Accelerated Fermentation of High-Gravity Worts and Its Effect on Yeast Performance

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ABSTRACT

Brewery worts of 16, 18, and 20° P were fermented with two yeast strains (J-3015 and J-2036), at three temperature programs (initial temperature of 10° C and maximum temperatures of 16, 18, and 20° C). The attenuation curves were normal with the three wort gravities. The pitching rate had to be increased proportionally to the increase in gravity. The percent of dead cells in the yeast collected after each fermentation was proportional to the gravity of the wort, and this effect was more pronounced when the yeasts were in contact with beers with an alcohol content above 4% by weight at high temperatures (18–20° C). The collected yeast was used in subsequent fermentations, adjusting the pitching rate to compensate for the percent of dead cells. The total vicinal diketone (VDK) curves were different, with strain J-3015 producing higher levels of VDK precursors during fermentation when a maximum temperature of 16° C was used. Both strains showed a tendency to produce lower levels of total VDK as the maximum temperature of fermentation was raised to 20° C.

Key words: Diacetyl-reducing activity, Fermentation temperature, High-gravity brewing, Yeast viability

Today's need to produce good quality beer in a short time and in the least expensive way has prompted many breweries to use high-gravity brewing. Every day more and more breweries are making beer using this technology as a preferred option to conventional brewing.

Some of the main reasons for using this technology include increased production, significant savings in energy, the possibility of using a higher proportion of adjuncts, and a more stable beer. Some disadvantages are a lower brewhouse yield, an increase in the level of esters in the beer, and possible differences in flavor from beer obtained by the conventional process.

Few reports exist in the literature on the use of worts with gravities above 16° P. Schaus (19) in 1971 reported fermentations of worts with gravities up to 26.7° P and a maximum fermentation temperature of 12° C. Longer fermentation times are needed to reach the desired attenuation, suggesting the necessity of using high pitching rates. The beers obtained from such fermentations showed high concentrations of residual α-amino nitrogen, leaving the possibility of using large amounts of adjuncts in the brewhouse. Casey and Ingledew (4) in 1983 reported fermentations of worts up to 39° P (gravities raised by the use of syrups) and observed drastic losses in yeast viability during the first 12 hr after pitching. Owades (16) reported the negative effect of a high osmotic pressure and also attempted to establish an upper limit for high gravity of the worts according to the resistance of yeast to high osmotic pressures. Panchal and Stewart (17) observed excessive excretion of glycerol by yeast subjected to high osmotic pressures. Considering that the use of high-gravity worts will give rise to high concentrations of ethanol at the end of fermentation (over 6% by weight), Nagodawitana and co-workers (14) visualized a possible inhibitory effect of ethanol, when present in concentrations above 5% v/v, by feedback to the glycolytic pathway. Anderson and co-workers (3) also mentioned the noxious effect of ethanol on yeast viability, reporting large percentages of dead cells when yeast was subjected to high concentrations of ethanol.

Another factor directly related to yeast physiology is temperature. Hough et al (9) and Lund (13) wrote excellent discussions on the positive effect of temperature on yeast physiology, determining optimum temperatures for growth and metabolic activity. On the other hand, Epstein (5) reported the negative effect of temperature on metabolic activity.

In brewing, the maximum fermentation temperature has been related to beer quality and, in some instances, with problems in fermentation characteristics such as early yeast flocculation, incomplete attenuation of wort, and accumulation of noxious metabolic substances during the aerobic step. One of the compounds that causes problems in beer quality when present in quantities above its flavor threshold is diacetyl. Many studies have been conducted on the factors that are directly related to the reduction of diacetyl during fermentation and storage. Among them are the relation between the number of yeast cells in suspension and the speed of reduction of vicinal diketones (VDK) (8,10,11,12), the effect of maximum fermentation temperature on yeast flocculation (7), and the differences among yeast strains in their diacetyl-reducing activity (8).

The purpose of this work was to observe the behavior (fermenting activity, ethanol tolerance, flocculence, and diacetyl-reducing activity) of two yeast strains (J-3015 and J-2036) inoculated into brewery worts of 16, 18, and 20° P during six consecutive generations, using three different temperature programs during the fermentations.

