Mixed Culture Fermentation of Cucumber Juice with 
Lactobacillus plantarum and Yeasts

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ABSTRACT

Saccharomyces cerevisiae and Saccharomyces roesi were tested for use in mixed culture fermentation of cucumber juice with Lactoba-
cillus plantarum. Extent of sugar utilization and the ratio of products formed (lactic acid:ethanol) were influenced by time of inoculation 
and cell numbers of each microorganism. Sugar fermentation was 
complete within 6 days at 28°C when similar numbers of bacteria and yeasts were added simultaneously, or when yeasts were added before 
the bacteria. Sugar remained when only L. plantarum was added, or 
when yeasts were added in low numbers. Glycerol was produced when 
yeasts were present, the concentration being directly related to NaCl 
concentration. Results suggest advantageous uses of yeasts to com-
plete fermentation and to modify acidity.

INTRODUCTION

LACTIC ACID BACTERIA are mainly responsible for the 
primary fermentation of brined vegetables such as cucumbers. 
Fermentative yeasts may also grow during the primary fer-
mentation stage, and may grow exclusively during the sec-
ondary stage if fermentable sugars remain after growth by lactic 
acid bacteria ceases (Fleming, 1982). Fermentative yeasts tra-
titionally have been viewed as undesirable in cucumber fer-
mentations because of their CO₂ production and implication in 
blotter damage (hollowness) of the cucumbers (Etchells and 
Bell, 1950).

Acetic buffers have been added to cucumber brines to as-
sure complete sugar utilization during primary fermentation 
(Etchells et al., 1973, 1975). Neutralization of acids during 
fermentation with a pH controller has also been used to assure 
complete sugar utilization (Fleming et al., 1983). However, 
only with cucumbers, green beans and peppers was complete 
sugar utilization accomplished.

Purging of CO₂ from fermenting cucumbers by bubbling air 
or N₂ through the brine is an effective means for preventing 
blotter damage to the cucumbers (Fleming et al., 1973a, 1973b;
Costilow et al., 1977). Thus, the intentional use of fermenta-
tive yeasts in cucumber fermentations may be advantageous 
with the advent of purging. Selected yeasts might remove a 
portion of the fermentable sugars during primary fermentation, 
thereby assuring more rapid and complete utilization of fer-
mentable sugars. By preventing residual sugar after primary 
fermentation, the time necessary to continue purging could be 
shortened and purging costs reduced. Also, by converting a 
portion of the sugars to ethanol by yeast fermentation, unde-
irable high lactic acid concentrations from exclusively lactic 
acid fermentations could be prevented. Mild acidity and other 
flavor characteristics imparted by the yeasts could be highly 
desirable. In this investigation, cucumber juice was used as a 
model fermentation system to represent cucumber fermenta-
tions.

The objectives of this investigation were: (1) to screen se-
lected species of yeasts for their ability to ferment cucumber 

juice at varying concentrations of salt and acids, (2) to deter-
mine conditions necessary to complete cucumber juice fer-
mentation using a mixed culture of a selected yeast with a 
lactic acid bacterium, and (3) to determine the effect of mixed 
culture fermentations on end product concentrations.

MATERIALS & METHODS

Microbial strains

Saccharomyces cerevisiae Y-635, Torulopsis etchellsii Y-6651, 
Saccharomyces baillii Y-2227, Saccharomyces rouxii Y-2547, and 
Candida utilis Y-900, which were used in preliminary screening 
experiments, were obtained from the USDA-ARS, Northern Regional 
Research Laboratory (Peoria, IL). An additional 13 strains, including 
Saccharomyces roesi, were isolated from fermenting cucumbers and 
red pepper mash in our laboratory. Identification of natural isolates 
was done using the physiological tests suggested by Barnett and Par-
khurst (1974). The lactic acid bacterium, Lactobacillus plantarum, 
WSO, is from the Food Fermentation Laboratory (author's labora-

Media

Yeasts were maintained in YM (Difco) broth and lactic acid bacteria 
in MRS (Difco) broth. Cucumber juice for growth studies was pre-
pared by freezing fresh pickling cucumbers at −20°C overnight and 
then partially thawing and blending them to a homogenous slurry. The 
slurry was brought to boiling and rapidly cooled to approximately 
25°C. Successive filtrations were done through cheesecloth, Whatman 
o. 1 filter paper, and sterile, 0.2μ Millipore filters. Juices were 
diluted twofold with sterile distilled water prior to use. Varying con-
centrations and combinations of NaCl (0, 3, and 5% w/v), acetic acid 
(0 and 0.16% w/v) and lactic acid (0, 0.1, 0.25, and 0.50% w/v) 
were added to cucumber juice to simulate different cucumber brine 
conditions. Inoculated media were incubated in static culture in 16 
mm disposable test tubes with Kim Kap enclosures. In testing for 
glycerol production, anaerobic conditions were achieved with BBL 
Gas Pak jars using a carbon dioxide-hydrogen generator.

Growth tests

Relative comparisons of growth among strains in various cucumber 
juice formulations were determined by measuring the specific growth 
rates, and generation times (Drew, 1981). Carbon recoveries were 
determined as described by Wood (1961).

Chemical assays

Reducing sugar concentrations were determined by the colorimetric 
method of Sumner and Somers (1944). Lactic acid, ethanol, and glyc-
erol concentrations were measured using high performance liquid 
chromatography according to the procedures of McFeeters et al. (1984).

