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ALCOHOLIC FERMENTATION

Reduction of Aldehydes during Alcoholic Fermentation. Application to Processing of Heads

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Aldehydes are effectively reduced by the action of yeasts during alcoholic fermentation; this provides a simple and effective method for processing heads separated during the distillation of wine into brandy. Acetal was used as the aldehyde source to characterize the ability of 14 species or strains of fermentative yeasts to reduce aldehydes. All yeasts were able to complete the fermentation of grape musts after acetal additions equal to 0.23% aldehyde and all except two at the 0.46% added aldehyde level. The effectiveness of reduction varies, but in all cases of completed fermentations amounts to 90% or more of the quantity added with a corresponding supplement of ethyl alcohol production.

NUMEROUS MINOR CONSTITUENTS are contained in fermented alcoholic liquids. These minor compounds may be regarded as impurities or as flavor materials, depending on their nature and concentration and the intended use of the product. The compounds less volatile than ethyl alcohol accumulate at the top of concentrating columns during distillation. Customarily a low-boiling fraction, termed heads, is separated from the main product during the distillation of wine into brandy.

In the production of brandy, the principal impurities in the heads fraction are acetaldehyde, acetal, ethyl acetate, and acetaldehyde–sulfurous acid, if sulfites have been used during fermentation. In the California wine and brandy industry, sulfur dioxide or bisulfites are commonly used during fermentation, especially in the distilling material used for the production of neutral brandy for addition to dessert wines.

The heads fraction generally contains less than 1% of volatile compounds, but in plants that use an aldehyde-concentrating column it may contain as much as 10 to 15% aldehydes. In either case, ethyl alcohol constitutes the major portion of the liquid, and its recovery in usable form has been a problem in processing procedures. Various procedures, including chemical treatment and further concentration by distillation, have been and still are in use. They generally cost too much or are ineffective in removal of impurities.

Acetaldehyde and its compounds are generally the most objectionable impurities. They impart sharp odors and hot tastes to alcoholic beverages. Ethyl acetate may often constitute a larger percentage of the nonalcohol compounds than the aldehydic ones, especially from liquids fermented without sulfites. However, it is less objectionable and can be considered to have a higher tolerance in alcoholic beverages.

This report includes studies underlying a procedure for the successful removal of the aldehydic portions of heads by reduction during alcoholic fermentation. Heads collected during distilling operations are recycled into subsequent active fermentations of distilling material. Under suitable conditions removal or reduction of aldehydes is complete and a corresponding supplement of alcohol is produced from the aldehydes.

The ability of yeast to reduce numerous aldehydes including acetaldehyde was summarized by Harden (*9*). Acetaldehyde added to a fermenting medium should be reduced to ethyl alcohol in view of the well-established concept that acetaldehyde is the direct precursor of ethyl alcohol in alcoholic fermentation (*2*). Genevois, Peynaud, and Ribéreau-Gayon (*4*) and Ribéreau-Gayon, Peynaud, and Lafon (*11*) have shown that progressive additions of acetaldehyde to fermenting grape juice or must produce increased amounts of minor constituents, including acetic, lactic, and succinic acids and 2,3-butylene glycol, as well as ethyl alcohol. Gade (*3*) patented a process for recycling the forerun or heads from distillation of fermented sulfite liquor into subsequent fermentations. Sundman (*12*) recommended the addition of heads to subsequent sulfite liquor fermentations in order to bind the free sulfite in the liquor and inhibit the formation of more aldehydes, and thus increase the yield of ethyl alcohol.

Guymon and Nakagiri (*5*) showed that the aldehydes in heads from brandy distillations were removed by adding them to fermenting grape musts, most effectively when addition was delayed until the end of the yeast growth phase. These authors (*6*) also reported on the separate effects and limiting concentrations of acetaldehyde, acetal, and ethyl acetate, three principal components of heads, added to grape must both before and during fermentations. These results were applied to the processing of heads in pilot scale and wine industry experiments by Guymon and Pool (*8*).

A particular strain of *Saccharomyces cerevisiae* var. *ellipsoideus*, a wine yeast called Montrachet and widely employed in the California wine industry, was used in these studies. Its behavior constitutes a basis of comparison with that of 13 other yeast species or strains.

Acetal affects fermentations in the same manner and degree as acetaldehyde, if the concentrations used are expressed on an equivalent aldehyde basis (*6*). Hence, acetal, somewhat easier to prepare and handle quantita-

tively than acetaldehyde, was used as the aldehyde source.

