Journal of Industrial Microbiology, 11 (1992) 19–22

Published by Elsevier



SIM 00444

# Localization of volatile acidity reducing factors in grape

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(Received 23 October 1991; revision received 30 June 1992; accepted 6 July 1992)

*Key words:* Grape musts; Clarification; Acetic acid; Wine yeasts

## SUMMARY

Must clarification processes cause an increase in the acetate content of wine at the end of the alcoholic fermatation process, this phenomenon being particularly noticeable when fermentation is obtained by means of the so-called 'high acetate-producer' yeast strains. The influence of different must fractions (free run juice, pressed juice, skins and seeds) on acetate production in white grape was investigated, and the addition of skins and seeds to a synthetic nutritive medium (MNS) was seen to cause a considerable reduction in acetate production. Strain-related differences become evident when the grape bunch is subjected to heat shock (90 °C) before musting. In such conditions, acetate content after fermentation is approximately the same as that of the control specimen (not heat treated) for the low acetate-producer strain (S191c) and higher for the high producer strain (S22b). This suggests the presence of some thermolabile factor that is responsible for inhibiting acetate production. In order to determine the chemical nature of this factor, a series of tests was performed on two substances contained in grape skins and seeds, i.e., polyphenolic compounds and unsaturated fatty acids. A reduction in acetate production was observed in the presence of both substances, their effect being greater when used in connection with high acetate-producer yeast strains.

## INTRODUCTION

During the alcoholic fermentation of white grape or red must subjected to the addition of charcoal and clarification, average acetate production is between 0.2–0.7 g/l. Small amounts of ethyl acetate (20 to 40 mg/l) are also produced [3]. Although the presence of ethyl acetate cannot be detected by olfactory and gustative means, other than in concentrations of greater than 180 mg/l [8], it is commonly believed that in good quality wine acetate content should be as low as possible and should never exceed 0.7 g/l. When acetate formation is higher than this, there are grounds to suspect bacterial contamination (*Acetobacter* or *Gluconobacter* spp.) or the intervention of *Kloeckera* spp. The yeast strains employed in the fermentation process are also thought to play a role [4].

Paradoxically, a must produced by means of modern technologies designed to obtain highly refined white wine of superior taste and quality will often give rise to a final product with a higher than average acetate content. An exceedingly high level of volatile acidity, which is absent in pressed juice, in fact, is seen to be present in clarified white free run juice, and it reappears in pressed juice after a strong clarification treatment [1]. Clearly, the 'muddy material' removed by the clarification treatment contains important factors, both for yeast growth and fermentation purposes [5–7], and for controlling the level of volatile acidity [9]. Since gelatin acts by forming colloidal complexes with wine cathechins and tannins, as does bentonite (by absorbing anthocyans) [10], we formulated the hypothesis that these phenolic compounds might represent the fraction of the 'muddy material' that plays a crucial role in inhibiting acetate production.

The aims of this investigation were to verify the foregoing assumption by assessing the role of polyphenols and to localize the fraction containing the volatile acidity reduction factors in the grape bunch.

## MATERIALS AND METHODS

### *Yeast strains*

Two yeast strains were employed: a low acetate-producer strain, S191c (= *Saccharomyces cerevisiae* f.r. *cerevisiae*), and a high acetate-producer strain, S22b (= *Saccharomyces cerevisiae* f.r. *bayanus*). Both strains were supplied by Istituto Sperimentale per l'Enologia of Asti. In laboratory tests, the inoculum of the two strains was $10^4$ cells/ml; in wine cellar tests, it was 2% w/v.

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*Medium*

Industrial tests were carried out on natural must obtained from Riesling grape. Industrial processes are illustrated in Fig. 1.

In one test, the grape bunch was subjected to heat-shock (90 °C) before must production.

Laboratory tests were also performed on Riesling grapes. A flow chart of the testing procedures is shown in Fig. 2. All must fractions (30 g/150 ml) were added to a synthetic nutritive medium (MNS) of the following composition:

(a) vitamins: pyridoxine hydrochloride (400 μg/l), thiamine hydrochloride (400 μg/l), inositol (2000 μg/l), biotin (20 μg/l), calcium pantothenate (400 μg/l), nicotinamide (400 μg/l), *p*-amino benzoate (200 μg/l).

