THE TESTING OF SOME ORGANIC SUPPORTS FOR YEASTS IMMOBILIZATION TECHNOLOGY USED IN SPARKLING WINE PRODUCTION

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Abstract. Various support and immobilization techniques have been proposed and tested for application in alcoholic beverage production. Methods of yeast immobilization included various supports: inorganic supports, organic supports and natural supports. In this paper are presented data obtained with three immobilized preparations using a selected yeast strain and three organic supports which two are synthetic supports, cross-linked, macromolecule, form of beads (AS-68, AS-80) and gellan gum, polysaccharide produced by fermentation. Aim of this work was to obtain preparations of yeasts immobilized in order to exclude the operation of riddling of sparkling wine production technology. Yeasts immobilized on acrylic supports, in the second fermentation in bottles, have led to the production of sparkling wines that requiring riddling operation, because they were separated from the beads. Yeasts immobilized on a support of gellan gum remained included in beads, which led to the production of clear sparkling wine, eliminating riddling stages.

INTRODUCTION

The second fermentation of the wine in bottles, a process that stands at the basis of producing sparkling wines through the “champenoise” method, is of a long period, approximately 3-6 months. At the end of the fermentation process there will form a yeast deposit which has to be brought at the neck of the bottles through a riddling process. This operation lasts 25-30 days and needs space in order to work, experienced operators or the acquisition of automat riddling installations. All of these things raise the cost of the final product.

The elimination of the riddling process can be made using immobilized materials of oenological yeasts. Such information can be found in the papers of the following authors: Fumi M.D. and collaborators (1998), Yokotsuka K.M. and collaborators (1997), Duteurte B. and collaborators (1987), Godia F. (1991), Tiţă O. (2005), Silva S. (2002), Efremenco Elena and collaborators (2006), Tataridis P and collaborators (2005). The mentioned authors have tested different materials as a support for the immobilization of the oenological yeasts, following the activity of these biocatalysts in the second alcoholic fermentation in bottles.

In this paper there have been used two methods of achieving the immobilized materials: by using macromolecular acrylic pearls and the gellan gum jellifying agent.

MATERIAL AND METHODS

Yeast strain used in the experiment, MO14 codified, was isolated from Iaşi’s vineyard, Copou wine- growing center, from grape must being from Muscat Ottonel breed. Yeast cell biomass was obtained on a cultural site having the following recipe: 4% glucoses, 1% peptone, K_2HPO_4 2g/L, 2g/L $MgSO_4$ and 0, 5% yeast extract. After keeping it 48 hours at 25° C on the agitator, the cells were centrifuged at 4° C at 5000 rpm for 15 minutes and washed with sterile distilled water, twice. From the yeast biomass they made a 10% suspension that was used in the process of immobilization on the AS-68, AS-80 and gellan gum base.

In order to obtain the immobilized materials there were taken 100 g of beads from the AS-68 and AS-80 bases which were introduced in 100 mL suspension having 10g of humid biomass. The beads were in contact with the suspension of yeasts for 6 hours and they were slightly shaken, a few times, afterwards the yeast suspension was eliminated and the beads were washed with sterile distilled water in order to be clear off the yeast cells that were not immobilized. The determination of the quantity of the immobilized cells on the acrylic basis was made gravimetric at 105°C for an hour, weighing a gram from the testate basis, before and after the immobilization.

The immobilized product in gellan was obtained using the following recipe: 0,4 g gellan gum (purchased from *Sigma*), 31 mL calcium bentonit suspension (c = 0,035 g/mL), 12 mL suspension 10% from MO14 yeast and 40 mL sterile distilled water. After obtaining the gellan gum/ suspension blending of yeast cells, it was made the extrusion of the gell through a capillary in order to obtain the beads that were introduced in a 2% substance of $CaCl_2$. After 24 hours the obtained beads were washed with sterile distilled water until the elimination of the calcium ions.

The physicochemical analysis of the draft mixture and of the sparkling wines obtained was done according with the O.I.V methods (Office International de la Vigne at du Vin).

RESULTS AND DISCUSSIONS

In this paper there were tested in the second fermentation in bottles, three accelerators from which two were obtained through the absorption of the yeast cells on the acrylic basis as beads, codified AS-68-MO14 (*figure 1a)* and AS-80-MO14 (*figure 1b)* and the third, gellan-MO14 (*figure 2*) obtained by putting it in a gel.