RESULTS & DISCUSSION

INITIALLY, five yeast species were selected for screening 
based upon their described properties of being salt-tolerant (>5% 
w/v) and their established use in food fermentations. The initial 
screening tests (data not shown) consisted of comparing the 
ability of the yeasts to grow and ferment sugars in cucumber 
juice containing various concentrations of NaCl and lactic acid. 
Under these conditions, S. cerevisiae Y-635 was selected for
further experiments because of its ability to grow in the presence of comparatively high concentrations of lactic acid (0.5% w/v). A second group of 13 yeasts that we originally isolated from active cucumber fermentations was screened using tests similar to that used with the first group. One isolate, identified as S. rosei, was chosen for further study because, like S. cerevisiae, it grew in the presence of a relatively high concentration of lactic acid (0.5% w/v).

Saccharomyces cerevisiae and S. rosei, were compared to test their ability to ferment cucumber sugars in the presence of various concentrations of lactic acid. The cucumber juice used was supplemented with acetic acid (0.16% w/v), which represents the concentration used in controlled cucumber fermentations. In the absence of lactic acid, S. rosei was able to ferment more than 95% of the sugar in 3 days, whereas S. cerevisiae needed 7 days to ferment 80% of the sugar (Fig. 1).

In the presence of 0.1% lactic acid, the same general pattern existed except for a slight inhibition of both species. At the 0.25% lactic acid concentration, S. rosei was severely inhibited in its ability to use the sugar, whereas S. cerevisiae was not. At 0.5% lactic acid, neither species grew.

Subsequently, the growth rates of the yeasts and of L. plantarum in cucumber juice containing 5% salt and 0.16% w/v acetic acid were determined. Under these conditions, S. rosei was competitive in terms of growth rate (generation time = 148 min) with the L. plantarum (165 min). Saccharomyces cerevisiae was the slower growing of the two yeasts (200 min), and lagged behind the growth of L. plantarum.

Both yeasts were used with L. plantarum in mixed culture fermentations of cucumber juice. In these experiments the inocula consisted of one concentration of L. plantarum and various concentrations of the yeast. In addition, the time of inoculation of each species was varied.

By varying the inoculum level of S. cerevisiae it was possible to manipulate the product ratios of lactic acid to ethanol and at the same time to ferment all of the sugar (Table 1). In addition, the final acidity was reduced, with a higher final pH, when S. cerevisiae was added with L. plantarum, as compared to L. plantarum alone. A similar pattern was observed when S. rosei was added with L. plantarum (Table 2) except that greater amounts of ethanol were produced, which probably reflects the faster growth rate of this yeast under the test conditions. Varying the time of inoculation of the yeast and L. plantarum had a significant effect on the end-product ratios. When inoculation with yeast preceded that of L. plantarum, a

![Fig. 1](image_url)  
Sugar fermentation by yeasts in cucumber juice at 30°C containing 5% w/v NaCl, 0.16 w/v acetic acid and various concentrations of lactic acid. ▲ = S. cerevisiae; ♦ = S. rosei.
greater proportion of ethanol to lactic acid was formed as compared to simultaneous inoculation or inoculation of the yeast 24 hr after L. plantarum.

Carbon recovery varied considerably in completed mixed culture fermentations employing yeasts and L. plantarum. Values were calculated under the assumption that the yeast quantitatively converted haxose to ethanol and CO₂. Lower recovery values were observed with S. cerevisiae than with S. roseei. Lactobacillus plantarum, when grown alone in the same cucumber juice medium, had carbon recovery values greater than or equal to 100%. We have found carbon recoveries to exceed 100% in the fermentation of whole cucumbers by L. plantarum (Fleming et al., 1988). Various explanations were offered for such high carbon recoveries, some of which may be applicable to this study. These included the possibility that not all substrates were accounted for; cell wall material, for example, could have degraded during brine storage to yield fermentable substrates. Also, malic acid, which is present in cucumbers (McFeeters et al., 1982), may have been degraded to lactic acid, but it was not included as a possible substrate.

Several explanations may exist for the low carbon recoveries with yeasts: (1) Since the cultures were grown in static culture, a proportion of the hexoses may have been respired as CO₂ + H₂O; (2) the yeasts may have produced products that we did not measure initially such as glycerol, which some yeasts are capable of producing (Phaff et al., 1966).

Glycerol production by S. cerevisiae was tested in cucumber juice under anaerobic and static incubation conditions. The data show (Table 3) that greater amounts of glycerol and lower amounts of ethanol were produced as the concentration of salt was increased in the cucumber juice. This was observed for both anaerobic and static conditions of growth. Carbon recoveries were consistently higher under anaerobic conditions compared with static conditions, regardless of the salt concentration.

The production of glycerol from glucose under high NaCl conditions was demonstrated by Wei et al. (1982) with Bakers yeast grown in a gelatin matrix. A possible mechanism for glycerol production was presented by Unemoto et al. (1967), who proposed that high NaCl levels inhibited pyruvate carboxylase, which forced phosphoglyceraldehyde to be a substitute electron acceptor resulting in glycerol formation.

Glycerol, like ethanol, would be a neutral fermentation product and would not contribute acidity to a fermentation. It is not known whether glycerol would be a stable product or be used as a substrate by other microorganisms present in mixed culture fermentations.

**SUMMARY**

FROM THIS STUDY we can conclude that: (1) S. cerevisiae and S. roseei are suitable for use in mixed culture fermentations of cucumber juice; (2) complete fermentation of cucumber juice can be achieved by using a yeast in mixed culture with L. plantarum without having to add buffer; and (3) the final pH and product concentrations in such fermentations can be manipulated by varying the inoculum size and the time of inoculation of each species. When N₂ is used in purging cucumber fermentation tanks to prevent foamer damage due to CO₂ accumulation, it is possible that selected yeasts can serve useful purposes in cucumber fermentations.

**REFERENCES**


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Ms received 9/8/87; revised 12/14/87; accepted 12/16/87.

This investigation was supported in part by a research grant from Pickle Packers International, Inc., St. Charles, Ill.
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