Experimental

Materials and Media. Acetal, $CH_3CH(OC_2H_5)_2$, was prepared before each experiment by fractional distillation of Eastman's reagent with collection of a middle cut boiling at 102–3° C. The fermentation media were untreated juices or musts from Thompson Seedless and Emperor grapes. The Thompson Seedless must had been initially stabilized with sulfur dioxide and stored 4 months at 0° C. The initial Brix was 19.3°. Cold storage Emperor grapes were crushed and lightly pressed to produce a must of 17.5° Brix. No sterilization nor sulfite addition was used.

Yeast cultures were started from agar slants and transferred in must at least three times before use as inoculants. Individual fermentations consisted of 1-liter quantities in half-gallon bottles at room temperature (about 75° F.). A 2% inoculation was used.

Procedure. A required volume of must was seeded with active yeast culture. At the end of the growth phase or the start of the active fermentation phase (Brix had decreased to about 12°), the must was subdivided into 1-liter portions for the separate fermentation test lots. All treatments were made in duplicate and consisted of direct additions of acetal. The rate of fermentations was measured by daily checks with a Brix-reading hydrometer.

Analyses were made as each individual lot was completed, to avoid the slow increase in aldehydes after the end of fermentation as reported by Lafon (*10*) and observed by the authors. The methods of analysis were generally those of the AOAC (*1*). Aldehyde determinations were made by the Jaulmes and Dieuzede procedures as given by Guymon and Nakagiri (*7*).

Results and Discussion

The maximum tolerance of *S. cerevisiae* var. *ellipsoideus*, Montrachet strain, to increasing levels of added acetal was sought. Additions of 0, 5, 10, 15, 20, 25, or 30 ml. of acetal per liter of actively fermenting Thompson Seedless must were used. The progress of the fermentations from the time of acetal addition is shown in Figure 1.

Table I. Effect of Acetal on Fermentation of Grape Must by *Saccharomyces cerevisiae* var. *ellipsoideus* (Montrachet)

Acetal Added, Mg./Liter	Acetaldehyde, Equivalent	Mg./100 Ml. Found	Ethyl Alcohol, Vol. %	Volatile Acid, G. HAc/100 Ml.	Time for Completion, Days
		Thompson Seedless			
0	0	10.7	11.0	0.037	2
5	154	12.1	11.3	0.039	3
10	308	14.5	11.6	0.054	5
15	462	26.3	11.7	0.069	15
		Emperor			
0	0	2.2	9.55	0.020	2
7.5	231	4.5	10.39	0.034	2
10.0	308	8.0	10.80	0.035	2
12.5	385	11.4	10.39	0.041	2.5
15.0	462	19.7	11.05	0.058	7

Analytical data are shown in Table I for successful fermentations. The residual quantity of aldehydes increased slightly with higher acetal concentrations, but the conversion up to the 10-ml. level of addition was 99% complete if allowance is made for the residual in the control (10.7 mg. per 100 ml.). This residual level in the control is somewhat higher than that normally found, and probably was caused by the sulfite initially added to stabilize the must.

Ten-liter quantities of must, freshly prepared from stored Emperor grapes, were separately inoculated with cultures of the Montrachet and Tokay strains of *S. cerevisiae* var. *ellipsoideus* and strain No. 1 of *Saccharomyces beticus*. At the time the degree Brix of the three separate media had fallen to approximately 12, subdivisions were made into 1-liter portions as before. Additions of acetal of 0, 7.5, 10.0, 12.5, or 15 ml. per liter of must were made in duplicate. The results of the prior experiment indicated that a maximum of 15 ml. of acetal per liter was sufficient for this survey of the ability of various yeasts to utilize acetal.

A few days later a nearly identical must of 17.5° Brix was prepared for testing the remaining 11 yeasts toward acetal. At the time the Brix of the media receiving each of the separate yeasts had decreased by 3° to 4°, 1-liter duplicated subdivisions were made. The levels of acetal additions were limited to 0, 7.5, or 15 ml. per liter of must.

The pattern of the fermentation rate curves for most of the 14 yeasts was very similar (Figure 2). Only a few yeasts showed any significant difference in pattern (Figure 3).