EXPERIMENTAL

Microorganisms

Two production strains of *Saccharomyces uvarum (carlsbergensis)* (J-3015 and J-2036) were used. Both strains, previously maintained in wort-agar slants, were propagated by the conventional method using 16° P worts.

Wort

Wort was obtained from the first runnings in our Monterrey plant. The wort was hopped and boiled in the laboratory until the specific gravity was about 1.0830 (20° P). The time required to attain this gravity was about 2 hr. The 18 and 16° P worts were obtained from this wort and with brewing water diluent. All worts were cooled and aerated to saturation before being pitched with yeast.

Fermentation

Fermentations were conducted in 2-L Erlenmeyer flasks containing 1.6 L of wort. The flasks were incubated in a low-temperature incubator (SP-815) calibrated and regulated to follow the temperature programs shown in Figure 1. After the maximum fermentation temperature was reached, the flasks were kept at that temperature until the 15th day. The yeast was cultured in 1% ammonium sulfate aqueous solutions containing increasing concentrations of sucrose (15–20° P). Yeast growth was followed by measuring transmittance (T) of suspensions at 600 nm and plotting (100-%T) versus time. The effect of ethanol concentration on yeast growth and consequently on the rate of attenuation of the wort was determined by conducting fermentations with 16° P worts with increasing additions of ethanol (0–8% by weight) at the beginning of incubation. The samples were incubated at 28° C for 10 days, and wort gravity and the number of dead cells at the end of the procedure were determined.

Determination of Dead Cells

The percent of dead cells was determined by the ASBC methylene blue method (1).

Beer Analysis

Beers were analyzed by using the ASBC methods of analysis (2).

Vicinal Diketone Determination

The VDK content of fermenting wort was determined spectrophotometrically by the method of Garza-Ulloa et al (6).

RESULTS AND DISCUSSION

Results of a series of fermentations that were run to observe the fermentation pattern of carbohydrates by strain J-3015 are shown in Table I. This strain has an excellent fermentative capacity for fructose and sucrose and good fermentative capacity for maltose, glucose, and maltotriose. The pattern was the same regardless of the gravity of the wort, with the percent of transformation to fermentation products being similar in all cases.

The effect of carbohydrate concentration on the growth rate of yeast is shown in Figure 2. Note that the number of cells after 10 days of fermentation was similar for all the carbohydrate concentrations.

Having observed the capacity of strain J-3015 to ferment worts of 16, 18, and 20° P and its resistance to high concentrations of sugars, we proceeded to determine the effect of the number of generations on yeast behavior. Freshly propagated yeast was used to inoculate 16, 18, and 20° P worts. The pitching rate in all cases was kept at 18×10^6 cells per milliliter. The fact that a slow start in the second generation was more evident in the 18 and 20° P worts was corroborated several times by starting again with freshly propagated yeast.

If the percent of transformation to fermentation products is constant for the 16, 18, and 20° P worts, levels of ethanol in the order of 4.7, 5.7, and 6.5% by weight in the corresponding beers should be obtained. To determine if ethanol concentration has an effect on the yeast and causes slow starts in the fermentations that follow, an experiment was done to study the effect of ethanol on yeast activity. The results (Fig. 3) show the noxious effect of ethanol on the yeast. Lower attenuations were obtained at higher

