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THE USE OF IMMOBILIZED YEASTS IN BOTTLE-FERMENTED MOSCATO BIANCO SPARKLING WINE

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The entrapment in alginate gels of yeasts and bacterial cells is a simple, mild immobilization technique introduced by Hackel et al. (1). The use of immobilized cells has been also suggested to the wine industry (2,3,4,5). Usually Moscato Bianco is processed as sparkling wine by using the Charmat method that appears to be the most suitable process for aromatic wines. Promising results have been obtained (6)with free yeast cells by the bottle-fermentation process; an improved method has been studied too, Saccharomyces cerevisiae being immobilized within coated alginate beads have been employed.
This study was undertaken to obtain a bottle-fermented Moscato Bianco, free of viable yeast cells, biologically stable with an improved composition of the aromatic constituents.

MATERIALS AND METHODS

Saccharomyces cerevisiae were entrapped within coated alginate beads as described by Vorlop et al. (7). To each bottle, with base wine filtered in 0.22 µ pore size membrane filters, 4.5 g gel beads containing 2.18×10^6 cells/ml wine were added. Bottles were placed on vertical stacks at 13°C, the secondary fermentation for a period of 60 days was followed with an aphrometer. The wine samples were then aged on immobilized yeasts at 4-7°C, blanks with the same free cell amount went through the same process. Alcohol, sulphur dioxide and reducing sugars were determined following the Italian standard methods, turbidity was monitored at $O.D._{420}$ and viable cell counts were performed on TJB containing 2% agar as previously described (8).

Higher alcohols and volatile compunds on Carlo Erba HRGC 5300 gas chromatograph equipped with two capillary columns (HP-20 M fused silica 50 m x 0.32 mm I.D.x 0.3 µm film tickness and DB-WAX fused silica 30 m x 0.32 mm I.D. x 0.5 µm film tickness) were evaluated.

Wine samples at intervals of 2 and 8 months were analyzed.

RESULTS AND DISCUSSION

Both the free and immobilized yeasts reached a suitable value (about 7.0 atm) of carbon dioxide pressure in a period of 60 days. The pressure was controlled, to prevent the bottle fractures, by lowering the cellar temperature. The residual reducing sugars decreased from 95.00 (g/l) to 64.00 (g/l) for both thesis after 2 months and to 52.50 (g/l) and 40.90 (g/l) for wine samples with free and immobilized yeasts respectively, after 8 months. In the latter case, however, the residual sugar content is considered low for a commercial muscat wine. The immobilized yeast samples displayed lower turbidity values than free yeast ones (O.D. 0.077 and 0.098) (Table 1) and in agreement with literature data (3,9). No viable cells and 900 viable cells/ml wine in bottles with immobilized yeasts after 8 and 2 months were revealed (Table 2), showing a satisfactory behaviour of the used immobilization system to obtain a biologically stable sweet sparkling wine. With regard to aroma substances similar content of most volatiles considered as important factors for the

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organoleptic quality of sparkling wines, was found (Table 3, 4). No significant differences in terpenic constituents were observed; isoamyl and n-hexyl acetate and diethylsuccinate behaved as reported (5). The increase of ethyl lactate and ethyl pyruvate after 8 months of contact on free and immobilized yeasts would depend on malo-lactic fermentation while ethylacetate values were in agreement with published data (10) (Table 3).The muscat sparkling wine samples with immobilized yeasts showed unchanged levels of acetaldehyde (about 65 mg/l) at 2 and 8 months and of the same magnitude of the base wine (Table 3) and besides an acetoin decrease from 1.87 mg/l to 1.26 mg/l after 8 months. The corresponding free ones, on the contrary, had amounts increasing both for acetaldehyde and acetoin from 64.50 to 144.60 mg/l and from 2.93 to 19.40 mg/l, respectively. Acetaldehyde and acetoin formation depends on yeast strain and conditions on fermentation medium (11), therefore it would seem that the microenvironment have positively influenced the metabolism of the immobilized yeast cells (12).

REMARKS

From our studies it appears that the secondary fermentation of bottle-fermented Moscato Bianco sparkling wine by Saccharomyces cerevisiae free and immobilized within coated alginate gel beads produced a stable product without showing differences in terpenes. On the contrary some fermentation volatile compounds presented interesting data such as lower production of acetaldehyde and acetoin in immobilized system. Looking at sparkling winemaking technology, the immobilization technique indicates: (i) it is possible to promote a simple disgorging without twirling and using a cooled liquid; (ii) the feasibility for the winaries to make a secondary fermentation without an expensive equipment.

