentrations of alginate were not significant.

The quality of the meads produced using strains QA23 and ICV D47 immobilized with 2 or 4% Ca-alginate was assessed in terms of the physicochemical characteristics (pH, volatile acidity, titratable acidity, final nitrogen, total SO_2 and ethanol), and is presented in Table 2. The pH has been noted in the past as one of the causes of sluggish or premature fermentation arrest in alcoholic beverages *(6)*, which is why this parameter was determined in all of the experiments. As expected from previous

Table 2. Physicochemical characteristics of honey-must and meads fermented by *S. cerevisiae* QA23 and ICV D47 immobilized cells in 2 or 4% alginate

Honey-must	
pH	3.71 ± 0.00
°Brix (%)	23.20 ± 0.14
Titratable $acidity_{tartaric\ acid}$ (g/L)	3.43 ± 0.03
Initial $nitrogen_{YAN}$ (mg/L)	353.50 ± 4.95

Meads	Strain QA23		Strain ICV D47		Significance		
	2% Alginate	4% Alginate	2% Alginate	4% Alginate	Alginate (A)	Strain (S)	A × S
pH	3.66 ± 0.02	3.67 ± 0.03	3.62 ± 0.01	3.63 ± 0.01	n.s.	0.031	n.s.
Volatile $acidity_{acetic\ acid}$ (g/L)	0.63 ± 0.00	0.65 ± 0.02	0.51 ± 0.04	0.54 ± 0.04	n.s.	0.007	n.s.
Titratable $acidity_{tartaric\ acid}$ (g/L)	5.18 ± 0.00	5.14 ± 0.16	4.99 ± 0.21	5.10 ± 0.05	n.s.	n.s.	n.s.
Final $nitrogen_{YAN}$ (mg/L)	52.50 ± 4.95	42.00 ± 9.90	43.75 ± 2.47	45.50 ± 4.95	n.s.	n.s.	n.s.
Total SO_2 (mg/L)	23.68 ± 0.91	23.68 ± 0.91	21.12 ± 0.91	21.12 ± 0.91	n.s.	0.016	n.s.
Ethanol (% vol)	10.54 ± 0.94	11.20 ± 0.57	11.50 ± 0.14	11.40 ± 0.14	n.s.	n.s.	n.s.

Lack of superscript indicates no significant difference between the alginate concentrations for each strain ($p < 0.05$). n.s., No significant difference at $p < 0.05$.

work on mead production *(3,6,7)*, the pH of the meads was lower than that of honey-musts (3.71 ± 0.00) for both strains and alginate concentrations. The reduction of pH during mead fermentation is probably caused by the production of acids by yeasts *(3)* and the low buffer capacity of honey-musts *(6)*. However, meads fermented by strain QA23 presented a significantly higher pH than those from strain ICV D47 ($p = 0.031$). Control of volatile acidity is a critical issue for the industrial manufacture of fermented beverages. Indeed, the production of acetic acid, by far the most abundant volatile acid, can have a dramatic effect on the quality of the final product. In addition to undesirable aromas, high levels of acetic acid are toxic to yeast and can lead to stuck alcoholic fermentations *(22)*. The volatile acidity ranged from 0.51 ± 0.04 to 0.65 ± 0.02 g/L of acetic acid and was within the values reported for wine *(23)* and the results obtained previously for mead produced without an immobilization system *(3,5–7)*. For this parameter and for total SO_2, which varied between 21.12 ± 0.91 and 23.68 ± 0.91 mg/L, no differences were detected between meads obtained with either of the alginate concentrations. However, significant differences were observed between the strains ($p = 0.007$ for volatile acidity and $p = 0.016$ for total SO_2), with the lowest concentrations found in meads produced with strain ICV D47. Similar concentrations of titratable acidity, around 5 g/L of tartaric acid, were found in all meads irrespective of the strain or alginate concentration. Higher titratable acidity was found in meads, when compared with the honey-must, indicating the production of acids by the yeast. Different results were obtained during the fermentation of a fruit wine from cagaita, where a reduction of titratable acidity from 0.5% in must to 0.3% in wine was observed *(24)*. The ethanol content ranged from 10.54 to 11.50% (v/v) with no remarkable differences in meads fermented with immobilized cells in 2 or 4% Ca-alginate. Different results are reported in the literature. Najafpour *et al*. *(25)* found that immobilization in 2% alginate was more suitable for ethanol production, based on the activity of the beads. Similar amounts of ethanol have already been reported in fermentations of mead with the same initial °Brix and produced with free cells *(6,7)*. A concentration of residual nitrogen remained in all meads independent of the yeast strain and concentration of alginate used for immobilization. As reported previously, some of the residual nitrogen could correspond to the concentration of the amino acid proline, present in honey, which is not assimilated by yeast during the fermentation *(7)*.