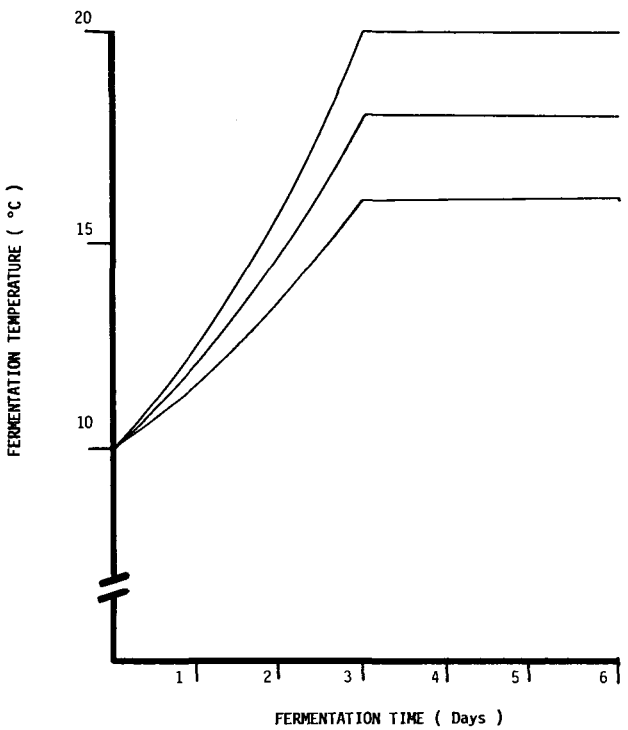

Fig. 1. Temperature programs used in fermentations of high-gravity worts.

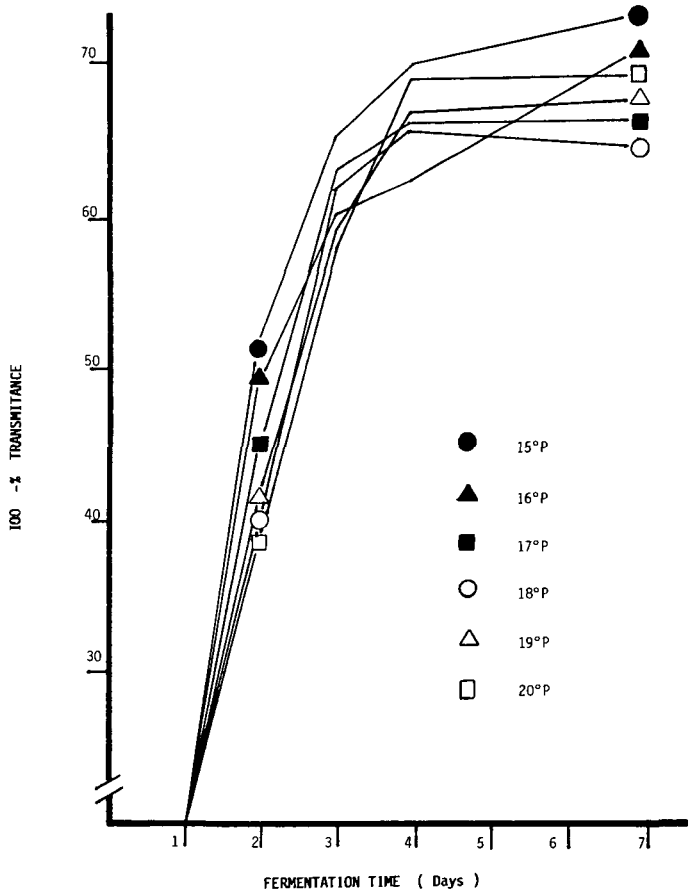

Fig. 2. Effect of carbohydrate concentration on growth of *Saccharomyces uvarum* J-3015.

TABLE I
Transformation of Carbohydrates in Worts to Fermentation Products by *Saccharomyces uvarum* J-3015[a]

	16°P Wort		18°P Wort		20°P Wort	
Compound	**Nutrients**	**Products**	**Nutrients**	**Products**	**Nutrients**	**Products**
Fructose	0.002075		0.002275		0.0025	
Glucose	0.057615	0.00064	0.062790	0.000400	0.0690	0.00054
Sucrose	0.000668		0.000728		0.0008	
Maltose	0.345356	0.00974	0.376376	0.003840	0.4136	0.00423
Maltotriose	0.156288	0.00591	0.170261	0.009560	0.1871	0.00653
Ethanol		0.25430		0.278960		0.30956
CO_2		0.12710		0.139480		0.15478
Higher alcohols and esters		0.00397		0.004822		0.00475
Total	0.561942	0.40366	0.612430	0.437060	0.6730	0.48039
Percent of transformation		71.83		71.30		71.43

[a] Data = moles of carbon.

