

Effect of Various Microorganisms on the Vitamin and Amino Acid Content of Cucumber Brines¹

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A study of the availability of the vitamins and amino acids essential for *Lactobacillus plantarum* in cucumber fermentations (Costilow and Fabian, 1953b) indicated that the concentrations of some of these nutrients were influenced by the microflora present. Therefore, a survey was made of two species of bacteria and four yeast species that have been found to be most active in cucumber fermentations (*L. plantarum*, a coliform organism, *Torulopsis holmii*, *Torulospore rosei*, *Torulopsis caroliniana*, and *Hansenula subpelliculosa*) as to their effect on the concentrations of these nutrients in brine.

MATERIALS AND METHODS

The brine used for testing the effect of the various microorganisms was nonfermented cucumber brine. The preparation of this brine was described previously (Costilow and Fabian, 1953b). Test flasks were prepared by filtering the brine through cheese cloth to remove dirt particles, dispensing it in 500 ml flasks, 250 ml per flask, and sterilizing the flasks at 15 pounds pressure (121 C) for 20 minutes.

The bacteria and yeast cultures used in the tests were all isolated from the commercial cucumber fermentations studied by Costilow and Fabian (1953a), except *Torulopsis caroliniana*. This culture was originally isolated by Etchells and Bell from cucumber fermentations and was supplied to this laboratory by the Northern Regional Research Laboratory, Peoria, Illinois.

The cultures to be tested were grown in micro-inoculum broth (Difco) for 24 hours, centrifuged, and the cells resuspended in 5 ml of sterile isotonic saline. One drop of this suspension was used to inoculate a flask of brine. Each organism was inoculated into one flask of the sterile brine. One flask was not inoculated and served as a control.

After five days' incubation at 30 C, heavy growth was noted in all of the inoculated flasks as evidenced by turbidity and/or sediment. Since several days were required to run all of the microbiological assays, the flasks of brine were autoclaved at 15 pounds pressure for 10 minutes to prevent further activity.

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Microbiological assays for the vitamins—niacin, biotin, and pantothenic acid, and for the amino acids—leucine, isoleucine, valine, tryptophane, glutamic acid and cystine were run by the same methods used by Costilow and Fabian (1953b).

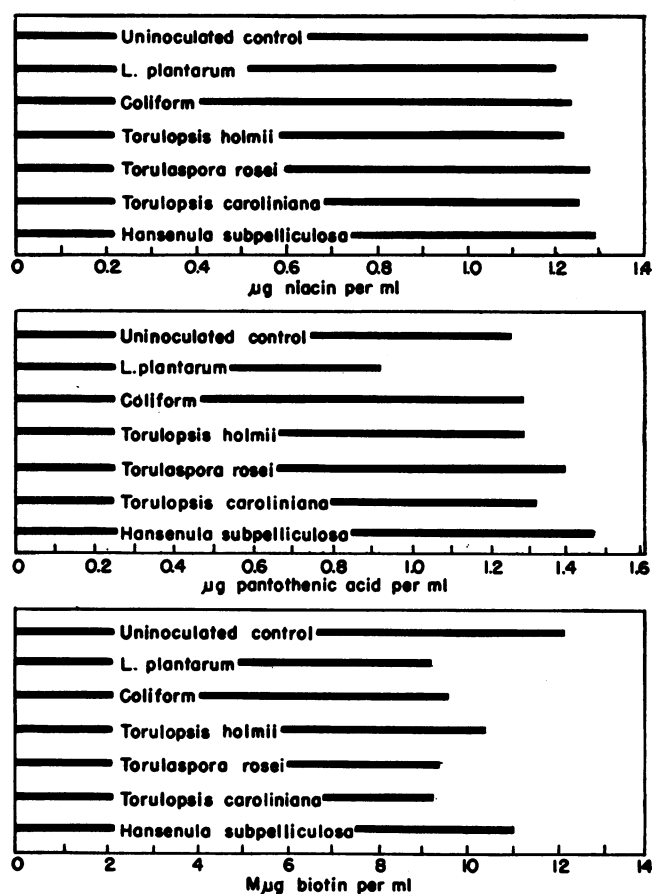


FIG. 1. The effect of various microorganisms on the available niacin, pantothenic acid and biotin content of cucumber brine.

RESULTS AND DISCUSSION

The effects of the various microorganisms on the vitamins studied are shown in figure 1. It is readily apparent that the bacteria and yeasts tested had little or no effect on the niacin content of the brine. The niacin concentrations of the various inoculated lots of brine varied less than 5 per cent from that of the uninoculated control. However, *L. plantarum* produced a

significant reduction in both the pantothenic acid and biotin content of the brine. The coliform organism and the four yeasts failed to lower the pantothenic acid level, but all of these organisms except *Hansenula subpelliculosa* reduced the biotin content by considerably more than 10 per cent. A synthesis of pantothenic acid was indicated in the brines inoculated with *Hansenula subpelliculosa* and *Torulasporea rosei*.