In summary, at the end of the fermentation the number of cells entrapped in beads was higher than the number of free cells in the medium. Independent of the strain, the number of cells in the medium was similar for both concentrations of alginate. Considering the quality of the meads, the results showed that the yeast strain had more influence than the concentration of alginate used for yeast entrapment. Indeed, the parameters of pH, volatile acidity and total SO_2 were significantly different between the two yeast strains.

Although the alginate concentrations tested did not prevent the phenomenon of cell leakage, the entrapment agent did not cause negative effects on mead production, since no remarkable differences were observed in fermentation performance and mead quality compared with mead produced previously with free cells. Fermentation length was 120 h and the characteristics of the final product were not influenced by the alginate concentration. Since no differences were found between the two alginate concentrations, for economic reasons using 2% of alginate for immobilization of yeasts for mead would be more advantageous. The current study also suggests that, considering the low volatile acidity produced by strain ICV D47, it appears be the more suitable yeast for immobilization.

Acknowledgements

The research presented in this paper was partially funded by the Fundação para a Ciência e Tecnologia, and by the PTDC project (contract PTDC/AGR-ALI/68284/2006). A.P.P. is the recipient of a PhD grant from FCT (SFRH/BD/45820/2008).

References

1. Gupta, J. K., and Sharma, R. (2009) Production technology and quality characteristics of mead and fruit-honey wines: A review, *Nat. Prod. Radiance 8*, 345–355.
2. Ramalhosa, E., Gomes, T., Pereira, A. P., Dias, T., and Estevinho, L. M. (2011) Mead production: Tradition versus modernity, in *Advances in Food and Nutrition Research* (Jackson, R. S. Ed.), pp. 101–118, Academic Press: Burlington, VA.
3. Sroka, P., and Tuszyński, T. (2007) Changes in organic acid contents during mead wort fermentation, *Food Chem. 104*, 1250–1257.
4. Navrátil, M., Šturdík, E., and Gemeiner, P. (2001) Batch and continuous mead production with pectate immobilised, ethanol-tolerant yeast, *Biotechnol. Lett. 23*, 977–982.
5. Pereira, A. P., Dias, T., Andrade, J., Ramalhosa, E., and Estevinho, L. M. (2009) Mead production: Selection and characterization assays of *Saccharomyces cerevisiae* strains, *Food Chem. Toxicol. 47*, 2057–2063.
6. Mendes-Ferreira, A., Cosme, F., Barbosa, C., Falco, V., Inês, A., and Mendes-Faia, A. (2010) Optimization of honey-must preparation and alcoholic fermentation by *Saccharomyces cerevisiae* for mead production, *Int. J. Food Microbiol. 144*, 193–198.
7. Pereira, A. P., Mendes-Ferreira, A., Oliveira, J. M., Estevinho, L. M., and Mendes-Faia, A. (2013) High-cell-density fermentation of *Saccharomyces cerevisiae* for the optimisation of mead production, *Food Microbiol. 33*, 114–123.
8. Qureshi, N., and Tamhane, D. V. (1986) Mead production by continuous series reactors using immobilized yeast cells, *Appl. Microbiol. Biotechnol. 23*, 438–439.
9. Park, J. K., and Chang, H. N. (2000) Microencapsulation of microbial cells, *Biotechnol. Adv. 18*, 303–319.