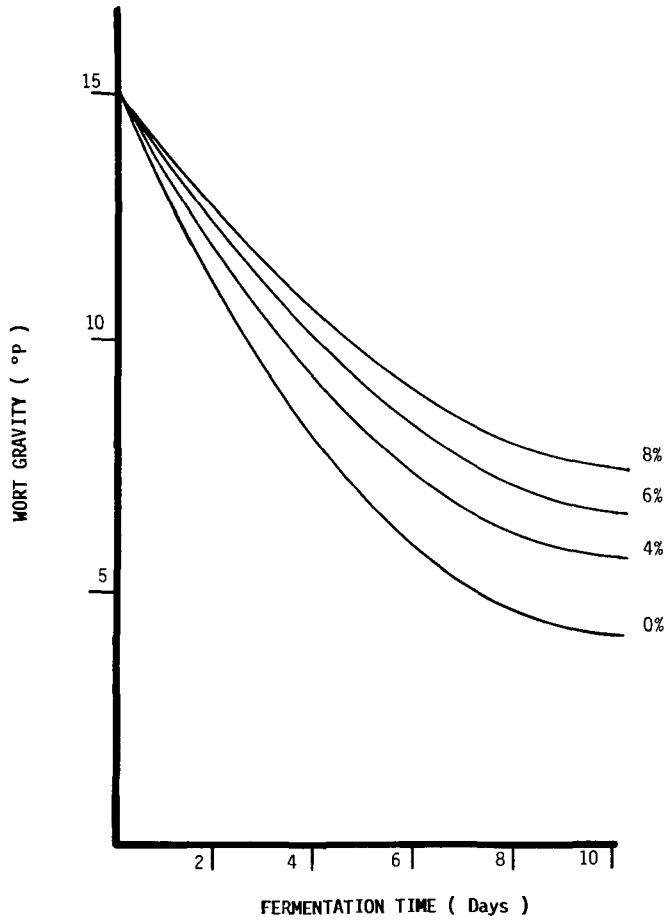

Fig. 3. Effect of ethanol concentration on the fermentative capacity of *Saccharomyces uvarum* J-3015.

concentrations of ethanol. Table II shows a direct relationship between the number of dead cells and ethanol concentration.

Because of the results, we increased the pitching rate proportionally to the increase in gravity, being sure that the cells pitched were all viable (Table III). With adjustments to the pitching rate, fermentations with strains J-3015 and J-2036 proceeded well during six successive generations. There were no slow starts, and rates of attenuation were good.

The attenuation curves obtained with the three wort gravities, fermented at three different temperatures, are shown in Figure 4 for strain J-3015 and in Figure 5 for strain J-2036. The rates of attenuation were normal, which attests to the effectiveness of the pitching rate increase. The slight stimulatory effect of temperature caused the capacity of strain J-3015 to reach maximum attenuation in a shorter time for all wort gravities. When 20° C was maximum

TABLE II
Effect of Ethanol Concentration on Yeast Viability

% Ethanol (w/v)	Total Cells after 10 Days of Aerobic Incubation[a]	Dead Cells	% Dead Cells
0	10.2×10^7	1.08×10^7	10.65
4	5.0×10^7	0.833×10^7	16.66
6	1.33×10^7	0.333×10^7	25.06
8	1.0×10^7	0.333×10^7	33.33

[a] Yeast cells at pitching = 1.8×10^7 cells/ml.

TABLE III
Pitching Rates Used in Fermentations of High-Gravity Worts

Wort Gravity (°P)	Pitching Rate (cells/ml)
16	18.00×10^6
18	20.25×10^6
20	22.50×10^6

