THE VITAMIN A, B, AND C CONTENT OF ARTIFICIALLY VERSUS NATURALLY RIPENED TOMATOES.*

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INTRODUCTION.

The use of ethylene in the ripening of fruits and vegetables has received considerable publicity and has been used to a limited extent commercially during the past 3 years. This method possesses distinct advantages over the older method in that it materially reduces the time required for the preparation or finishing for market, is relatively inexpensive, and ripens the product more uniformly. With a consuming public demanding fruits and vegetables at any and all seasons of the year the use of ethylene as a ripening agent has a valuable commercial aspect.

The consumer, however, is interested not alone in the exterior appearance of the final product, but in its nutritive constituents as well. Since fruits and vegetables are eaten largely because of their vitamin value, it is of interest to know the effect this new commercial method of ripening has upon the vitamin content. Accordingly, the vitamin A, B, and C content of tomatoes, a foodstuff commonly subjected to this treatment, was investigated.

HISTORICAL.

The artificial coloring of citrus fruits for the market has been practiced for some time. According to the old method the fruit was placed in rooms or tents heated with kerosene stoves. It was thought that the temperature and humidity thus produced hastened the ripening process, until Sievers and True (1), proved that the combustion products of the kerosene were

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the factors affecting the ripening. In an attempt to determine the particular gaseous constituent responsible for the coloring, Denny (2) found that a number of substances were effective. Many of these were not practical for one reason or another; cost, effect on the fruit, the color produced, the physiological effect on man, etc. Ethylene seems to be the best suited to the process and has come into rather general usage.

Ethylene causes an increase in the respiratory rate according to Denny (3), Rosa (4), and Chace and Denny (5), the general consensus of opinion being that ethylene "acts as a stimulus to the normal oxidative processes in the fruit" (4).

According to Rea and Mullinix (6) ethylene can act as a catalyzer converting pure dry starch to reducing sugars in the absence of the enzyme amylase. This conversion can also be brought about by saturating a starch solution with ethylene and allowing the mixture to stand.

Chace and Church (7) found that there was no significant change in the composition of the edible portion of citrus fruits when testing for soluble solids, reducing sugars, sucrose, and citric acid. They noted among other observations that the astringency of persimmons is destroyed by ethylene.

Experiments on the vitamin B content of board-blanced celery and ethylene-blanced celery have been reported by Babb (8). The difference in vitamin content observed was in favor of the ethylene-blanced celery but was not large enough to be considered significant.

Morgan and Smith (9) have tested the vitamin A content of green tomatoes, of tomatoes ripened in diffused light, ripened in ethylene, and ripened in the dark with the exception of three 30 minute periods of ultra-violet irradiation. They report that green tomatoes are relatively poor in vitamin A but that the ripened fruits contain about equal amounts of this vitamin. Their conclusions are based upon observations of two to five animals per lot. It is not possible from the data given to ascertain whether their results are statistically significant or not.

EXPERIMENTAL.

The tomatoes used were of the Bonny Best variety. Twice each week the vines were inspected and three-fourths of the tomatoes that had reached the "green mature" stage (as judged by size, color, and firmness of the fruit) were picked and brought to the laboratory, the other one-fourth being left on the vines to ripen completely. The green tomatoes brought to the laboratory were divided into three lots, the first being fed at the "green mature" stage. The second lot was ripened in air at room temperature (22–25°), and the third lot treated with an ethylene-air mixture. The fourth lot consisted of the tomatoes that were allowed to ripen on the vines.

The third lot (ethylene-treated) was placed on a rack in a tall
bell jar. The jar was provided with inlet and outlet tubes. A constant supply of an ethylene-air mixture, approximately 1:800, was kept passing through the jar at all times. This was done in order that the respiration products might not accumulate and become a factor influencing the speed of the process. Rosa (4) found that a lower concentration, namely 1:4300 (ethylene to air), was somewhat more effective in ripening tomatoes than the higher concentration of 1:800. The higher concentration was used in this study as the object of the experiment was to determine the effect of the ethylene, rather than to decrease the time of ripening to a minimum.

The experimental animals used for the vitamin A and B tests were albino rats 28 days old and weighing between 40 and 50 gm. The technique used was that of Ferry (10), each rat being kept in an individual all metal cage with a false bottom. Daily food consumption records were kept and the animals were weighed once each week or more often. Guinea pigs 6 to 8 weeks old were used for the vitamin C tests. The guinea pigs were weighed twice each week and carefully observed for symptoms of scurvy. Autopsies were performed to determine the severity of the disease.

Vitamin B.—The basal diet used in the vitamin B test consisted of vitamin B-free casein 18, starch 50, butter fat 8, lard 18, salt mixture 4 (11), and cod liver oil 2. The cod liver oil was fed separately from the ration. The rats were divided into four groups of twenty or more animals each, ten males and ten females in each group. No more than four animals of any one litter were assigned to a group. Three animals were used as negative controls and two as positive controls. One group of rats received 4 gm. of green tomato daily for a period of 8 weeks. The second group received 4 gm. of air-ripened tomato, the third 4 gm. of tomato ripened in the ethylene-air mixture, and the fourth 4 gm. of tomato ripened on the vines. By feeding 4 cc. of canned tomato juice Sherman and Grose (12) were able to maintain animals for 8 weeks with an average loss in weight of 2.2 gm. It was estimated that 4 gm. of fresh tomato would support growth near a maintenance level and thus render the method sufficiently sensitive.