10. Kourkoutas, Y., Bekatorou, A., Banat, I. M., Marchant, R., and Koutinas, A. A. (2004) Immobilization technologies and support materials suitable in alcohol beverages production: A review, *Food Microbiol. 21*, 377–397.
11. Tsakiris, A., Bekatorou, A., Psarianos, C., Koutinas, A. A., Marchant, R., and Banat, I. M. (2004) Immobilization of yeast on dried raisin berries for use in dry white wine-making, *Food Chem. 87*, 11–15.
12. Bezbradica, D., Obradovic, B., Leskosek-Cukalovic, I., Bugarsk, B., and Nedovic, V. (2007) Immobilization of yeast cells in PVA particles for beer fermentation, *Proc. Biochem. 42*, 1348–1351.
13. Vilela, A., Schuller, D., Mendes-Faia, A., and Côrte-Real, M. (2013) Reduction of volatile acidity of acidic wines by immobilized *Saccharomyces cerevisiae* cells, *Appl. Microbiol. Biotechnol. 97*, 4991–5000.
14. Divies, C., and Cachon, R. (2005) Wine production by immobilised cell systems, in *Applications of Cell Immobilisation Biotechnology*, (Nedović, V., and Willaert, R. Eds), pp. 285–293, Springer, Dordrecht.
15. Inal, M., and Yiğitoğlu, M. (2011) Production of bioethanol by immobilized *Saccharomyces cerevisiae* onto modified sodium alginate gel, *J. Chem. Technol. Biotechnol. 86*, 1548–1554.
16. Liouni, M., Drichoutis, P., and Nerantzis, E. T. (2008) Studies of the mechanical properties and the fermentation behaviour of double layer alginate-chitosan beads, using *Saccharomyces cerevisiae* entrapped cells, *World J. Microbiol. Biotechnol. 24*, 281–288.
17. Pires, J., Estevinho, M. L., Feás, X., Cantalapiedra, J., and Iglesias, A. (2009) Pollen spectrum and physico-chemical attributes of heather (*Erica* sp.) honeys of north Portugal, *J. Sci. Food Agric. 89*, 1862–1870.
18. Mendes-Ferreira, A., Barbosa, C., Falco, V., Leão, C., and Mendes-Faia, A. (2009) The production of hydrogen sulphide and other aroma compounds by wine strains of *Saccharomyces cerevisiae* in synthetic media with different nitrogen concentrations, *J. Ind. Microbiol. Biotechnol. 36*, 571–583.

19. Göksungur, Y., and Zorlu, N. (2001) Production of ethanol from beet molasses by Ca-alginate immobilized yeast cells in a packed-bed bioreactor, *Turk. J. Biol. 25*, 265–275.
20. OIV. (2006) *Recueil des méthodes internationales d'analyse des vins et des mouts*, Organisation International de la Vigne e du Vin: Paris.
21. Aerny, J. (1996) Composés azotes des moûts et des vins, *Rev. Suisse Vitic. Arboric. Hortic. 28*, 161–165.
22. Luo, Z., Walkey, C. J., Madilao, L. L., Measday, V., and Van Vuuren, H. J. J. (2013) Functional improvement of *Saccharomyces cerevisiae* to reduce volatile acidity in wine, *FEMS Yeast Res. 13*, 485–494.
23. Nikolaou, E., Soufleros, E. H., Bouloumpasi, E., and Tzanetakis, N. (2006) Selection of indigenous of *Saccharomyces cerevisiae* strains according to their oenological characteristics and vinification results, *Food Microbiol. 23*, 205–211.
24. Oliveira, M. E. S., Pantoja, L., Duarte, W. F., Collela, C. F., Valarelli, L. T., Schwan, R. F., and Dias, D. R. (2011) Fruit wine produced from cagaita (*Eugenia dysenterica* DC) by both free and immobilised yeast cell fermentation, *Food Res. Int. 44*, 2391–2400.
25. Najafpour, G., Younesi, H., and Ismail, K. S. K. (2004) Ethanol fermentation in an immobilized cell reactor using *Saccharomyces cerevisiae*, *Bioresour. Technol. 92*, 251–260.