(b) microelements: $Na_2MoO_4 \cdot 2H_2O$ (200 μg/l), $ZnSO_4 \cdot 7H_2O$ (400 μg/l), $CuSO_4 \cdot 5H_2O$ (40 μg/l), $H_3BO_3$ (500 μg/l), KI (100 μg/l), $FeCl_3 \cdot 6H_2O$ (400 μg/l), $MnSO_4 \cdot H_2O$ (400 μg/l).

(c) macroelements: $CaCl_2$ (0.1 g/l), NaCl (0.1 g/l), $KH_2PO_4$ (1.0 g/l), $MgSO_4 \cdot 7H_2O$ (0.5 g/l), $(NH_4)_2SO_4$ (0.944 g/l), $(NH_4)_2HPO_4$ (0.943 g/l), tartrate (3.0 g/l), KOH (enough to obtain pH = 3.0), sucrose (180 g/l).

Additional tests were performed by adding 278.7 mg/l of anthocyans and 1 ml of Tween 80 (dissolved in ethanol) to 145 ml of MNS.

*Analytical methods*

Acetic acid was determined enzymatically, according to the instructions of Boehringer. Ethanol was calculated from the weight loss in the fermentation bottles [2], a procedure that was chosen because of the limited amount of samples necessary for the analysis, and because it is in keeping with the distillation methods prescribed by EC standards on wine control. Furthermore, both determination methods exhibit a close correlation with enzymatic determination (Boehringer Kit) ($R = 0.42$; $n = 58$; $P = 0.01$) [2].

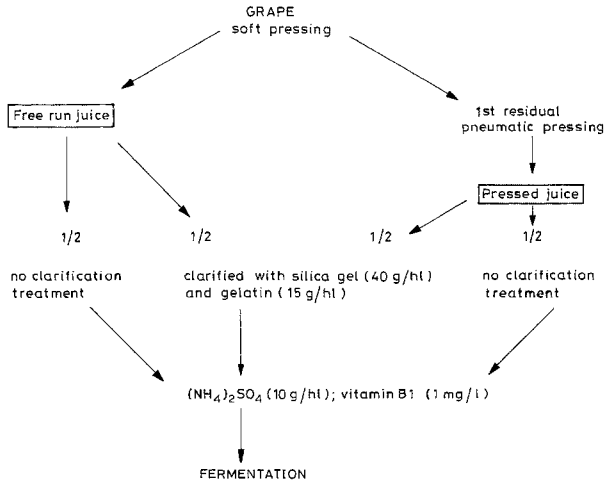


Fig. 1. Flow-chart showing must production and processing up to fermentation in industrial tests.

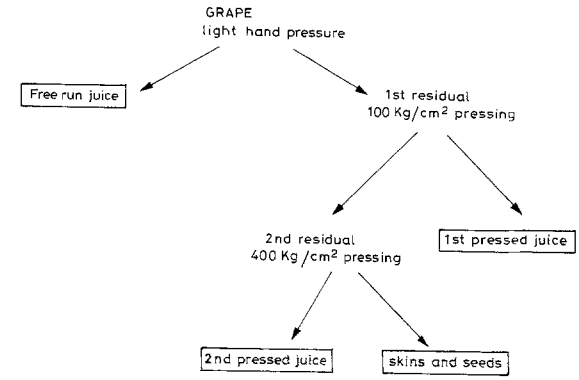


Fig. 2. Flow-chart showing the origin of the different must fractions employed in laboratory tests.

## RESULTS

Fig. 3 shows the influence of the industrial clarification treatment on acetate production during alcoholic fermentation with a high acetate-producer strain (S22b). A gradual increase in acetate content is observed as we go from