These results were in general agreement with the studies made by Rosen and Fabian (1953) on the

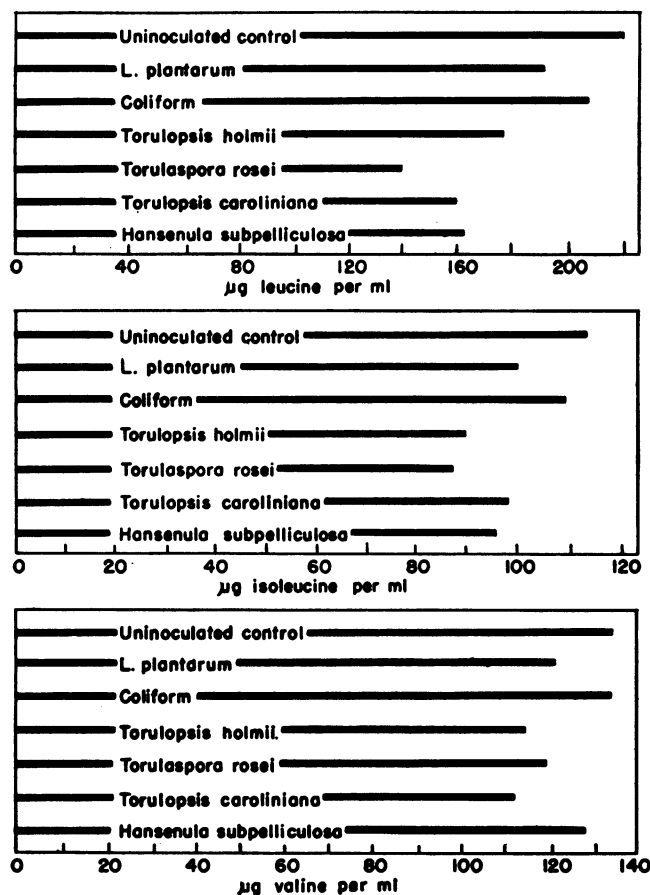


FIG. 2. The effect of various microorganisms on the available leucine, isoleucine and valine content of cucumber brine.

effect of these microorganisms on the niacin, pantothenic acid, and biotin content of cucumber juice. However, a much greater reduction in the biotin content of the brine used in these experiments was noted with *L. plantarum* than in the diluted cucumber juice as tested by Rosen and Fabian. The extent of growth and acid production in the two different media as well as the difference in the strains tested are variable factors which may account for this significant difference in biotin utilization by *L. plantarum*.

The results of this experiment were quite similar with regard to the influence of the various microorganisms on the leucine, isoleucine, valine and tryptophane content of the cucumber brine (figures 2 and 3).

Thus, *L. plantarum* and the four yeasts tested were noted to lower the levels of all these amino acids, although the decreases in valine and tryptophane contents due to *Hansenula subpelliculosa* were too small to be significant. The coliform isolate failed to affect the concentrations of these four amino acids to a significant extent.

In the case of glutamic acid, about the opposite relationship was noted. The coliform isolate lowered the glutamic acid content by more than 50 per cent,

