

Alcohol Production by Yeast in Whey Ultrafiltrate

INTRODUCTION

Deproteinized ultrafiltrate whey—a by-product of cheese manufacture—has become a serious pollution problem. The major component of ultrafiltrate whey solids is lactose. Utilization of whey as a fermentation substrate has been studied extensively. Different processes utilize whey as a substrate for the production of yeast,¹⁻³ alcohol,⁴ and beverages.⁵ A major problem for the production of alcohol has been the fact that relatively few yeasts are able to ferment lactose. Yoo⁶ found that a strain of *Kluyveromyces fragilis* was the most efficient lactose fermenter when the dry weight of the whey is less than 10%. However, only 55% of the lactose was fermented. O'Leary et al.^{7,8} investigated the potential use of lactose-hydrolyzed whey as a fermentation medium for alcohol production by *Saccharomyces cerevisiae*; they showed that a diauxic fermentation pattern occurred with the result that the fermentation time increased beyond that of conventional whey. We have shown in a survey of 40 lactose-assimilating yeasts that various strains produced a high alcohol yield (12%).⁹

We undertook the study here in order to determine the typical alcohol yield that might be obtained from the fermentation of deproteinized whey permeate by two strains of yeast.

MATERIALS AND METHODS

Organisms

Kluyveromyces fragilis CBS 397 and *Candida pseudotropicalis* IP 513 were maintained on yeast extract agar (Difco) glucose slants at 4°C. Active cultures for inoculation were prepared by growing the organisms in 0.5% yeast extract (Difco), 0.5% glucose at least twice for 24 hr with shaking [80 oscillations/min (opm)] at 28°C.

Deproteinized Whey Ultrafiltrate

The whey was fractionated by ultrafiltration under the industrial process conditions of the Bel France plant. The deproteinized ultrafiltrate was then concentrated to 75% total solids. Whey permeate fractions were diluted to various lactose concentrations with distilled water as needed for use in the fermentation studies.

Cultural Conditions for Ethanol Production

The maximum amounts of alcohol produced from glucose and lactose by the two strains of yeast were determined in medium described by Harris and Morgan.¹⁰ Another test was performed in whey permeate containing different lactose concentrations. Inoculation was at an initial cell concentration of approximately 30×10^6 cell/ml. The fermentation was carried out in sterile flasks under nitrogen atmosphere to maintain anaerobic conditions.

Analytical Procedures

Yeast cell counts

The enumeration of cell populations during growth and fermentation was carried out by pour plate counts on yeast extract (Difco) glucose. Plates were incubated at 28°C and counted after 48 hr.

Alcohol analyses

Alcohol was determined by gas chromatography using a Girdel 300 chromatograph.

Total reducing sugar

Total reducing sugar was measured with 3-5*N* dinitrosalicylic acid reagent using the Bernfeld method.¹¹ A standard curve prepared from dilutions of lactose was used to calculate the reducing sugar in the fermentation media. All readings were taken at 450 nm using a Klett-Summerson colorimeter.

RESULTS AND DISCUSSION

The growth characteristics of *Kl. fragilis* CBS 397 and *C. pseudotropicalis* IP 513 in Harris-Morgan medium or in whey permeate are identical. The maximum population obtained was 350×10^6 cell/ml in two days and the maximum amounts of ethanol are similar in all cases and averaged 12%. The data show that the sterols, considered as an anaerobic nutritional requirement for the growth of yeast,¹² are present in whey permeate. The whey permeate also contains all the vitamins required for the growth of *Kl. fragilis*.