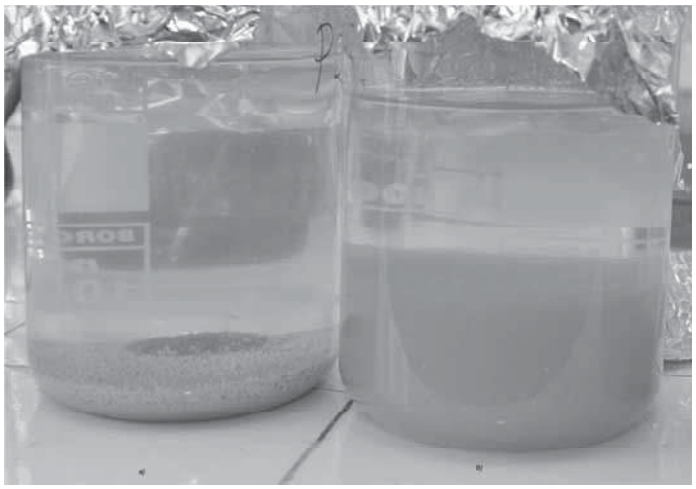

Figure 1. Biocatalyzators used in the second alcoholic wine fermentation.
a) AS-68-MO14; b) AS-80-MO14.

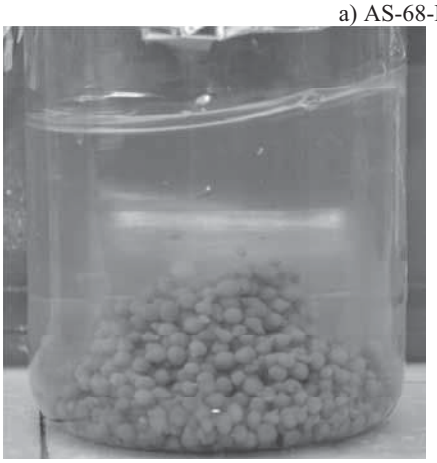

Figure 2. Gellan-MO14 biocatalyzator.

The quantity of yeasts absorbed by the acrylic beads was different. AS-68 retained 0,025 g of cells/g of beads and AS-80 retained 0,441 g of cells/g of beads. The quantity of yeast biomass per gram of gellan gum beads was of 0,027 g.

In order to obtain the carbonated wines, in this experiment were produced three lots of bottles with a capacity of 750 ml, where was introduced the draft mixture and the specific biocatalyst for each lot. The draft mixture contains the basic wine for carbonated wines, the draft liquor and the tested biocatalyst. In order to prepare the draft mixture, it was chosen as raw material wine, a wine from the variety Feteasca regală. The physico-chemical characteristics of the raw material wine for sparkling wines are described in table 1.

Table 1

The physico-chemical characteristics of the raw material wine

Alcohol % vol	Sugars g/L	Total acidity g/L H_2SO_4	Volatile acidity g/L CH_3COOH	SO_2 free mg/L	SO_2 total mg/L	pH
10,6	1,5	4,5	0,32	26	78	3,41

The physico-chemical properties of the raw material wine are situated, from the values'point of view, in the recommendations for obtaining carbonated wines. The alcohol concentration doesn't surpass the maximal value of 12% volume, consequently it will allow the tested yeasts to initiate the second alcoholic fermentation. The volatile acidity of 0,32 g/L of acetic acid is situated under the accepted maximum of 0,5 g/L acetic acid. A higher value of the raw material wine's volatile acidity can inhibit the yeasts' activity in classic fermentation, affecting the quality of the carbonated wine.

The draft liquor was prepared using the same raw material wine, to which was added sucrose, in order to obtain a concentration in total sugars of 500 g/L. The draft liquor provides the yeasts with the nutritional sublayer necessary for initiating and finishing the second alcoholic fermentation produced in the bottles.

The draft liquor volume was established according to the volume of raw material wine used and to the need to provide the optimal quantity of sugars so that, at the end of the second fermentation, it will be reached a pressure of approximately 6 bar at 10^oC. In each glass bottle of the three lots were added 10 g of catalyzing beads. The physico-chemical properties of the draft mixture are indicated in table 2.

Table 2.

