TESTING GELLAN-GUM BIOPOLYMER AS MATRIX FOR INCLUSION OF YEAST USED IN THE SPARKLING WINES PREPARATION

TESTAREA BIOPOLIMERULUI GELAN-GUM CA MATRICE PENTRU ENTRAPAREA LEVURILOR UTILIZATE ÎN PREPARAREA VINURILOR SPUMATE

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Abstract. *Obtaining sparkling wines through the champenoise method, using free yeast cells, is made through several stages, from which the operation of riddling requires greater time and qualified staff. The operation of riddling can be removed using different biocatalysts obtained by immobilization in different matrices such as alginate, carragenan, polyvinyl alcohol. However, producers of sparkling wines show a reluctance to using the obtained biocatalysts, arguing the possibility of some residues from the matrices used as support for inclusion of yeasts involving in the wine. We believe that this can be overcome by using the gellan-gum biopolymer as a matrix, which is allowed by FAO in food, pharmaceuticals and cosmetics. In this paper, we present the efficiency of biocatalysts obtained with gellan-gum biopolymer used as a matrix, in the preparation of sparkling wines through the champenoise method.*
Key words: yeasts, gellan-gum, inclusion, sparkling wine

Rezumat*. Prepararea vinurilor spumante prin metoda champenoise, folosind celule de levuri libere, parcurge mai multe etape, din care operațiunea de remuaj necesită o perioadă de timp mare şi personal calificat. Operațiunea de remuaj se poate elimina folosind biocatalizatori obținuți prin imobilizarea în diferite matrici ca alginat, carragenan, polivinil alcool. Însă, producătorii de vinuri spumante manifestă o reținere pentru utilizarea biocatalizatorilor obținuți, motivând posibilitatea antrenării în vin a unor reziduuri din matricele utilizate ca suport pentru entraparea levurilor. Acest aspect credem că poate fi depăşit prin utilizarea ca matrice a biopolimerului gelan-gum care este admis de FAO în industria alimentară, farmaceutică şi cosmetică. În această lucrare, prezentăm eficiența biocatalizatorului obținut prin utilizarea biopolimerului gelan-gum ca matrice, în procesul de preparare a vinurilor spumante prin metoda champenoise.*
Cuvinte cheie: levuri, gelan-gum, imobilizare, vinuri spumante.

INTRODUCTION

Studies regarding the use of gellan as a matrix for the immobilization of yeasts were done by Yuguchi et al. (2002), Desimone et al. (2002), Sun et al. (2007). The mentioned authors did researches on the role of cations in the aggregation

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and stabilization of the gellan-gum matrix, the resistance of the gel to enzymes, to the degradation generated by heat, to extreme pH and microorganisms. Sook et al. (2011) studied the feasibility of the immobilization of yeast cells in gellan-gum, by means of emulsification. The authors evaluated the efficiency of the bio-catalyser obtained in the continuous alcoholic fermentation for the achievement of bio-ethanol as well as the possibility of using it in several cycles. This information from the specialized literature led to the idea of preparing and testing a bio-catalyser with yeast cells immobilized in gelan-gum, with the purpose of using it in preparing sparkling wines.

The purpose of researches was of demonstrating the possibility of preparing a bio-catalyser with cells immobilized in gelan-gum and the efficiency of using it in preparing sparkling wines in bottles, as the qualities of the bio-catalyser manifest themselves in the alcoholic fermentation process and especially in the riddling operation, which can be excluded. This aspect was also approached by authors that used for the immobilization of yeasts different matrices such as alginate, carrageenan, polyvynil alcohol: Fumi (1998), Godia (1991), Țiță (2003), Silva (2002), Kourkouta (2004), Efremenco (2006).

MATERIAL AND METHOD

The yeast strain used in the experiment, with the code MNO14, was isolated from the vineyard in Iaşi – Copou viticultural centre. The biomass of yeast necessary for the immobilization in gelan-gum was obtained using the liquid culture environment made of: glucose 4%, peptone 1%, K_2HPO_4 0.2%, $MgSO_4$ 0.2% and yeast extract 0.55. For 72 hours the temperature was maintained at 25^oC, the yeast cells were centrifuged at 5,000 rpm and washed with sterile distilled water. A 10% suspension was made of the biomass of yeast cells, which was used in the inclusion in the gellan matrix.

The GG-MNO14 biocatalyser (cells included in gellan-gum) was made by using a 0.5% solution of gelan-gum and 16 mL 10% yeast cell suspension. After the homogenization of the gelan-gum gel/ yeast cell suspension, it was extruded through a capillary in order to obtain pearls with a 1.5-2 mm diameter. In order to be stabilized, the pearls were introduced in a 2% $CaCl_2$ solution. After six hours, the pearls were washed with sterile distilled water to remove the bivalent calcium ion.