Maximum Alcohol Produced from Glucose Lactose and Whey Permeate

The maximum amounts of ethanol produced from glucose and lactose in Harris-Morgan medium or from whey permeate at sugar concentrations in the 5-30% range are presented in Table I. The optimal sugar concentration for maximum alcohol production is 20-25% for the two strains. Yoo⁶ and O'Leary et al.,⁸ in studies using *Kl. fragilis* NRRLY 1109, also observed that the optimal glucose concentration for maximum alcohol production was 20-25%, but that the optimal lactose concentration was only 10%. In contrast to these reports our results show that glucose and lactose are fermented at the same rate by *Kl. fragilis* CBS 397 and *C. pseudotropicalis* IP 513. These results are in agreement with those of Rogosa et al.⁴

The percentage of theoretical alcohol yields was calculated by dividing the actual yields by the theoretical yield as calculated for the amount of lactose present in the whey permeate. The alcohol production efficiency in whey permeate is presented in Table II for a lactose concentration in the range of 5-30%. The data show that

TABLE I
Maximum Ethanol Production (%) by *Kl. fragilis* and *C. pseudotropicalis* in the Fermentation of Glucose, Lactose, and Whey Permeate (WP)

Substrate conc. (%)	<i>Kl. fragilis</i>			<i>C. pseudotropicalis</i>		
	glucose	lactose	WP	glucose	lactose	WP
5	3.0	3.0	3.1	3.0	3.1	3.1
10	5.9	5.8	6.0	5.7	5.7	5.8
15	8.8	8.8	8.7	8.6	8.8	8.9
20	12.4	10.5	11.4	12.0	11.8	11.5
25	12.3	11.7	11.4	11.7	11.5	11.3
30	9.2	11	10	9.2	11	11

TABLE II
Efficiency of Alcohol Production in Whey Permeate

Substrate conc. (%)	<i>Kl. fragilis</i> CBS 397	<i>C. pseudotropicalis</i> IP 513	<i>Kl. fragilis</i> ^a CBS 5795
5	90	90	90
10	90	87	91
15	87	89	53
20	86	86	30
25	68	68	12
30	50	55	—

^a Strain studies by O'Leary et al.^{7,8}

although more lactose was used as the content increased up to 20%, an over 85% yield was obtained. Yoo,⁶ in a study of alcohol production in whey, reported that *Kl. fragilis* NRRL Y 1109 showed the greatest percentage of conversion of lactose

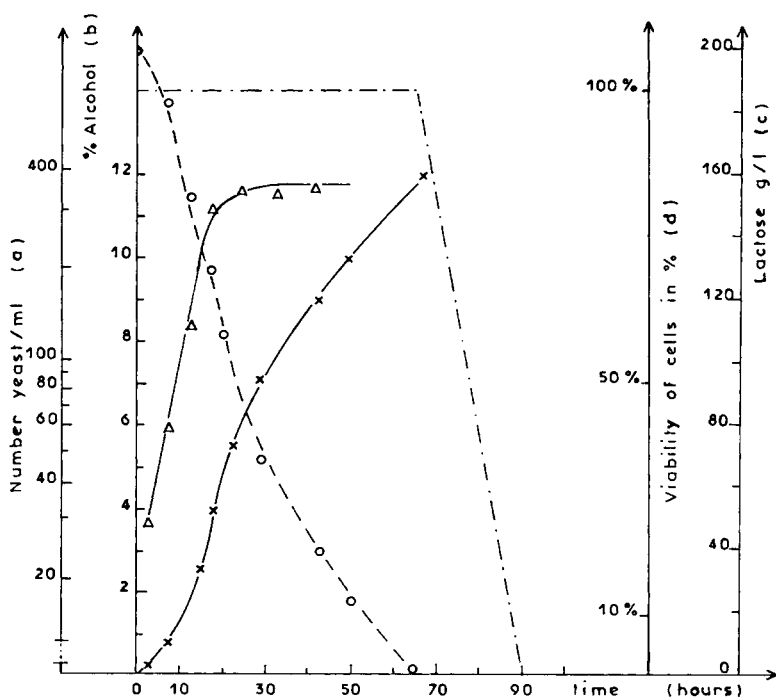


Fig. 1. Kinetic study of alcoholic fermentation of *C. pseudotropicalis* IP 513. (Δ) a; (x) b; (o) c; (— · —) d.

to alcohol in whey solutions containing low lactose concentrations (3–5%). O'Leary et al.⁸ showed that beyond 10%, the conversion efficiency declined rapidly. Thus our results indicate that it is possible to produce alcohol from whey at high lactose concentrations with the two strains tested.