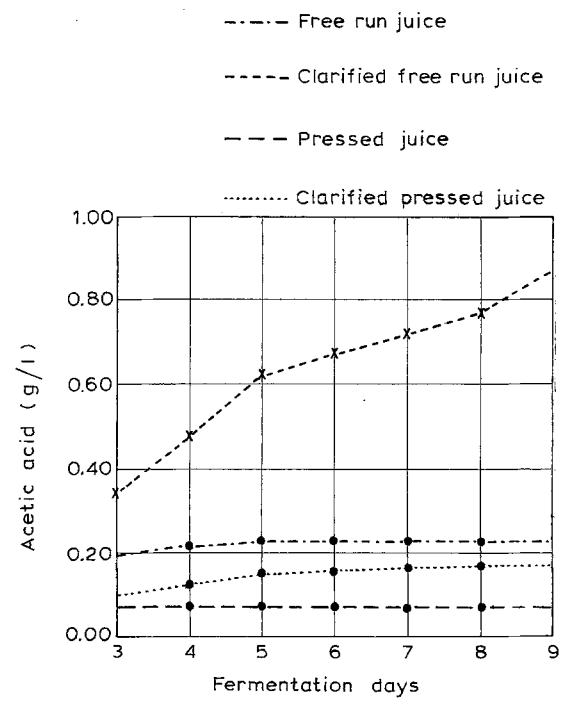


Fig. 3. Acetic acid production of strain S22b (high acetate producer) in different must conditions.

pressed juice to clarified pressed juice and from free-run juice to clarified free-run juice.

The main difference between pressed juice and clarified must lie in the cell composition: biological fractions, such as external pulp cells, skin cells and seeds, in fact, are present in sizeable amounts in the former, but are absent in the latter. Our hypothesis is that the factors inhibiting acetate production may be located in the seeds, skins and external pulp cells.

To explore this hypothesis, we carried out some in vitro experiments with a high acetate-producer strain, S22b, by adding different must fractions to the MNS. The results, as summarized in Table 1, strongly suggest a central role of grape skins and seeds in the reduction of acetate production.

The 1st and 2nd fractions of pressed juice are also involved in this phenomenon: the differences between the 1st pressed juice fraction and the skin and seed fraction are seen to be significant, while there are no significant differences between the 2nd pressed juice fraction and the skin and seed fraction. An explanation for this might lie in the different time of contact between must and skin, a longer time enabling the extraction of some compounds (from skin to must) to take place.

In order to identify the chemical nature of the factor that is responsible for reducing the acetate content of wine, the grape bunch was subjected to heat shock (90 °C) before fermentation. As shown in Fig. 4, results are totally different depending on whether we consider the high acetate-producer (S22b) or the low acetate-producer strain (S191c). The fermentation juice obtained from grape heated to 90 °C shows higher acetate production when strain S22b is used, while the heat treatment makes no difference with strain S191c.

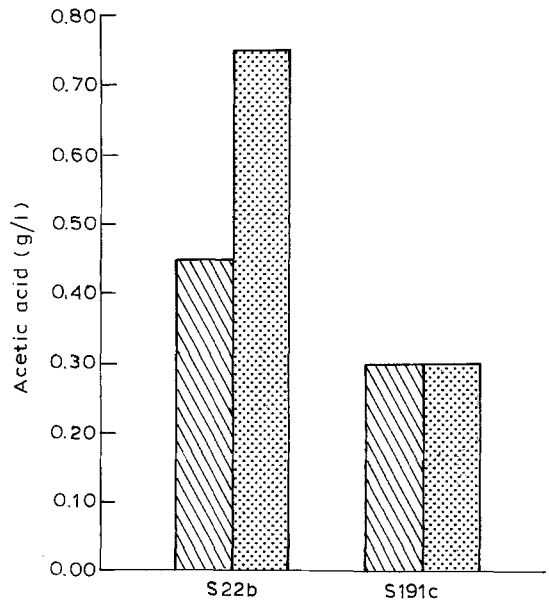


Fig. 4. Influence of grape heat shock (90 °C) before fermentation. S191c = *Saccharomyces cerevisiae* f.r. *cerevisiae* (low acetate-producer). S22b = *Saccharomyces cerevisiae* f.r. *bayanus* (high acetate-producer). Heat shock (90 °C), ⊡ ; not heat treated, ▨.

Since the main components of grape skin are polyphenolic compounds and the predominant constituents of seeds are unsaturated fatty acids, laboratory tests were performed by adding both of these substances to the same culture medium. The tests were carried out on both the high and the low acetate producer strains. Table 2 shows

TABLE 1

Acetic acid and ethanol production at the end of fermentation (13th day) in a synthetic nutritive medium (MNS) with different must fractions added

| Medium | Acetic acid (g/l) | Ethanol (% v/v) |
|---|---|---|
| MNS | 0.77 | 9.42 |
| MNS | 0.71 | 10.08 |
| MNS + free run juice | 0.32 | 11.09 |
| MNS + free run juice | 0.29 | 11.17 |
| MNS + first pressed juice | 0.38 | 10.99 |
| MNS + first pressed juice | 0.47 | 11.05 |
| MNS + second pressed juice | 0.29 | 10.91 |
| MNS + second pressed juice | 0.27 | 10.96 |
| MNS + skins and seeds | 0.22 | 10.77 |
| MNS + skins and seeds | 0.22 | 10.67 |

Yeast strain employed: S22b, *Saccharomyces cerevisiae* fr. *bayanus* (high acetate producer).