The obtained biocatalyser was tested in the second alcoholic fermentation, thus determining the behaviour of the pearls during the alcoholic fermentation in cylinders, the time (days) of completion of the fermentative processes, the time (days, seconds) for the achievement of the rotation and the physical-chemical characterization of the sparkling wines obtained. The physical-chemical determinations were carried out according to the methods of the International Organization of Vine and Wine. The pressure in cylinders was determined with an aphrometer.

RESULTS AND DISCUSSIONS

In this experiment, two lots of 35 cylinders with the volume of 750 mL, namely a control lot in which to the mix draft free yeast cells were added and an experimental lot in which pearls were introduced, namely the GG-MNO14 biocatalyser with cells immobilized in gellan-gum.

In order to appreciate the catalytic activity of the yeast strain with free cells and with immobilized cells, in the secondary alcoholic fermentation, identical fermentation conditions were ensured. In preparing the draft mix for the control lot, base wine was used to which the quantity of draft liquor was added in order to obtain the concentration of 24 g sugars/bottles and 2 mL of 10% yeast cell suspension, which may ensure the yeast concentration in the experimental lot for the preparation of 10 g of pearls with immobilized cells.

The draft mix for the experimental lot was made of the same raw matter wine of the variety Fetească regală, to which the same quantity of draft liqueur and 10 g of pearls of the GG-MNO14 catalyser were added.

In order to measure the pressure in the bottles and monitor it in time, an aphrometer was attached to one cylinder of each lot. Taking into account the fact that the pressure created by the accumulation of carbon dioxide in cylinders does not affect the evolution of the fermentative process, it was considered that the records of this parameter for 18 days, every day, and then every three days, during the experiment, offer information regarding the time (in days) necessary for the activation of alcoholic fermentations and the time (in days) in which these processes were finalized. On the same time periods, a cylinder was taken out from each lot and its concentration of sugars was determined.

The physical-chemical characteristics of the base wine are mentioned in tab. 1.

Table 1

Physical-chemical characteristics of the base wine

Alcohol % vol	Sugars, g/L	Total acidity, g/L $C_4O_6H_6$	Volatile acidity, g/L CH3COOH	Free sulphurous anhydride, mg/L	Total sulphurous anhidride, mg/L	pH
10,6	1,5	6,9	0,32	26	78	3,41

The physical-chemical composition of the draft mix is rendered in table 2.

Table 2

The physical-chemical composition of the draft mix

Alcohol % vol	Sugars, g/L	Total acidity, g/L $C_4O_6H_6$	Volatile acidity, g/L CH3COOH	Free sulphurous anhydride, mg/L	Total sulphurous anhidride, mg/L	pH
10,4	24,0	7,3	0,31	25	75	3,35

The outcomes of this experiment offer the possibility of characterizing the catalytic activity of the yeast strain in the secondary alcoholic fermentation in bottles, using free cells or immobilized cells.

Figure 1 reveals the dynamics of the consumption of sugars and the evolution of the pressure in bottles during the alcoholic fermentation, for 41 days.

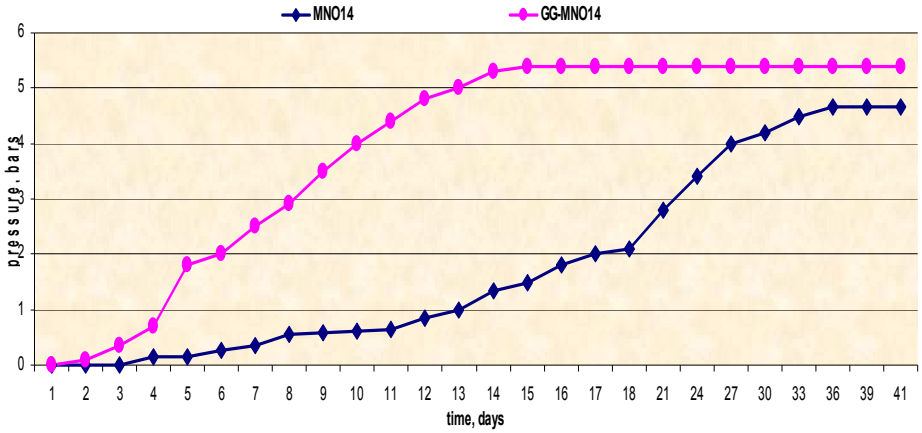

Fig. 1 – The dynamics of the accumulation of pressure in cylinders, during the alcoholic fermentations achieved by the free cells MNO14 (control) and the GG-MNO14 biocatalser

The graphic representation of data regarding the consumption of sugars and the record of the pressure values reveals, in comparison to the control, that the activation of the alcoholic fermentation in cylinders happened faster than with the biocatalyser with immobilized cells, namely after two days. The process of alcoholic fermentation in the cylinders with free yeast strain occurred after three days. This behaviour in the catalytic evolution of the immobilized yeast was determined by the protection offered by the gellan-gum matrix against the stress of the high concentration of alcohol from the draft mix, which was not ensured for the free cells.

