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Immobilization of yeast on dried raisin berries for use in dry white wine-making

A. Tsakiris [a], A. Bekatorou [b], C. Psarianos [b], A.A. Koutinas [b], R. Marchant [c], I.M. Banat [c,*]

[a] *Department of Oenology and Spirits Technology, Faculty of Food Technology and Nutrition, Technological Educational Institution of Athens, Ag. Spiridonos Str, Egaleo, GR-12210 Athens, Greece*
[b] *Food Biotechnology Group, Department of Chemistry, University of Patras, GR-26500 Patras, Greece*
[c] *School of Biological and Environmental Sciences, University of Ulster, Cromore Road, Coleraine, Co. Londonderry, Coleraine, Northern Ireland BT52 1SA, UK*

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Abstract

Cells of a commercial *Saccharomyces cerevisiae* strain (*Uvaferme 299*) were immobilized on dried raisin berries (*Sultanina* variety) to produce an immobilized biocatalyst for use in dry white wine-making. The immobilised biocatalyst was found to be suitable for wine making at ambient temperatures (15–25 °C). The wines produced had low volatile acidities and low methanol and acetaldehyde contents, while volatile by-products showed no statistically significant differences from wines produced by free cells. The immobilized cell system had a good operational stability for more than 4 months. Sensory evaluation revealed differences between wines produced by immobilized and free cells.

Keywords: Wine making; Immobilization; Dried raisins; Volatile by-products; Scanning electron microscopy; Sensory evaluation

1. Introduction

Cell immobilisation for wine-making has been extensively studied during the past three decades due to a number of technical and economic advantages over free cell systems. Nevertheless, full industrial use has not been reported, apart from a few specific applications such as the production of sparkling wines (Colagrande, Silva, & Fumi, 1994; Fumi, Trioli, Colombi, & Colagrande, 1988). Full-scale applications of immobilized cells for alcoholic beverage production have been reported, mainly for the production of alcohol-free beer (Van Iersel, Meersman, Swinkels, Abee, & Rombouts, 1995) or the secondary fermentation for rapid maturation of beer (Yamauchi et al., 1995). Various supports have been proposed for potential use in main or secondary fermentation in wine. Inorganic supports, such as mineral kissiris (Bakoyianis, Kanellaki, Kalliafas, & Koutinas, 1992) and γ-alumina have been investigated (Loukatos et al., 2000) since they are abundant, are of low cost and have unlimited reuse possibilities. However, they do not meet the prerequisites for food grade purity, due to mineral residues found in the final product. Wine making, using yeast immobilised in gel-forming materials, such as Ca-alginate, agar, κ-carrageenan, cellulosic materials and pectic acid, has also been extensively reported (Colagrande et al., 1994; Kotoyoshi & Koki, 1987; Nakanishi & Yokotsuka, 1987).

Food grade, natural supports, such as delignified cellulosic materials (Bardi & Koutinas, 1994) and gluten pellets (Bardi, Bakoyianis, Koutinas, & Kanellaki, 1996) were successfully used as immobilisation supports for ambient and low-temperature wine-making, producing wines by rapid fermentation and with improved characteristics compared to wines produced by free cells. In order to satisfy the demand for food grade purity and combine it with consumer acceptance, some researchers have proposed the use of fruit pieces as cell immobilisation

* Corresponding author. Tel.: +44-2870-323062; fax: +44-2870-324911.
E-mail address: im.banat@ulster.ac.uk (I.M. Banat).

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carriers for wine or beer production (Bekatorou et al., 2002; Kourkoutas, Komaitis, Koutinas, & Kanellaki, 2001) and reported products with fine taste and aroma and a distinct fruity character.

Taking into account that the raw material for wine-making is grapes, it was thought that it would be interesting to use grape products, such as residual grape skins (Mallouchos et al., 2002), as a support for the immobilisation in wine-making. The use of raisins (dried seedless grapes) as a support for the immobilisation of yeast cells in wine-making has not been reported. This support is of food grade purity and abundance. Given that this support material is directly related to the main raw material of wine-making, it was thought that the use of raisins as a support for cell immobilisation would possibly lead to a final product with improved taste and aroma, and a more mature character, due to the transfer of some constituents into the wine. Furthermore, in the case of raisins, consumer acceptance will not be an issue, even though wine is considered a traditional product and any novelty concerning its production techniques is resisted by consumers.

Therefore, the aim was to investigate the suitability of raisins as immobilisation supports, suitable for wine-making at ambient and low temperatures, that would lead to a dry white wine of improved aroma profile that could be characterised as novel.

