

Potentiality of gravitational field-flow fractionation using a modified glass channel for the characterization of active dry wine-making yeasts

BY RAMSÉS SANZ, PIERLUIGI RESCHIGLIAN, LLUÍS PUIGNOU, AND M-TERESA GALCERAN

GRAVITATIONAL FIELD-FLOW fractionation (GFFF)¹ is applied here for the characterization of wine yeasts. It is simple, non-destructive, inexpensive, and easy to implement in a standard HPLC system. Active dry wine yeasts are now used throughout the industry, and quality control of the cells is mandatory before fermentation and during the winemaking process. A previous study² reported the suitability of GFFF for the analysis of this kind of sample. The methodology developed earlier was improved by modifying the separation channel walls to decrease sample adsorption, and the new conditions were applied to the same yeast strain before testing for comparison. The GFFF profiles could be used to characterize yeast samples because of differences in their morphological features; in this study, the use of silylated glass channels to improve the analysis of yeast samples was investigated. A Coulter counter technique was also used to assess the correlation of GFFF results with size distribution of the sample.

Experimental

Methods and equipment

To improve on the earlier method,² the nature of the channel walls was modified by silylation of the glass wall. Silylation was performed by immersion of the glass plates in a 10% solution of hexamethyldisilazane (HMDS) (Sigma, St. Louis, MO) in toluene (Merck KGaA, Darmstadt, Germany) for 24 hr. After the conditioning step, the volatile organic solvent was allowed to evaporate at room temperature prior to assembling the channel. The dimensions of the resulting ribbonlike channel were 0.0109 cm thick, 2 cm wide, and 30 cm long. Experiments were performed using an HP model 1050 LC (Hewlett Packard, Palo Alto, CA) with an HP model 1050 UV-VIS spectrophotometer detector. The channel outlet was connected to the UV-VIS spectrophotometer through a polyetherether ketone (PEEK) union. The channel void volume, V_o , was 0.601 mL. Other experimental conditions were the same as those previously described,² thus, an injection time of 45 sec with an injection flow of 0.2 mL/min⁻¹ was applied. The stop flow time was 6 min. A carrier solution composed of 0.5% Triton® X-100 (Fluka, Buchs, Switzerland) in MeOH/H₂O (20:80) was used. The carrier flow rate was 0.2 mL/min⁻¹. All the fractograms were obtained at 330 nm and the analyses were performed at room temperature.

Coulter counter size measurements were carried out through a Multisizer II (Coulter

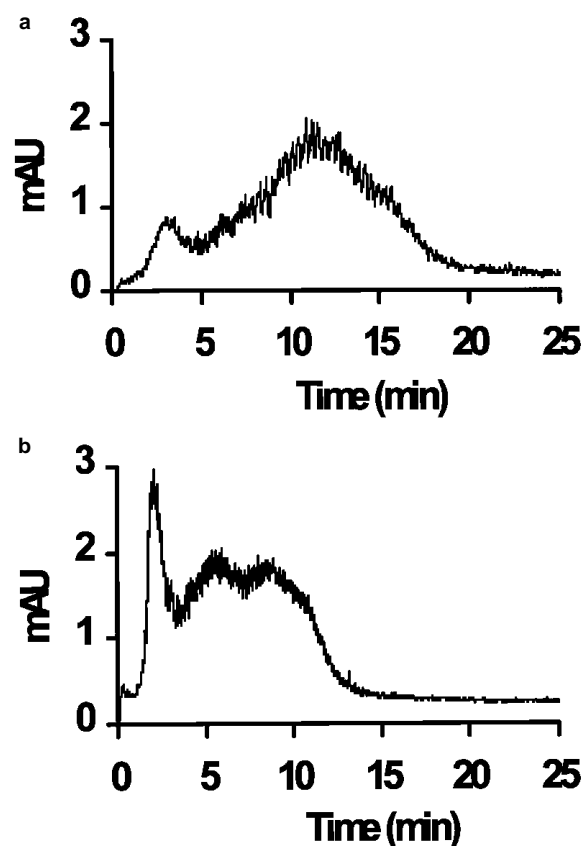


Figure 1 a) Fractogram of FERMOL Bouquet obtained for a GFFF channel with polar walls. b) Fractogram of FERMOL Bouquet obtained for a GFFF silylated channel. Experimental conditions described in text.

Corp., Hialeah, FL) set for 256-channel analysis. Aperture size was 70 μ m (measure rank: from 2 to 60% of nominal aperture size). Instrumental calibration was performed with Calibration Standard Polystyrene Latex of 18.5 μ m (Coulter Electronics, Luton, U.K.) according to the instruction manual of the instrument. Dispersions of 0.05% (wt/vol) of yeast sample were diluted 1:1000 with a specific isotonic reagent (Isoton II, Coulter Corp.). The analytical volume was 500 μ L. All measurements were replicated three times.