The analytical data in Table I for the Montrachet strain in the Emperor must are to be compared with those for the stored Thompson Seedless must. For the freshly prepared must (Emperor) containing no sulfur dioxide, the residual aldehyde level of the control was only 2.2 mg. per 100 ml., compared to 10.7 for the first experiment. Correspondingly lower aldehydes were found at comparable levels of acetal additions. The rate of fermentation at comparable acetal levels was also improved in the fermentations of the Emperor must. The supplement of ethyl alcohol from utilization of the acetal is

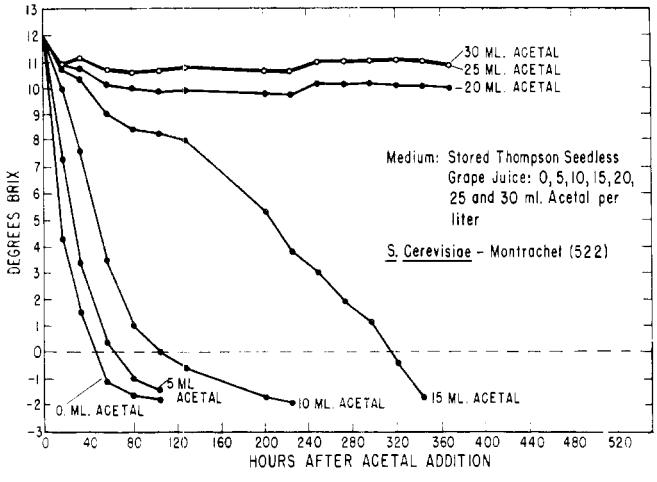

Figure 1. The rate of fermentation decreases with increasing amounts of acetal

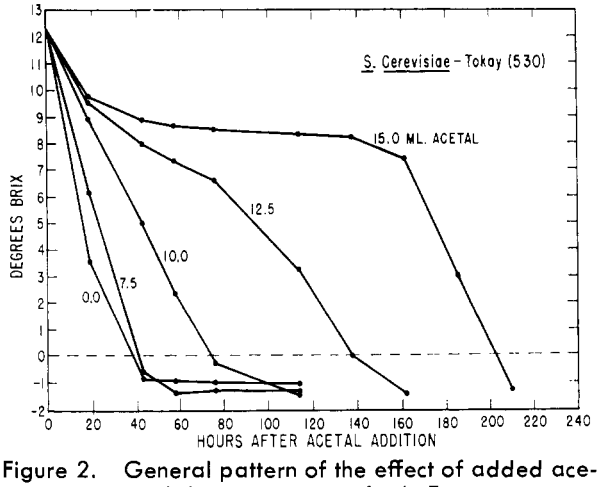

Figure 2. General pattern of the effect of added acetal on rates of fermentation in fresh Emperor grape juice media