2. Materials and methods

2.1. Yeast

Dried *Saccharomyces cerevisiae* of *Uvaferme 299*, which is widely used in industrial wine-making, was used in the present study. *Saint Denis* grape must for white wine-making was used, which does not contain sucrose, pigments or preservatives. It was adjusted to an initial 11.3 °Be density or 192 g of sugar per litre. Dried raisin berries of the Greek variety *Sultanina* were used as support for yeast immobilisation.

2.2. Preparation of support and immobilization of cells

150 g of *Sultanina* raisins (apparent volume 200 ml, real volume excluding air spaces 100 ml) were placed in a 1 L glass cylinder and covered with 400 ml of warm water (30 °C) in which 10 g of dried yeast were suspended. The total bioreactor volume was 500 ml. The raisins absorbed about 100 ml of water and their volume was correspondingly increased. The system was allowed to ferment the extracted sugars at 30 °C. The amount of cells immobilized on raisin berries at this stage was calculated by an indirect gravimetric method, comparing two fermentation systems containing the same amounts of yeast with and without the presence of raisins. It was calculated that approximately 2.5 g of yeast were immobilized on 150 g of raisins (1.67 g of yeast per 100 g of raisins).

2.3. Fermentations

Yeast was immobilized on 150 g of dried raisins, as described above, and the biocatalyst was placed in a 1 L glass cylinder for alcoholic fermentation. In the first batch fermentation, the sugars contained in the raisins were the fermentation substrate. When the °Be density reached 0 the fermented liquid was removed, and for the second batch fermentation, the residual raisin sugars plus 350 ml of sterilized grape must of 11.3 °Be density were used as substrate. After that, all raisin sugars were extracted and fermentations were performed using (exclusively) the grape must as substrate. After the end of each batch fermentation, the wine produced was collected and the immobilized biocatalyst was washed with fresh grape must, and 350 ml of grape must were added for the next batch fermentation. From this point, the raisins reached a maximum swelling and the total bioreactor volume became 600 ml.

In a similar way, batch fermentations were carried out with free yeast cells, adding 350 ml of grape must and 2.5 g of yeast in a 500 ml glass cylinder.

2.4. Analytical methods

Fermentation kinetics were obtained by measuring the °Be density at various time intervals. The final products were collected and analysed for ethanol, residual sugar, total acidity, volatile acidity and volatile by-products. Ethanol concentration was determined by gas chromatography (GC) and high performance liquid chromatography (HPLC) as ml of ethanol per 100 ml of wine (% v/v or ''alcoholic degrees''). Ethanol productivity was calculated as grammes of ethanol per litre liquid volume produced per day (g/l/day). Wine productivity was calculated as grammes of wine per litre total volume produced per day (g/l/day), considering that wine density is 1 g/ml. Residual sugar and ethanol were determined using HPLC on a SHIMADZU LC-9A Liquid Chromatograph. A Shim-pack (SCR-101 N) column, a refractive index detector, three times distilled and filtered water with a flow rate of 0.8 ml/min as mobile phase and 1-butanol, as internal standard, were used. The column temperature was 60 °C.

Volatile by-products were determined by means of GC using a Shimadzu GC-8A Gas Liquid Chromatograph, with a stainless steel column packed with Escarto-5905 consisting of 5% squalene, 90% Cabowax-300 and 5% di-2-ethyl-hexyl sebacate, with N_2 as the carrier gas (20 ml/min) and a FID detector (Cabezudo, Gorostiza, & Herraiz, 1978). The injection port and detector temperatures were 210 °C and the column temperature was

70 °C. The internal standard was 1-butanol at a concentration of 0.1% (v/v). Samples of 4 μl of wine were injected directly into the column and the concentrations of the above compounds were determined using standard curves. Methanol and ethanol were determined by a Shimadzu GC-8A Gas Liquid Chromatograph, with a column packed with Porapac-S, N_2 as the carrier gas (20 ml/min) and an FID detector. The injection port and detector temperatures were 210 °C and the column temperature was programmed between 140 and 180 °C. 1-Butanol was used as the internal standard and samples of 2 μl of wine were injected directly into the column.

2.5. Scanning electron microscopy

A piece of the immobilized biocatalyst (yeast cells immobilized on raisin berries) was washed with deionized water and dried overnight at 30 °C. The sample was coated with gold in a Balzers SCD 004 Sputter Coater for 3 min and examined in a JEOL model JSM-6300 (Japan) scanning electron microscope (Fig. 1).