The alcoholic fermentation achieved in the presence of the biocatalyser with immobilized cells was intense between the 6th and the 12th day, being completed after 14 days, when the maximum pressure of 5.5 bars is achieved.

The secondary alcoholic fermentation in the control cylinders, after three days from the activation, evolved slowly for 11 days, after which it crossed two stages, between the 12th and the 18th day and the 18th and 33rd day with an intense evolution, and the process completed in the 36th day from the experiment, at a pressure of 5.1 bars.

The first ascertainment of this experiment is that, due to the immobilization of the yeast cells in gellan-gum, the time of secondary alcoholic fermentation is reduced by 60%, in comparison to the time of completion of the alcoholic fermentation achieved with free cells.

The biocatalyser pearls did not lead, from the time of their addition to the draft mix and during the process of the alcoholic fermentation, to the apparition of floating pearls, which is a very important aspect, as their presence would make the riddling and degorging operations impossible.

The riddling operation for the bottles from the experimental lot was carried out in a few seconds, and the pearls formed a stable deposit on the cylinder cork. For the cylinders with free cells, the same operation was carried out after nine

days, as the deposit of fine beads deposited on the cylinder cork needed every three days operations typical for this stage. The degorging operation was carried out in optimum conditions in both bottles lots.

The gross sparkling wines obtained in the alcoholic fermentations with free cells or immobilized cells had a clear crystalline brilliant aspect.

The physical-chemical tests for the characterization of gross sparkling wines were carried out on three bottles from each lot. The average values of the studies parameters are graphically represented in fig. 2.

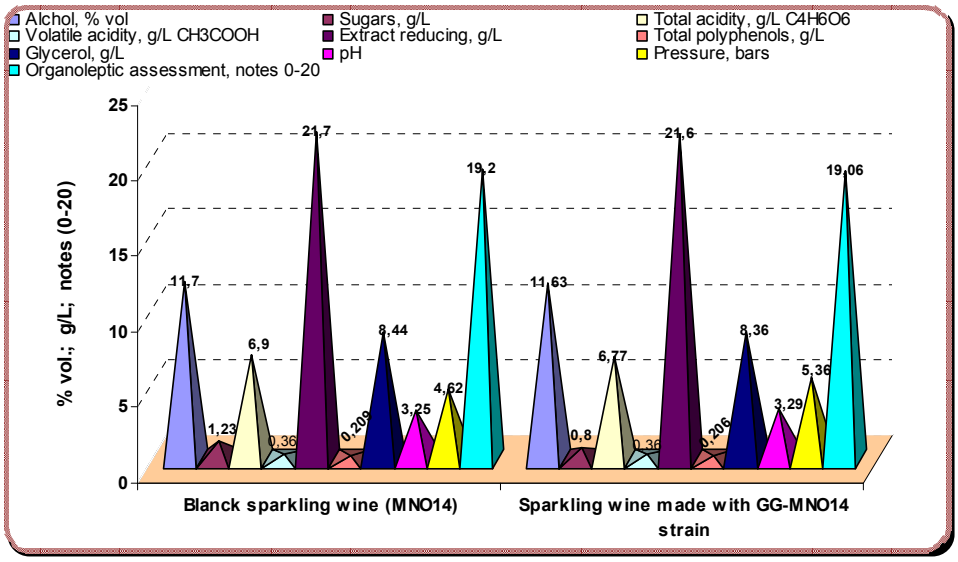

Fig. 2 – The quality of sparkling wines obtained by using free MNO14 cells and GG-MNO14 immobilized cells

According to the graphic representation of data, there are average values higher as regards the alcohol concentration in the gross sparkling wine obtained with free cells of yeasts, but the difference is not significant. Also, the tested parameters: total acidity, volatile acidity, pH, pressure, had different average values insignificant in the two lots of bottles, which was also asserted by others authors, such as: Tataridis et al. (2005), who did a compared study using free cells and the biocatalyser with cells of yeasts immobilized in alginate.

In exchange, there was an increase in the concentration of glycerol in both lots of gross sparkling wines, in comparison to the value determined in the base wine, namely 8.44 g/L and 8.36 g/L. This result is in accordance with the data obtained by Ciani et. al. (1996), Ferraro et. al. (2000).

CONCLUSIONS

1. The gellan-gum gelling agent can serve as a matrix for the immobilization of the yeast cells in order to obtain a biocatalyser corresponding to the criteria for its use in the preparation of sparkling wines.

2. By the immobilization of the yeast cells in gellan-gum, the time of the

secondary alcoholic fermentation is reduced by 60% in comparison to the time of completion of the alcoholic fermentation achieved with free cells.

3. The riddling operation for the bottles for which the biocatalyser with immobilized cells was carried out in a few seconds, which led to optimum conditions for the degorging operation.

4. The use of free yeast cells and yeast cells immobilized in gellan-gum does not modify the composition characteristics of the gross sparkling wines obtained, which means that they were very close, which is a remarkable criterion as regards the inclusion of the immobilized products in the technology of making sparkling wines.

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