The physico-chemical properties of the draft mixture

Alcohol % vol	Sugars g/L	Total acidity g/L H_2SO_4	Volatile acidity g/L CH_3COOH	SO_2 free mg/L	SO_2 total mg/L	pH
10,4	24	5,1	0,30	25	76	3,30

The draft mixtures were distributed in bottles, after which were performed the operations of putting corks and labeling. The bottles were placed in a special chamber, without natural light, at the temperature of 20^oC. The storage was made in horizontal position, on superposed rows, in order to ensure the second wine fermentation.

At the end of the experiment was evaluated the pressure resulted from the accumulation of carbonic gas in three bottles of each lot, also performing in this manner a control of the alcoholic fermentation. During the fermentation process were made periodical observations concerning the transparency of the draft mixture, the deposit of yeasts created, the presence or absence of the floating beads.

Moreover, at the end of the experiment were determined the physico-chemical characteristics of the sparkling wines. The data concerning the biocatalysts tested in the second alcoholic fermentation in bottles are presented in table 3.

Table 3.

The characteristics of the biocatalysts tested in the second alcoholic fermentation in bottles

Biocatalysts	Floating beads	Transparency of the sparkling wine	Yeast deposits	Riddling process
AS-68-MO14	absent	turbid	present	necessary
AS-80-MO14	absent	turbid	present	necessary
Gellan-MO14	absent	clear	absent	unnecessary

An initial observation on the data in table 3 is that, by introducing the biocatalyst beads in the draft mixture, don't appear floating beads. The presence of floating beads would lead to the exclusion of bottles from the process of preparing the carbonated wine, because the exclusion wouldn't be possible in the yeast removal stage.

The release of yeast cells in the wine mass of the draft mixture was seen only at the biocatalysts AS-68-MO14 şi AS-80-MO14. The presence of free cells in the draft mixture has led to three negative aspects concerning the alcoholic fermentation: (1) it prompted the prolongation of the lag period due to the stress induced by the high alcohol concentration on the yeast cells released in the draft mixture; (2) the second alcoholic fermentation was finished after 25 days; (3) in the bottles appeared a yeast deposit which entailed a riddling process *(figures 3 and 4).* In fact, the biocatalysts AS-68-MO14 and AS-80-MO14 behaved the same as in the free yeast cells fermentation.

Figure 3. Sparkling wine obtained using in the second fermentation of the AS-68-MO14 biocatalyst

Figure 4. Sparkling wine obtained using in the second fermentation of the AS-80-MO14 biocatalyst

The gellan-MO14 beads biocatalyst obtained by introducing the yeasts suspension in gel completed the fermentation of two bottles in 15 days without the yeast cells in the beads being released, resulting in the end a clear and crystalline sparkling wine *(figure 5).*

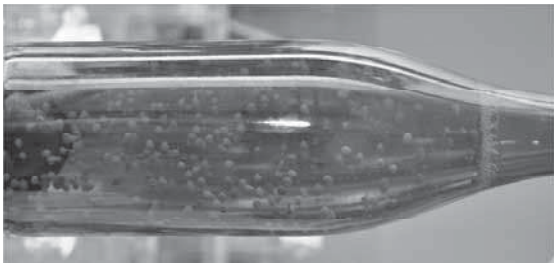

Figure 5. Sparkling wine obtained using in the second fermentation of the gellan-MO14 biocatalyst

The completion of the second fermentation ten days earlier compared to the biocatalysts AS-68-MO14 and AS-80-MO14 was possible because the cells introduced in gel were protected, progressing optimally, without being stressed by the alcohol found in the draft mixture. The riddling process was completed in a few seconds, positioning the bottle with the neck down, which allowed the collection of the yeasts found in beads, in the yeast removal stage. In this case, the riddling process allows a reduction in the time necessary to obtain the carbonated wines of 25-30 days, without influencing the sparkling wines quality.

The research on the gellan-MO14 biocatalyst will be continued, in order to determine the optimal quantity of beads per bottle, the operational stability and the possibility of reusing the beads eliminated at yeast removal in a new process of bottle fermentation.

CONCLUSIONS

The AS-68 and AS-80 acrylic gellan beads supports don't offer the possibility to obtain biocatalysts with favourable properties the production of sparkling wines.

The use of the gellan-gum agent in beads/yeast cell suspension processing using the inclusion method has led to the creation of a biocatalyst with genuine perspectives of being used in the sparkling wines production technology.

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