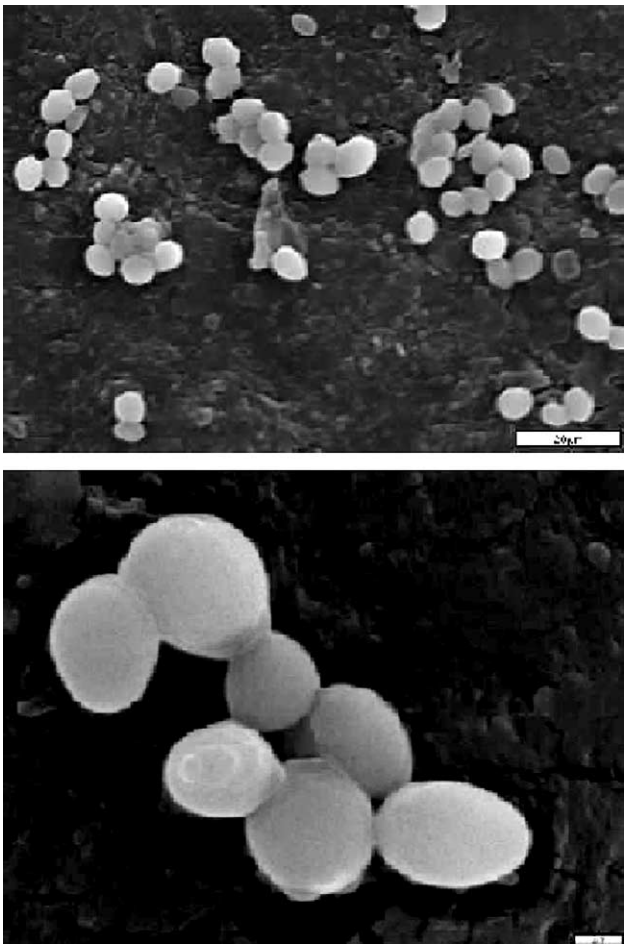

Fig. 1. Electron scanning micrograph of *S. cerevisiae* AXAZ-1 cells immobilised on the surface of raisin berries.

2.6. Sensory evaluation

Sensory evaluation was conducted by 15 trained oenologists using a triangle test (one-tailed, $p = 1/3$) as proposed by Seldom in 1979 (Amerine & Roessler, 1983) for pairs of samples (wines produced by free and immobilized cells).

3. Results and discussion

Cells immobilized on raisin berries (*Sultanina* variety) were found to be suitable for wine-making at ambient temperatures and the biocatalyst appeared to have good operational stability. Cell immobilization on grape raisins was proved by the ability of the immobilized biocatalyst to perform successive fermentations, although the support was washed after each batch to remove free cells. Electron microscopy examination (Fig. 1) showed yeast cells attached to the raisin surfaces. Cell immobilization on raisin berries takes place, either by natural entrapment, or chemical forces. In the first case, the cells enter the interior of the berry and the resulting cells have no way out, yet can multiply. As far as the surface is concerned, it is possible that cell wall proteins form bonds with tannins (polyphenols) of the raisin surface. The raisin production method includes a step of immersing in KOH solution just before the drying step, which operates as a deligrification medium (Georget, Guardo, Smith, & Waldron, 1997) allowing easier removal of water from the berry interior and easier entrance of yeast during the immobilization step.

Repeated batch fermentations were conducted with entrapped and free cells, separately, at different temperatures (6, 15 and 25 °C). The cell populations were such as to allow similar fermentation times for both free and immobilized cells. The results are summarized in Tables 1 and 2. Table 1 shows the ability of cells to ferment and the effect of temperature on fermentation times. Fermentation times were within desirable ranges for the production of wines, taking into account that the spontaneous must fermentation without extra addition of yeast takes 1–2 days (maturation period not included).

Ethanol and wine productivities were high and residual sugar traces (<0.1 g/l) were detected (Table 1), showing suitability for dry wine production. The immobilized cells were able to ferment completely at 15 and 25 °C, as were the free yeast cells, but fermentation, in both cases, slowed down at low temperature (6 °C) requiring fermentation times of at least 30 days. This was expected, as the strain used was not a cryotolerant one.