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Table 1. Chemicals in base wine and after 2 and 8 months of refermentation with free and immobilized yeasts.

Chemical (measure)		Base wine	2 Months		8 Months	
			A	B	A	B
		3.17	3.09	3.11	3.12	3.12
Alcohol	% v/v	6.20	8.03	8.06	8.78	9.45
Total alcohol	% v/v	11.90	11.87	11.90	11.93	11.90
Residual reducing sugars	g/l	95.00	64.00	64.00	52.50	40.90
Total SO_2	mg/l	76.00				
O.D. 420^{2}nm		0.069	0.088	0.077	0.106	0.098

A = Wine with free yeasts
B = Wine with immobilized yeasts

Table 2. Cell density in base wine and after 2 and 8 months of refermentation with free and immobilized yeasts.

		Base wine	2 Months	8 Months
Total yeast cells (Thoma's apparatus)	A	3.28×10^6	6.40×10^6	1.38×10^5
	B	4.20×10^6	650.000	0.44×10^5
Viable yeast cells (cfu/ml wine)	A	2.10×10^6	3.50×10^6	0.038×10^5
	B	2.20×10^6	900	0

A = Wine with free yeasts
B = Wine with immobilized yeasts

Table 3. Methanol, aceteldehyde, ethyl acetate and higher alcohols in base wine and after 2 and 8 months of refermentation with free and immobilized yeasts.

		Wine base	2 Months		8 Months	
			A	B	A	B
Methanol	mg/l	0.10	0.10	0.09	0.09	0.09
Acetaldehyde	"	64.50	121.00	66.4	144.60	64.40
Ethyl acetate	"	32.60	39.70	40.80	40.70	42.60
1-Propanol	"	31.80	41.50	32.80	32.60	32.60
2-Methyl-1-propanol	"	29.80	33.80	30.70	29.00	29.70
2-Methyl-1-butanol	"	26.10	26.60	28.30	10.70	12.60
3-Methyl-1-butanol	"	53.00	60.70	62.80	56.70	58.40
Total higher alcohols		140.70	159.60	162.60	129.00	133.30

A = Wine with free yeasts
B = Wine with immobilized yeasts

Table 4. Levels of some volatile components in base wine and after 2 and 8 months of refermentation with free and immobilized yeasts.

Volatiles	Base wine	2 Months		8 Months	
		A	B	A	B
Esters (mg/l)					
Isobutyl acetate	0.07	0.07	0.05	0.04	0.04
Isoamyl acetate	1.70	1.32	1.26	0.74	0.77
n-hexyl acetate	0.59	0.24	0.24	0.15	0.17
Ethyl butyrate	0.07	0.07	0.07	0.05	0.05
" caprate	0.03	0.02	0.02	0.02	0.02
" caprylate	0.08	0.07	0.09	0.08	0.08
" lactate	25.30	20.80	21.90	41.80	40.00
" pyruvate	0.49	0.24	0.024	1.27	2.07
Diethyl succinate	3.10	1.50	1.50	3.80	4.40
Alcohols (mg/l)					
Benzyl	0.10	0.08	0.08	0.10	0.09
B-Phenylethanol	6.80	7.00	6.00	8.00	9.00
1-hexanol	0.80	0.72	0.70	0.70	0.65
Acetoin (mg/l)	2.93	10.80	1.87	19.40	1.26
ɣ -butyrolactone (mg/l)	2.00	1.10	1.10	2.10	2.14
Terpenes (mg/l)					
Trans-linalool oxide (OXA)	0.06	0.07	0.06	0.07	0.06
Cis-linalool oxide (OXB)	0.05	0.04	0.04	0.04	0.04
Nerol oxide	0.02	0.01	0.01	0.01	0.01
Linalool	1.00	0.90	0.85	0.70	0.70
ho-Trienol	0.18	0.18	0.16	0.25	0.20
∝-Terpineol	0.65	0.75	0.80	0.95	0.98
Nerol	0.03	0.02	0.03	0.03	0.03
Citronellol	0.03	0.02	0.03	0.05	0.04
ho-Diol (I)	0.75	0.50	0.55	0.98	0.80
ho-Diol (II)	0.38	0.30	0.21	0.34	0.28

A = Wine with free yeasts
B = Wine with immobilized yeasts