Active dry wine yeast

The sample was a commercial active dry wine yeast from the *Saccharomyces cerevisiae* strain named FERMOL Bouquet (courtesy of Dr. J. Guasch, Group of Química Analítica Enológica i dels Aliments, University Rovira i Virgili, Tarragona, Spain). The sample was dispersed at 0.05% (wt/vol) in the carrier liquid and kept at 4–5 °C before the analysis. Sample treatment was sonication for 1 min in the carrier liquid and equilibration for 15 sec before injection in the GFFF channel. The amount injected was always 20 μ L.

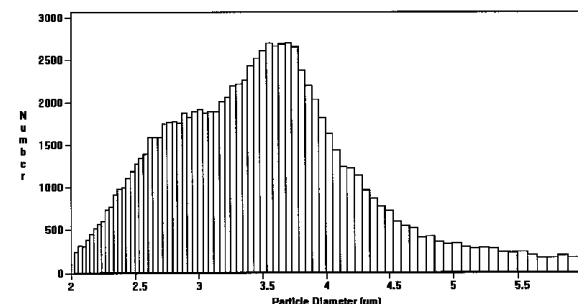


Figure 2 Cell size distribution obtained with Coulter counter technique for a FERMOL Bouquet yeast strain. Experimental conditions described in text.

Results and discussion

Interactions between cells and the glass channel wall can be produced due to the presence of polar groups on the wall surface; these interactions³ are responsible for both losses of reproducibility and increases in elution times. Polar interactions between cells and silylated groups on wall surfaces were prevented using the glass channel previously silylated with HMDS; thus, fractograms showing better resolution and lower elution times than those on nonsilylated glass channel were obtained. As an example, Figure 1a shows the fractogram of the FERMOL Bouquet sample using the glass channel before silylation, and Figure 1b shows the corresponding fractogram for the same sample when the silylated channel obtained under the conditions described in the experimental section was used. A clear bimodality can be observed on the elution profile when using the silylated channel, probably due to the different cell size population inside the sample, which produced the appearance of two poorly resolved peaks (Figure 1b).

In contrast, bimodality was not clearly observed in the fractogram obtained with the nonsilylated channel; in this case, an important fraction of cells seemed to be eluted together with the void volume (Figure 1a). Differences in the separation process were related to the changes of polarity properties of the modified channel surface, which reduced ionic interactions, thus preventing adsorption phenomena. In accordance with this fact, the elution time for the whole peak was significantly reduced. In the fractogram obtained with the silylated channel only 14 min was necessary, whereas with the nonsilylated one 20 min was needed (Figure 1), which indicated fewer interactions between yeast cells and channel wall surface when the channel has nonpolar walls.

continued

Because GFFF results depend in part on cell size, the Fermol Bouquet strain was also analyzed by the Coulter counter technique, obtaining the histogram of the total cell population, and so a correlation between them could be established. *Figure 2* shows the cell size distribution obtained from 2 to 6 μm for a cell population count of 99,626. Two different groups of cells can be observed, showing a mean size of 2.70 and 3.75 μm , respectively. The Coulter counter information was in agreement with the fractogram obtained in GFFF with the silylated glass walls; the bimodal profile could also be explained by the presence of these two populations of cells.

The stability of the silylated surface must be checked, and a periodic silylation process is necessary to obtain reproducible results. Actually, the quality of the surface was established by observing the retention behavior of benzoic acid as marker compound, and, after a run of 35–40 sample injections, the channel had to be silylated again. In spite of this, the applicability of this modified channel was stated, showing that the characterization of several types of yeast is possible with GFFF at very low cost and simplicity. Furthermore, this technique can

help not only in the cell morphology control of the yeast before its use, but also during fermentation processes.

Conclusion

The use of a separation channel with silylated glass walls has allowed improvement on the methodology developed in a previous work.² A commercial type of *Saccharomyces cerevisiae* wine yeast was analyzed using GFFF with a silylated glass channel, and more information and sample throughput than when using a glass channel were achieved. The yeast sample studied showed a bimodal elution profile due to differences in cell size distribution. To verify the GFFF results, another technique for particle sizing such as the Coulter counter was used. The obtained cell size distribution agreed well with the GFFF results, and makes it possible to confirm that the yeast population was not a homogeneous sample in size nor cell number. Therefore, the GFFF technique using silylated channels could provide a simple way to analyze active dry wine yeast before and during wine-making, which would be necessary for the qual-

ity assurance of the final product and to keep the process under control.

References

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Mr. Sanz, Dr. Puignou, and Dr. Galceran are with the Departament de Química Analítica, Universitat de Barcelona, Martí i Franquès, 1-11, E-08028 Barcelona, Spain; tel.: (34) 93 402 12 32; fax: (34) 93 402 12 33; e-mail: puignou@apolo.qui.ub.es. Dr. Reschiglian is with the Dipartimento di Chimica G. Cianciani, Università di Bologna, Bologna, Italy. This work has been partially supported by the Comissió Interdepartamental de Recerca i Innovació Tecnològica (SGR 99-49). Mr. Sanz wishes to thank the University of Barcelona for a grant used for this work (Beca de Formació en la Recerca i la Docència).