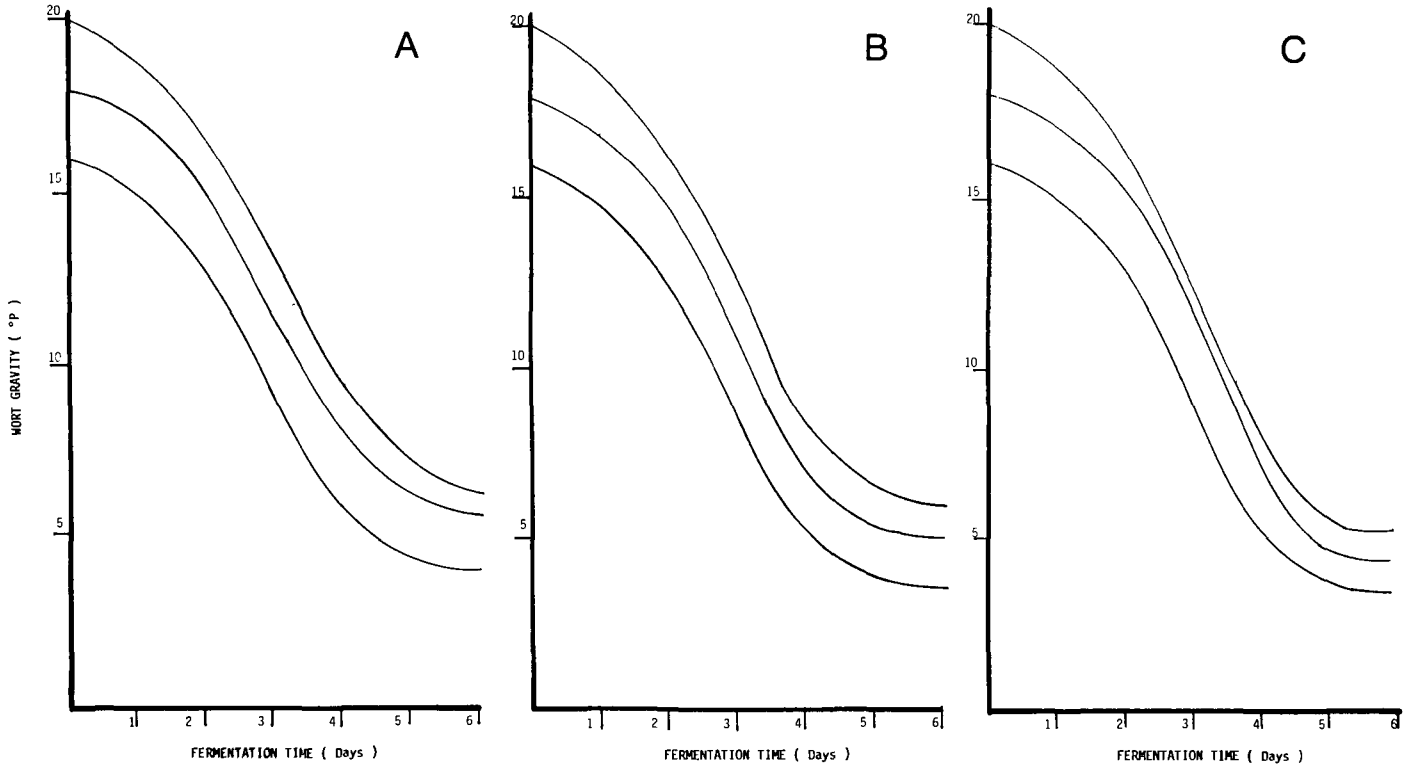

Fig. 4. Attenuation curves of high-gravity worts by *Saccharomyces uvarum* J-3015 at maximum temperatures of **A**, 16° C; **B**, 18° C; **C**, 20° C.

fermentation temperature, the final attenuation was obtained on the fifth day. With 18° C as maximum fermentation temperature, only the 16 and 18° P worts were completely attenuated, and at 16° C, only the 16° P wort was completely attenuated. This tendency was not observed with strain J-2036, which may be due to a lower growth capacity of this strain than that of strain J-3015. The maximum cell counts of both strains by the third day were 30–40 $\times 10^6$ cells per milliliter for J-2036 and 70–90 $\times 10^6$ cells per milliliter for J-3015.