Total acidity (expressed as tartaric acid) of all produced wines was ≈6.0 g/l. Volatile acidity was 0.3–0.34 g/l

Table 1
Kinetics of repeated batch fermentations of grape must (11.3 °Be) by yeast cells immobilized on dried raisin berries and free cells

Batch	Temperature (°C)	Fermentation time (days)	Ethanol concentration (%v/v)	Ethanol concentration (g/l)	Ethanol productivity (g/l/day)	Wine productivity (g/l/day)	Residual sugar (g/l)	Conversion (%)
Immobilised cells								
1, 2, 3, 4 and 5	25	3	11.5	91	30	194	Traces	100
6, 7, 8 and 9	15	5	11.5	91	18	117	Traces	100
10, 11 and 12	6	7–14	6.1–9.5	48–75	5–7	–	34–92	52–82
Free cells								
1, 2, 3, 4 and 5	25	3	11.5	91	30	333	Traces	100
6, 7, 8 and 9	15	5	11.5	91	18	200	Traces	100
10, 11 and 12	6	7–14	5.9–9.1	47–72	5–7	–	41–91	53–79

Table 2
Effect of temperature on formation of volatile by-products in the repeated batch fermentations of grape must by yeast cells immobilized on dried raisin berries and free cells

Batch	Fermentation temperature (°C)	Acetaldehyde (mg/l)	Ethyl acetate (mg/l)	1-Propanol (mg/l)	Isobutyl alcohol (mg/l)	Amyl alcohols (mg/l)	Methanol (mg/l)	Total volatiles (methanol excluded) (mg/l)
Immobilised cells								
1, 2, 3, 4 and 5	25	12–25	17–33	8–23	23–57	66–95	100–180	155–204
6, 7, 8 and 9	15	22–24	27–44	9–22	14–25	46–58	48–75	140–181
10, 11 and 12	6	20–60	21–25	22–27	8–10	31–44	90–95	124–151
Free cells								
1, 2, 3, 4 and 5	25	10–35	28–39	6–22	18–37	76–98	30–90	165–197
6, 7, 8 and 9	15	14–28	28–48	10–20	12–22	46–64	35–70	113–165
10, 11 and 12	6	30–65	7–22	15–17	4–6	15–42	30–32	96–117

(expressed as acetic acid) without significant differences between immobilized and free cells.

The wines produced were also analysed for the formation of the major volatile by-products (Table 2). In the first 5 batch fermentations, the methanol concentrations in wines produced by immobilized cells (100–180 mg/l) were higher than those of free cells (30–90 mg/l) but within normal limits, as expected from the presence of raisin berries. After that, a reduction in methanol concentration was observed. Acetaldehyde concentrations were low (12–25 mg/l), due to the fast start of alcoholic fermentation but higher in the case of samples that remained for a longer time in the fermentation vessel. The low acetaldehyde concentration is important and may lead to the possibility of wine production without or with minimum addition of SO_2, which is a fundamental practice in wine-making (Liu & Pilone, 2000). The ethyl acetate concentration was relatively low (17–44 mg/l) and propan-1-ol, isobutyl alcohol and amyl alcohols showed no statistically significant differences from wines produced by free cells (Table 2).

Samples from batches 4 and 7 of the immobilized cells remained in the fermentation vessel after the end of fermentation, for 21 days at 5 °C. This storage period did not affect the recovery of cells or their ability to ferment. This fact supports the ability of the system to tolerate stress conditions during prolonged periods of operational shutdown that normally occur on an industrial scale, for example due to lack of raw material.

The immobilized cell system operated for more than 4 months, taking into account the red wine-making fermentation batches that followed, during which the raisin berries remained intact. This is a significant observation but, on the other hand, the raisin cost is negligible, especially for those deriving from raisin syrup-producing plants, which are normally discarded after extraction of sugars.

Sensory evaluation was conducted using a triangle test (one-tailed, $p = 1/3$) judging between pairs of samples (wines produced by free and immobilized cells) and in all cases differences were observed (probability level: 1%). On the other hand, no differences were observed between samples produced by different batch fermentations using each type of biocatalyst. The preference between pairs of samples of wines produced by immobilized and free cells was not statistically significant except in the case of samples 4 and 7 (after storage) where a significant preference for wines produced by immobilized cells was observed. This was attributed to the H_2S smell in samples produced by free cells, due to the prolonged contact of wine with the yeast cells. No such odour was detected in the case of wines produced by immobilized cells.

4. Conclusions

Grape raisins are materials suitable for yeast immobilization. They are cheap, readily available, of food grade and their use needs no pretreatment. The immobilized biocatalyst shows good stability, which makes its use possible at industrial scale. The alcohol-tolerant yeast strain used was not negatively affected by the immobilization and the biocatalyst support produced wines with special flavour, improved quality due to low volatile acidity, low acetaldehyde and ability to decrease use of SO_2.

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