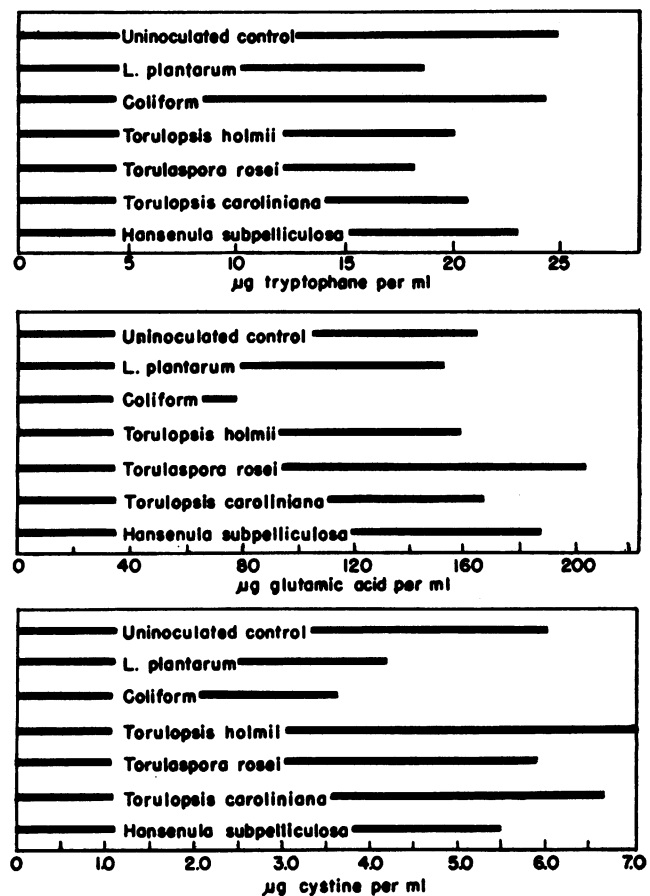


FIG. 3. The effect of various microorganisms on the available tryptophane, glutamic acid and cystine content of cucumber brine.

while the yeasts failed to decrease the level significantly. In fact, the growth of *Torulaspora rosei* and *Hansenula subpelliculosa* resulted in an increase in the available glutamic acid. Only a slight decrease due to *L. plantarum* was noted.

The concentrations of all of the amino acids except cystine in the uninoculated control lots of brine were approximately what were expected on the basis of the assays made on the nonfermented brine samples before sterilization. The cystine content, however, was considerably lower. The only plausible explanation for this was that much of the cystine was destroyed or rendered unavailable for *Streptococcus equinus* P-60 (*Leucono-*

stoc mesenteroides) by the sterilizing process. Similar observations have been made by Riesen *et al.*, (1947) and Sarkar *et al.*, (1950) on the cystine content of basal media. The former workers demonstrated that the effect of sterilization reduced the availability of cystine to *S. equinus* (*L. mesenteroides*) about 40 per cent but did not affect its availability to *Lactobacillus casei*. Sarkar *et al.*, (1950) noted that the loss in test material was proportional to that in the standard and, thus, did not result in inaccuracies in cystine assays.

To ascertain that the differences in cystine concentrations noted in the various inoculated lots of brine were not the result of the sterilization process, the experiment was repeated for this amino acid. The brine used in this experiment was also from the nonfermented control lots. It was filtered through cheese cloth and dispensed in 16 mm tubes, 10 ml per tube. One tube was saved as an unheated control and kept under toluene in the refrigerator. The other tubes were sterilized at 15 pounds pressure for 10 minutes, cooled, and inoculated with the various microorganisms. The preparation of the inoculum and the inoculations were carried out in the same manner as in the foregoing experiment except that the initial cell suspension was diluted 1-25 before making the drop inoculations. After five days' incubation at 30 C, microbiological assays for cystine were made immediately to prevent the necessity of resterilization.

On comparing the heated with the unheated controls, it was evident that the sterilizing process markedly reduced the available cystine content of the brine. The heated control contained 6.61 μg per ml and the unheated sample 11.93 μg per ml of cystine. However, the results obtained on the influence of the various isolates on the cystine content were quite consistent in both trials. Thus, it was believed that the reduction in cystine was incidental to this experiment.

The average values for the two trials with cystine are shown in figure 3. Both *L. plantarum* and the coliform isolate reduced the available cystine content of the brine markedly, by about 30 per cent and 50 per cent respectively. The growth of *Torulopsis holmii* resulted in an increase in the available cystine, while the other three yeasts failed to influence the concentration of this amino acid to a significant extent.

It should be pointed out that the utilization of cystine by the coliform group could be quite important in cucumber fermentations. This group of organisms have been noted to be active in some fermentations

the first few days after brining and it was at this period that the cystine concentrations in a few of the fermenting brines studied were noted to be relatively low.

SUMMARY

The effects of *Lactobacillus plantarum*, a coliform isolate, *Torulopsis holmii*, *Torulopsis rosei*, *Torulopsis caroliniana*, and *Hansenula subpelliculosa* on the concentrations in cucumber brine of three vitamins and six amino acids have been observed.

The niacin content of the brine was not measurably affected by any of the six microorganisms tested. *L. plantarum* was the only organism which reduced the pantothenic acid content, but biotin levels were lowered by both of the bacterial cultures and all four yeasts.

Growth of the yeasts and of *L. plantarum* resulted in decreases of varying degrees in leucine, isoleucine, valine, and tryptophane concentrations, while the coliform isolate had no measurable effect. Conversely, the coliform organism greatly lowered the concentrations of glutamic acid and cystine, while the yeasts had no effect or resulted in an increase in the levels of these two amino acids. *L. plantarum* had little effect on glutamic acid concentrations but lowered the cystine content of the brine considerably.

Synthesis of pantothenic acid and glutamic acid by *Torulopsis rosei* and *Hansenula subpelliculosa* were indicated, and increases in cystine content were noted with *Torulopsis holmii* and *Torulopsis caroliniana*.

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