Observing the high levels of ethanol produced in the fermentations of high-gravity worts and their negative effect on viability of yeast, we decided to study the influence on yeast viability of a prolonged fermenting step after the ethanol concentration was over 4% w/v and while the temperature was still

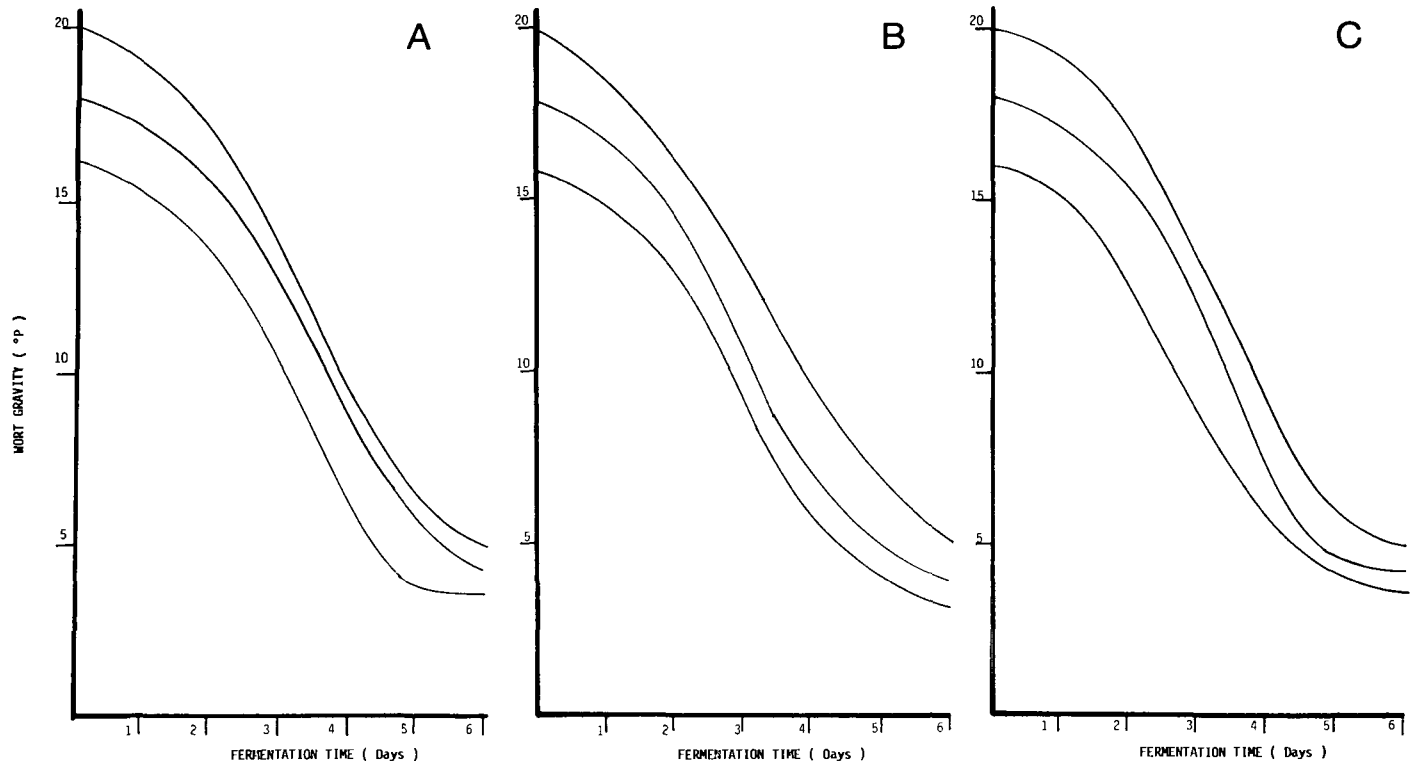

Fig. 5. Attenuation curves of high-gravity worts by *Saccharomyces uvarum* J-2036 at maximum temperatures of **A**, 16° C; **B**, 18° C; **C**, 20° C.