Kinetic Study of Alcoholic Fermentation

Figures 1 and 2 give the growth curve of the two strains, the alcohol production, the viability curve of yeast cells, and the lactose consumption. The entire study was carried out using concentrated whey permeate containing 20% lactose. As can be seen from this representation, the kinetics of alcohol production are similar for these two strains. The specific growth rate (μ) of the cells is 0.17 hr^{-1} ; the cell population never exceeded about $350 \times 10^6 \text{ cell/ml}$. The alcohol production is always close to 12% (v/v). It is interesting to note that the loss of cell viability appeared after termination of the fermentation. When the whey permeate used is not concentrated (5% lactose), the fermentation time is reduced from 48 to 12 hr and the production of alcohol is 3% (v/v).

In this case, the final cell population is not any different. However, we have observed an increase in final cell dry weight when the lactose in whey permeate is increased from 5 to 20%. The cells are much more voluminous. Roland and Alm¹³ reported that hydrolyzed whey permeate syrups prepared by ultrafiltration and

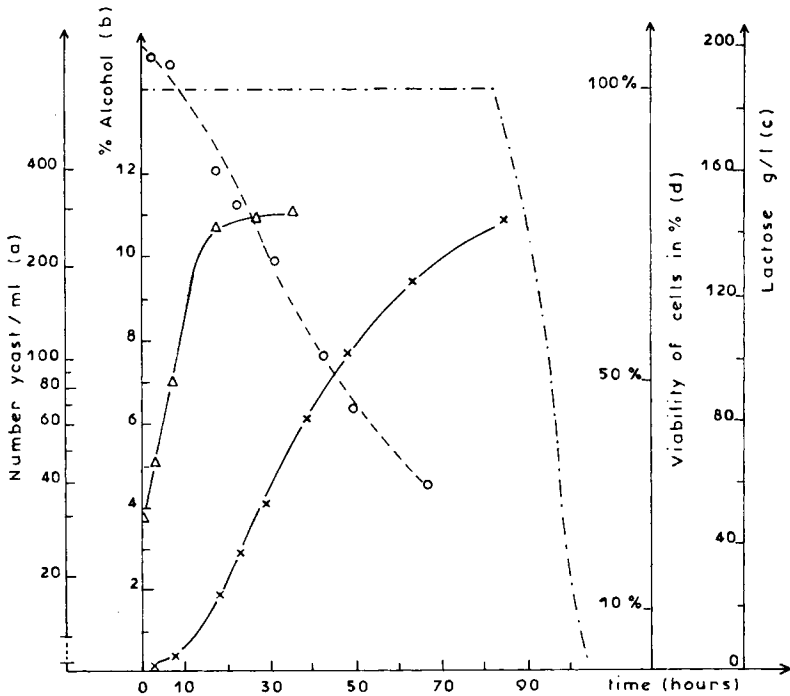


Fig. 2. Kinetic study of alcoholic fermentation of *Kl. fragilis* CBS 397. Symbols are the same as in Figure 1.

demineralization and fortified with different factors would be successfully fermented to up to 12.5% (v/v) beverage alcohol with a culture of *S. cerevisiae* var. *ellipsoideus*. Our data show that with an appropriate strain the same result can be obtained without the hydrolysis of whey.

CONCLUSION

Gray¹⁴ reported that different species of yeast and even different strains of the same species differed widely in their ability to produce alcohol. The data presented here show that the same remark can be made and can explain the great differences observed between our results and those of various other researchers. We have shown that high-efficiency alcohol production is possible from whey permeate concentrated up to 20%. The alcohol yield obtained from whey permeate shows that the prehydrolyzing of lactose is not indispensable.

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