Since this work was started the multiplicity of the factor known as vitamin B has been accepted. In this study, the term vitamin B refers to the complex.
to detect small differences in vitamin content. The fresh toma-
toes were eaten readily by the animals. The positive controls
received 200 mg. of dried brewery yeast daily.

The average daily consumption of the basal diet was practically
the same for all groups of animals. The rats fed green tomato
ate 3.2 gm. per day, those fed air-ripened tomato 3.2 gm., those
fed ethylene-ripened tomato 3.2, and those fed vine-ripened
tomato 3.1 gm.

The average gain in weight of the group of rats fed green tomato
was 3.2 ± 1.0 gm., of the group fed air-ripened tomato 4.7 ±
0.8 gm., ethylene-ripened tomato 4.7 ± 1.1 gm., and vine-
ripened tomato 6.4 ± 1.2 gm. The growth curves for each
group of rats are given in Chart I. Each curve is a composite
made by averaging the weights of the animals in each group.

The differences in growth in any of the four groups are not
statistically significant. The greatest difference observed was
that between the rats fed green tomato and those fed vine-ripened
tomato. In this case, the mean difference was 1.9 times as large as its probable error and according to Sheppard's probability tables, the chances are only nine to one, that a future trial would give results of the same sign. Since the differences in growth between the animals fed the four groups of tomatoes were not significant, it seems reasonable to conclude that the amount of vitamin B does not differ in green and ripe tomatoes and that the methods of ripening used did not measurably affect the vitamin B content.

**Vitamin A.**—The basal diet used in this experiment was that recommended by Sherman and Munsell (13) consisting of vitamin A-free casein 20, starch 70, yeast 5, salt mixture 4 (11), and sodium chloride 1. The diet was irradiated for 30 minutes at a distance of 2 feet in order to insure an adequate supply of vitamin D. In this laboratory the stock colony is maintained upon a slightly modified form of the ration recommended by Steenbock (14), 2 per cent of yeast and 10 per cent of wheat germ being substituted for 12 per cent of yellow corn-meal.

Albino rats 28 days old and of known nutritional history were maintained on this diet until their bodily store of vitamin A was depleted. At the end of the depletion period the rats were divided into four groups of twenty animals each. Their average weight at this time was 122 gm. The same precautions as to distribution among the litters and differences in sex were observed as previously. Tomatoes treated in the same manner as in the vitamin B tests were fed to the four groups of animals. Each group received 2 gm. of tomato per animal per day. Both positive and negative controls were used; the positive controls received 10 drops of cod liver oil daily. The average daily consumption of the basal diet by the rats fed green tomato was 10.3 gm., by those fed air-ripened tomato 10.1 gm., ethylene-ripened tomato 10.6 gm., and vine-ripened tomato 10.5 gm. Since the amount of the basal diet consumed was practically the same for all groups of animals it is not possible to ascribe the differences in growth to differences in the food intake.

The group of rats fed green tomato gained $38.5 \pm 3.8$ gm., those fed air-ripened tomato $57.1 \pm 5.7$ gm., ethylene-ripened tomato $58.4 \pm 5.8$ gm., and vine-ripened tomato $55.8 \pm 4.6$ gm. The growth curves for each group of animals are given in Chart II.
The curves are composites made by averaging the weights of the individual animals in each group.

It is apparent from Chart II that the differences in growth between any of the groups of rats fed ripened tomatoes were negligible. However, the growth of the group of rats fed green tomato was considerably less than that of any of the groups fed the ripened fruit. The mean difference in growth between the group of rats fed green tomato and those fed ethylene-ripened tomato is 3.05 times its probable error; between the green and vine-ripened tomato 3.00 times its probable error, and between green and air-ripened tomato 3.00 times its probable error. According to Shepard's probability tables the chances are approximately 49 to 1 that duplicate experiments would give results of the same sign.

Differences from 3 to 4 times their probable errors are regarded as showing a strict significance. Whether or not this is too severe a test for the type of biological data presented above is a matter of controversy (15). From these results it would seem reasonable
to conclude that there is the same amount of vitamin A in the ripened tomatoes, regardless of the method of ripening used, and that the amount found in green tomatoes is somewhat less than that found in the ripened fruit.