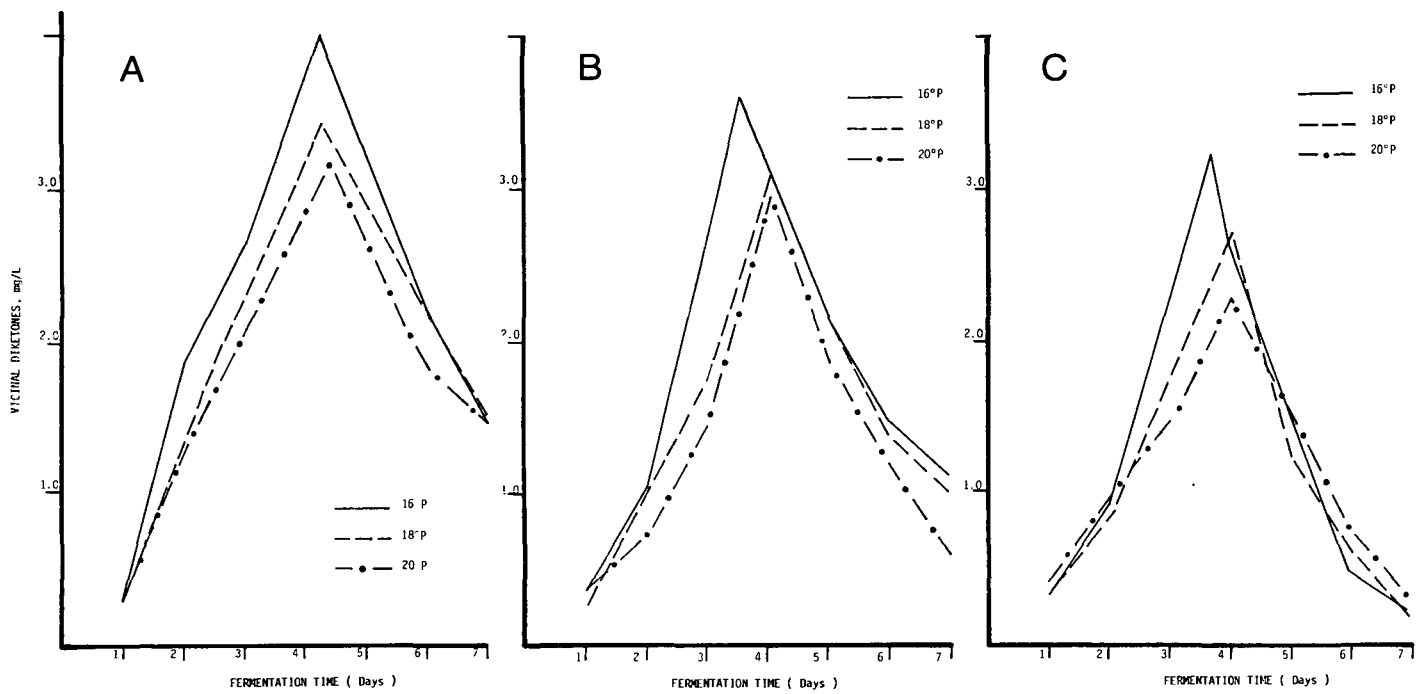

Fig. 6. Vicinal diketone profiles in fermentations of high-gravity worts by *Saccharomyces uvarum* J-3015 at maximum temperatures of **A**, 16° C; **B**, 18° C; **C**, 20° C.