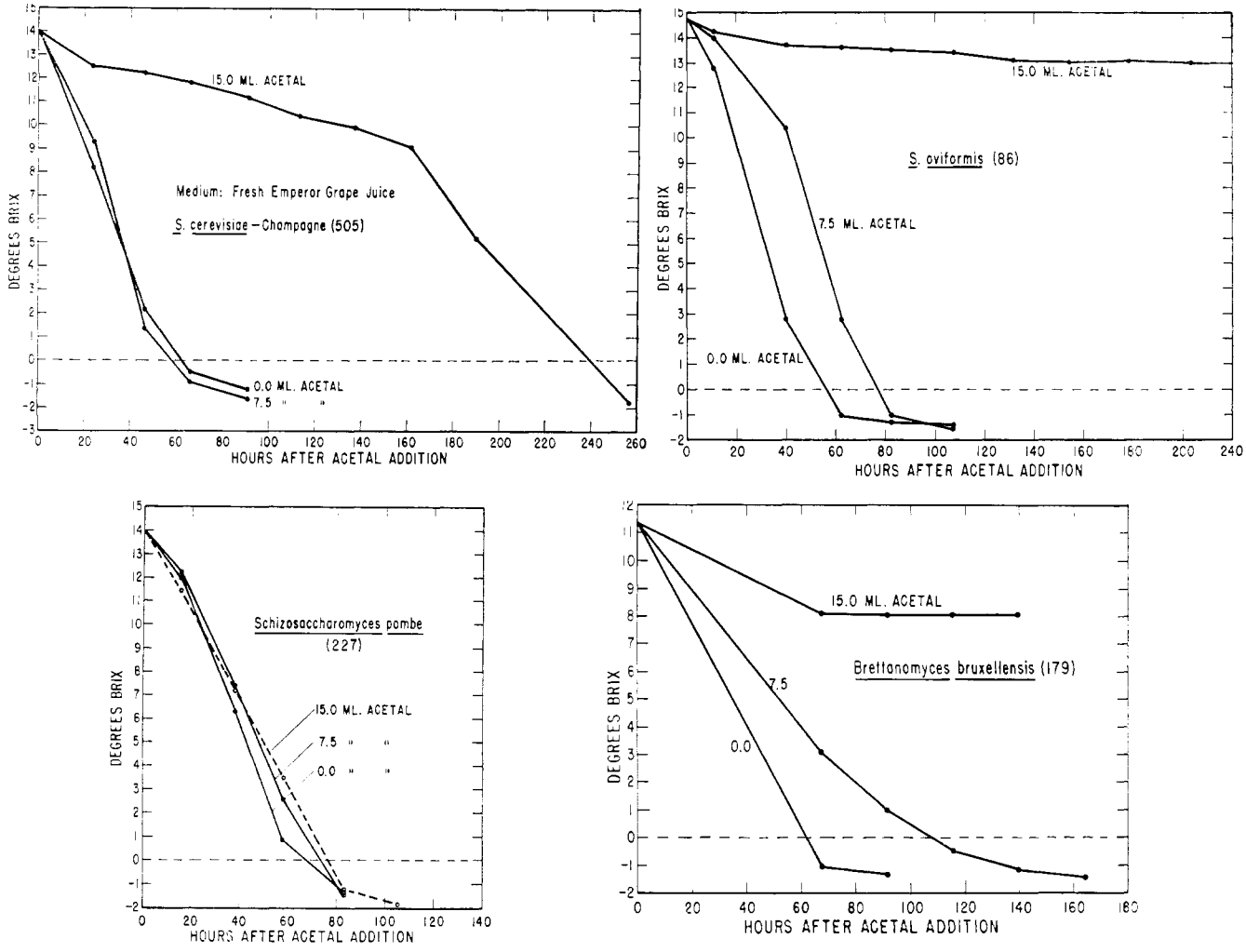

Figure 3. Effect of acetal on alcoholic fermentation by various yeasts

evident from the analytical data.

The aldehyde data in Table II indicate the relative ability of the yeasts to utilize acetal. Two of the wine yeast strains (Burgundy and Champagne) are comparatively ineffective based on these tests. On the other hand, *S. beticus*, strain No. 2, and *Schizosaccharomyces pombe* were very effective at the highest aldehyde levels used. Though the residual aldehyde from use of *Brettanomyces bruxellensis* was lowest of all the yeasts tried, it and *S. oviformis* were most inhibited by acetal; both failed to ferment with 15 ml. of acetal per liter. *S. oviformis*, on the other hand, had high residual aldehyde at the end of its fermentations.

Conclusion

The ability to utilize aldehyde, added as acetal, appears to be characteristic of a wide classification of fermentative yeasts. However, the completeness of utilization varies. A proper choice of yeast strain is important when relatively complete aldehyde reduction is expected in processing distillation heads by refermentation. The widely used Montrachet strain appears to be the best of the Saccharomyces strains tested with respect to completeness of aldehyde reduction, and its fermentation rate was not unduly inhibited by acetal in the medium. Attention to the choice of yeast strain for producing wines to be distilled should result in lower aldehyde in the brandy distillate or decrease the ratio of heads to product that need be withdrawn in practice.

Table II. Ability of Yeasts to Utilize Acetal in Emperor Must

Yeast	Acetal Added, Mg. CH_3CHO/100 Ml.		
	0	231	462
	Residual Aldehyde, Mg. CH_3CHO/100 Ml.		
S. cerevisiae			
var. *ellips.*, Montrachet	2.2	4.5	19.7
var. *ellips.*, Burgundy	17.5	18.3	12.5
var. *ellips.*, Champagne	15.4	15.7	31.4
var. ellips., Tokay	6.8	7.0	20.1
Bakers (1)	8.7	7.2	14.9
Bakers (2)	10.8	14.2	16.9
S. beticus (1)	5.6	8.9	14.5
S. beticus (2)	4.2	6.3	7.1
S. oviformis	17.6	23.6	...
S. chevalieri	6.5	12.7	12.6
Saccharomycodes ludwigii	4.8	5.6	14.0
Schizosaccharomyces pombe	2.7	3.8	6.7
Kloeckera africanus	3.4	15.9	15.0
Brettanomyces bruxellensis	1.3	1.6	...

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