**Vitamin C.**—Guinea pigs 6 to 8 weeks old and weighing between 250 and 350 gm. were placed on a basal diet consisting of a mixture of alfalfa meal and white flour in equal proportions by weight, oats, and water *ad libitum*. During the 1st week after the animals were brought to the laboratory, they were fed, in addition to the basal diet, sufficient amounts of green food to supply an abundance of vitamin C. All animals that did not show a normal growth during this period were discarded. The experiment was begun by withholding the fresh food and feeding weighed quantities of tomatoes as the sole source of vitamin C. From ten to twelve guinea pigs were used in each group. Five negative and four positive controls were used, the latter being fed 3 cc. of canned tomato juice daily (16). Group I received 4 gm. of green tomato, Group II 4 gm. of air-ripened tomato, Group III 4 gm. of ethylene-ripened tomato, and Group IV 4 gm. of vine-ripened fruit.

The animals were caged individually, daily food consumption records were kept, and the animals weighed twice each week. Autopsies were performed upon the completion of the experimental period or upon the death of the animal.

The protocols of four typical animals from each group are given. The initial weight, maximum weight, and final weight of the animals, the length of the survival period, food consumption, and autopsy findings are recorded. In Table I, hemorrhage of the joints, of the costochondral junctions, and the looseness of the teeth are indicated by the signs: − (not different from normal), ? (doubtful), and +, ++, and +++ for increasing degrees of severity.

It will be seen from an examination of Table I that the negative controls showed severe scurvy. The positive controls showed no symptoms of scurvy except in the case of the animal which refused to take the tomato juice. The group of guinea pigs fed green tomato showed very definite symptoms of scurvy, more apparent in many cases than in the negative controls. The small quantity of vitamin provided by the green tomato prolonged the life of the animals sufficiently to permit the full development of the characteristic
<table>
<thead>
<tr>
<th>Guinea pig No.</th>
<th>Weight</th>
<th>Duration of experiment</th>
<th>Autopsy findings</th>
<th>Average daily food consumption</th>
<th>Remarks</th>
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<td></td>
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<td>gm.</td>
<td>gm.</td>
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<tr>
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<td>228</td>
<td>38</td>
<td>+++</td>
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<tr>
<td>964</td>
<td>347</td>
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<td>210</td>
<td>37</td>
<td>+++</td>
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<tr>
<td>990</td>
<td>306</td>
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<td>311</td>
<td>77</td>
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<td>Fed 4 gm. of green tomato daily.</td>
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<td>996</td>
<td>268</td>
<td>484</td>
<td>484</td>
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<td>Fed 4 gm. of vine-ripened tomato daily.</td>
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<td>977</td>
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<td>560</td>
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<tr>
<td>Positive controls; fed 3 cc. canned tomato juice daily.</td>
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<td>82</td>
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<td>422</td>
<td>396</td>
<td>82</td>
<td>-</td>
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<tr>
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<td>273</td>
<td>414</td>
<td>411</td>
<td>65</td>
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TABLE I—Concluded.

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<th>Guinea pig, No.</th>
<th>Weight.</th>
<th>Autopsy findings.</th>
<th>Average daily food consumption.</th>
<th>Remarks.</th>
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<td>Final</td>
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<td>18 days</td>
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<td>954</td>
<td>312</td>
<td>347</td>
<td>190</td>
<td>23 days</td>
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<td>955</td>
<td>327</td>
<td>327</td>
<td>221</td>
<td>17 days</td>
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</table>

Negative controls; fed basal diet only.

symptoms. No appreciable difference could be detected between the groups fed the air-ripened or ethylene-ripened tomato. Some of the animals in both groups showed signs of incipient scurvy, others complete protection. They were, however, superior in general health to the group fed green tomato. The guinea pigs fed vine-ripened tomato were by far the healthiest and best conditioned animals of any group. Their bodies were firm and well fleshed, their hair sleek and smooth. Autopsies showed no indications of scurvy.

These data indicate that there is very little vitamin C in green tomatoes, confirming the observations of Hess (17) whose data are unpublished; tomatoes picked green and ripened either in air or in a mixture of ethylene and air contain considerably more vitamin C than the green fruit but not as much as the vine-ripened fruit.

SUMMARY AND CONCLUSIONS.

A comparison was made of the vitamin A, B, and C content of green, air-ripened, ethylene-ripened, and vine-ripened tomatoes. Twenty rats were used in each group for the vitamin A and B tests. Ten guinea pigs were used in each group for the vitamin C tests. Statistical treatment of the data has lead to the following conclusions:

1. The four lots of tomatoes showed no difference in their vi-
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tamin B content. It is evident that the methods of ripening used
did not alter the amount of vitamin B present in the green mature
fruit.

2. The vitamin A content of ripened tomatoes was found to be
greater than that of the green mature fruit. The same quantity
of vitamin A was developed in the tomatoes regardless of the
method of ripening used.

3. Green tomatoes were found to be relatively poor in vitamin
C. Air-ripened and ethylene-ripened tomatoes were richer in this
vitamin than the green fruit and vine-ripened tomatoes were
superior to either the artificially ripened or to the green tomatoes.

4. The commercial method of ripening tomatoes in an ethylene-
air mixture produces fruit which is equally as rich in the vitamins
A, B, and C as fruit which has been picked green and ripened in
air.

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