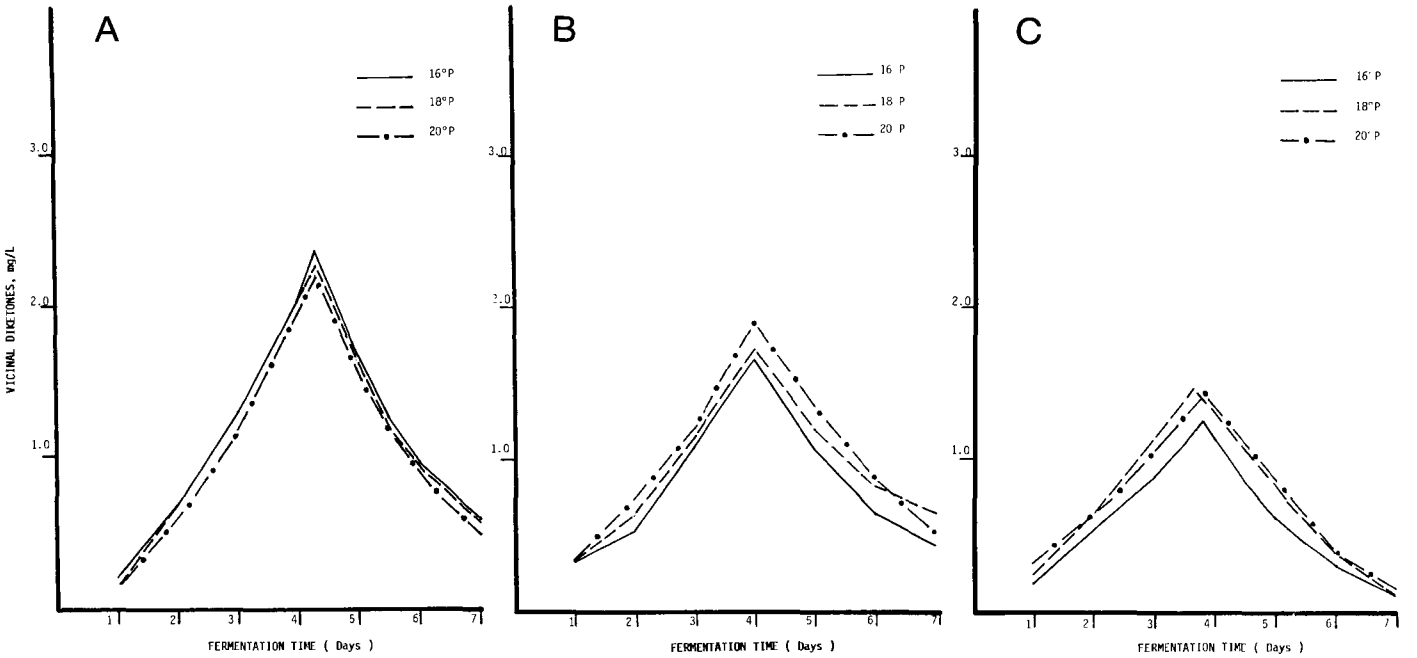

Fig. 7. Vicinal diketone profiles in fermentations of high-gravity worts by *Saccharomyces uvarum* J-2036 at maximum temperatures of **A,** 16° C; **B,** 18° C; **C,** 20° C.

high. The percent of dead cells in settled yeast on the 10th and 15th day of fermentation was determined. The average increases in dead cells varied from 30% at 16° C to 100% at 20° C. No homogeneity was observed, possibly because of the strong variations in the methylene blue method when the yeast samples had been subjected to different storge conditions (18).

The total VDK curves gave good information about the production of VDK precursors and the diacetyl-reducing activity of both strains. Figure 6 shows the VDK profiles of strain J-3015 and Figure 7 shows those of strain J-2036. In all cases, the maximum values of VDK were observed on the fourth day of fermentation, confirming the known relationship between accumulation of these compounds during fermentation and yeast growth (maximum cell counts were detected by the third day of fermentation). Both strains showed a well-defined tendency to produce lower levels of total VDK as the maximum fermentation temperature was raised to 20° C. The yeast strain had a definite influence on the speed of reduction of VDK (15). In all fermentations in which strain J-2036 was used, the levels of VDK were lower than those with strain J-3015, regardless of fermentation temperature or gravity of the worts.

No clear relationship was observed between the number of cells in suspension and the speed of reduction of VDK. The number of cells in suspension showed a strong decrease after the fifth day, whereas the speed of reduction of VDK was almost constant for each strain between the fifth and 10th days of fermentation.

CONCLUSIONS

The strains studied were of normally fermented worts of 16, 18, and 20° P. The pitching rate was increased proportionally to the increase in gravity.

The viability of the cells was strongly affected when the yeasts were in contact with beers with alcohol content above 4% w/v at high temperatures (18–20° C). The percent of dead cells in the yeast collected after each fermentation was proportional to the gravity of the wort. This percent of dead cells must be considered if the collected yeast is to be used in another fermentation.

The difference in the diacetyl-reducing activity between strains J-3015 and J-2036 was maintained in spite of changes in the fermentation temperature and gravity of the worts.

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