THE EFFECT OF FERMENTATION WITH SPECIFIC MICRO-
ORGANISMS ON THE VITAMIN C CONTENT OF
ORANGE AND TOMATO JUICE.*

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It has already been pointed out that both corn silage and
sauerkraut are free from vitamin C (1). Silage can be considered
as preserved green corn, yet it has been found devoid of vitamin
C even when prepared under carefully controlled laboratory con-
ditions where the temperature was not allowed to rise appreciably
above room temperature. Green corn itself is a very effective
antiscorbutic material—1 gm. of the green material fed daily (2)
being sufficient to protect the guinea pig from scurvy, while
1.5 gm. of fresh cabbage will do the same thing (3). In the case
of silage making it would seem unlikely that the destruction of
vitamin C was due to oxidation because large quantities of carbon
dioxide are produced by the biological and respiratory processes
going on in silage making. The same situation is true in sauer-
kraut making where there is a very rapid production of carbon
dioxide and displacement of a large part of the oxygen in the
interstices of the mass.

It was believed possible that microorganisms were instrumental
in the destruction of vitamin C although it was also considered
possible that the destruction was due to direct oxidation, the
oxygen not being completely used up or displaced during the
initial fermentation processes of silage or sauerkraut making.

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tural Station, Madison.
Further, in studying this problem we had in mind the possible use of organisms as agents in removing sugars and other carbohydrates from materials rich in vitamin C as a means of studying and purifying this substance. This part of the field has been particularly developed by Zilva (4) in his work on vitamin C preparations, through the use of yeast fermentations.

**EXPERIMENTAL.**

Before discussing the effect of fermentation by microorganisms on the stability of vitamin C, it is essential to show the effect of oxygen on the stability of this vitamin when its solutions are kept in test-tubes plugged with cotton. When orange juice is drawn under aseptic conditions into sterile test-tubes and plugged with cotton plugs, vitamin C is noticeably reduced in the course of about 2 weeks. Chart I gives the growth curves of guinea pigs fed sterile orange juice on the same day the orange juice was drawn; 7 days later; and 14 days later.

In all of this work guinea pigs of weights of from 200 to 250 gm. were placed on our scorbutic ration which consisted of 69 parts rolled oats, 25 parts alfalfa meal (steamed in the autoclave for 30 minutes at 15 pounds pressure), 5 parts casein, and 1 part common salt. On this ration most guinea pigs will show signs of scurvy, such as tender and swollen joints and difficulties in locomotion in 2 to 3 weeks. The criterion for the presence of vitamin C was the recovery type of experiment. When the animal showed signs of scurvy accompanied by loss in weight administration of the experimental material was then begun.

It is at once evident that when exposed to air through cotton plugs, feeding of the orange juice must begin on the same day it is drawn from the orange in order to cure guinea pigs of scurvy. On the other hand, if the orange juice is covered with vaseline as soon as drawn in order to keep the orange juice under strictly anaerobic conditions, then there appears to be little or no destruction of vitamin C when feeding is begun 18 days after the orange juice is drawn, as seen in Chart I. Animal 417 was cured of scurvy but died later of some cause other than scurvy. These results have been abundantly confirmed with tomato juice, as will be shown later.
The development of the technique of exclusion of the air through the use of a vaseline plug was of distinct advantage in studying this problem. The vaseline plug was from \( \frac{1}{4} \) to \( \frac{3}{4} \) inches in thickness and while it would be pushed up in the tube under certain conditions of gas production, yet it could be readily brought again into contact with the fluid of the test-tube by carefully melting.

The direct effect on the stability of vitamin C of oxygen removal by a fermentation is beautifully illustrated in Chart II where orange juice was fermented with yeast and ordinary cotton plugs, but no vaseline used. This yeast fermentation of orange juice shows at once that there has been no destructive effect on vitamin C—a result in harmony with the observations of Zilva (4).
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the contrary, the activity of vitamin C persisted for a very long time—being retained for 51 days. This preservative effect was due to the liberal amount of carbon dioxide produced which removed most of the oxygen from the juice in a physical manner; but also, in addition, to the direct utilization of the oxygen in the juice by the growing organism. After 126 days the orange juice was found inactive, due, very probably, to a termination in the carbon dioxide production and subsequent absorption of oxygen from the atmosphere.

In this experiment the orange juice was drawn into test-tubes under aseptic conditions, covered with vaseline, and plugged with cotton. These tubes were allowed to stand 13 days and those that showed contamination were discarded. The remaining sterile tubes were then inoculated with a pure culture of bread yeast, after removal of the vaseline plug.

With a realization of the lability of vitamin C in sterile juices
when exposed to the air and the great protective value of anaerobic conditions brought about by the use of vaseline or by a yeast fermentation, a reliable method for the investigation of the stability of vitamin C to various organisms was available. For these studies canned tomato juice especially prepared by Kemp Brothers of Kokomo, Indiana, was used. The seeds and pulp had been strained out and the juice canned in tins. To this firm we are greatly indebted for this aid.