TABLE 2

Acetic acid and ethanol produced at the end of fermentation (13th day) by two different yeast strains: S22b, *Saccharomyces cerevisiae* fr. *bayanus* and S191c, *Saccharomyces cerevisiae* fr. *cerevisiae* (low acetate producer) in the presence of Tween 80 and polyphenols

| Yeasts strains | Medium | Acetic acid (g/l) | Ethanol (% v/v) |
|---|---|---|---|
| S22b | MNS | 0.68 | 10.38 |
| S22b | MNS | 0.70 | 10.45 |
| S22b | MNS + Tw80 + polyphenols | 0.24 | 10.03 |
| S22b | MNS + Tw80 + polyphenols | 0.25 | 9.80 |
| S191c | MNS | 0.46 | 10.95 |
| S191c | MNS | 0.45 | 10.52 |
| S191c | MNS + Tw80 + polyphenols | 0.19 | 10.46 |
| S191c | MNS + Tw80 + polyphenols | 0.24 | 10.68 |

the acetate concentrations obtained at the end of the fermentation process: both strains exhibit a considerable reduction in acetic acid content in the presence of polyphenols + Tween 80. As expected, however, the reduction in acetate content was greater in the high acetate-producer strain (S22b) vs. the low-producer strain (S191c).

## DISCUSSION

We may draw several conclusions from these experiments:

(i) The addition of different juice fractions to a synthetic nutritive medium makes it possible to detect the presence of some 'unknown factors' inhibiting acetate production.

(ii) These 'unknown factors' seem to be located primarily in grape skins and seeds.

(iii) From a biochemical standpoint, the nature of such compounds is likely to be phenolic (skins) and lipidic (seeds). In vitro tests with polyphenols and Tween 80 (oleic acid) confirmed the role of such compounds in reducing the acetate content of the fermentation medium.

(iv) The high acetate-producer strain (S22b) generates more acetic acid when the grape bunch is heated to 90 °C before musting, suggesting that a thermolabile factor inhibiting acetate production is destroyed by the heat. It seems reasonable to assume that these thermolabile substances are the polyphenols themselves. Instead, in the case of the low acetate-producer strain (S191c), the preliminary heat treatment of the grape seems to make no difference.

As to the action of polyphenols and unsaturated fatty acids, we can make different hypotheses: unsaturated fatty acids may act at the cell membrane level, preventing the extracellular extrusion of acetate which can therefore be converted intracellularly into other metabolic products, even in anaerobic conditions. At the same time, it is also reasonable to assume that the presence of exogenous unsaturated fatty acids eliminates the need for yeast cells to synthesize such acids. Because of the anaerobic shift during the fermentation process, the biosynthesis of fatty acids stops at acetate formation so that acetate builds up in the cell and is then released into the external medium. By supplying the cell with unsaturated fatty acids, the addition of Tween 80 makes this metabolic pathway unnecessary and, therefore, prevents the overaccumulation of acetate, even in juice subjected to the clarification treatment.

On the other hand, polyphenols may exert an inhibitory effect on the acetaldehyde dehydrogenase, an enzyme which is probably genically overexpressed in high acetate-producer strains.

Further experiments are currently underway in our laboratories to evaluate the inhibitory effects of both polyphenols and skin-juice extracts on the activity of acetaldehyde dehydrogenase.

Considerable care is necessary to obtain good quality (low acetate content) wine from clarified must processed without skins. Recommended measures include: (i) employing low acetate-producer yeast strains, and either (ii) partly soaking the must with seeds and skins, or (iii) adding unsaturated fatty acids and polyphenolic compounds (anthocyans and catechins) which seems to be active factors contained in skins and seeds to the clarified must. The latter method, no yet tested at the industrial level and forbidden by the law, has yielded satisfactory results in laboratory tests.

By taking these measures, it is possible to reduce acetate production without having to forego the clarification treatment, which, needless to say, makes for better quality white wine.

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