The experimental procedure was as follows: Canned tomato juice was pipetted under aseptic conditions into sterile test-tubes, inoculated with the organism to be investigated, calcium carbonate added when the acidity of the tomato juice was too great for the organism under investigation to grow, and the juice immediately covered with the vaseline plug. Gas formers will produce enough gas to push up the vaseline plugs and in consequence these plugs must be melted down frequently during the active fermentation period.

The organisms used were those whose biological behavior had already been studied. Organism 118-8 was isolated from sheep’s manure by Fred, Peterson, and Davenport (5). It ferments xylose with the formation largely of lactic and acetic acids. It also ferments fructose, glucose, lactose, sucrose, and mannitol. It is active over a considerable range of acidity—of a pH varying from 3.0 to 8.6. The optimum temperature of growth is 30–35°C. This organism occurs in silage.

The organism *Streptococcus lactis* is so well known that its characteristics do not need to be described fully. It ferments glucose readily at room temperature. It also ferments lactose. It does not ferment pentoses, while 118-8 ferments pentoses as well as hexoses and disaccharides. These two organisms were considered especially valuable for study as to their effect on the stability of vitamin C. In addition the organism S-6, isolated from cheese, was studied. This organism was of a proteolytic type.

The following experiments were planned and the treated tomato juice allowed to ferment at room temperature and to stand for 2½ days before feeding; the fermented juice was fed at a level of 4 cc. per individual daily.
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Chart III.

The effect of fermentation on vitamin C in tomato juice when fermented with various microorganisms is shown in Chart III. The chart depicts the changes in vitamin C content over a period of time for different fermentation conditions. Each line represents a different fermentation condition, with the y-axis showing the vitamin C content and the x-axis showing the time in days.
1. Sterile tomato juice exposed to air that circulated through the cotton plugs.
2. Sterile tomato juice, plus vaseline.
3. Sterile tomato juice, plus vaseline, plus calcium carbonate.
4. Sterile tomato juice, plus vaseline, plus calcium carbonate, plus *Streptococcus lactis*.
5. Sterile tomato juice, plus vaseline, plus calcium carbonate, plus S-6.
6. Sterile tomato juice, plus vaseline, plus 118-8.
7. Sterile tomato juice, plus vaseline, plus normal NaOH solution to make n/20 alkalinity.

No calcium carbonate was added in Experiment 6 where organism 118-8 was used because this organism grew very well in the tomato juice without reduction of its acidity. As seen in Chart III tomato juice without vaseline failed to cure scurbutic guinea pigs, thus demonstrating again the destruction of vitamin C in sterile tomato juice when exposed to air in cotton-plugged test-tubes. Tomato juice plus vaseline with or without calcium carbonate cured scurbutic guinea pigs. Tomato juice fermented with organism 118-8 and S-6 under the conditions described effected a cure, indicating that these organisms do not destroy vitamin C, within the limits of the experiment.

The tomato juice fermented with *Streptococcus lactis*, though not effecting a cure, greatly prolonged the lives of the animals, thus indicating partial destruction of vitamin C when fed after 28 days of fermentation with this organism. On the other hand, when the tomato juice was fermented with this organism for but 7 days and then fed to the scurbutic guinea pigs, a cure of scurvy resulted. From these experiments it is difficult to decide whether this organism destroys vitamin C. It certainly does it slowly, if at all, and it is possible that the long time fermentation (28 days) may have allowed some access to oxygen. This hardly seems the case because the potency of the fermented mixture inoculated with S-6 and 118-8 was very distinct even after 24 days.

The destructive effect of an alkaline medium on vitamin C is well known. Orange juice made to a n/20 alkalinity loses practically all of its vitamin content in 24 hours (6). From the results secured (Chart III) it is evident that such alkaline tomato juice retains a considerable portion of its original vitamin C content even after 24 days when kept under strictly anaerobic conditions made possible by the use of vaseline. While the guinea pigs were
not completely cured of scurvy, their lives were very much prolonged.

There is no logical reason why microorganisms should not be found that will destroy vitamin C, but it is altogether probable that its disappearance from fermented corn (silage) and fermented cabbage (sauerkraut) is to be referred to a destruction through contact with oxygen, which is low in amount but seldom completely absent in the gases taken from the silage mass (7). This is especially true unless the silage is taken far from the surface or periphery of the silo. So far as we are aware the gases in a mass of fermented cabbage have not been investigated.

SUMMARY.

1. Attempts to determine the cause for the disappearance of vitamin C from fermented corn (silage) and fermented cabbage (sauerkraut) have been made. The action of several organisms occurring in silage have been studied, but it has not been shown that they destroy vitamin C.

2. For this study a technique involving the use of vaseline for exclusion of air from the fermenting mixture has been developed. With this technique, the destructive action of oxygen on vitamin C can be excluded.

3. While all of the organisms occurring in silage have not been included in these studies, yet it is very probable that the destruction of vitamin C in masses of fermenting plant tissue is to be referred to the oxygen still retained in the mass.

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