THE VITAMIN A, B, C, AND G CONTENT OF SULTANINA (THOMPSON SEEDLESS) AND MALAGA GRAPES AND TWO BRANDS OF COMMERCIAL GRAPE JUICE

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INTRODUCTION

During recent years a large annual surplus of grapes has been produced in the United States, and the development of new grape products to take care of this increasing supply is at present an important phase of the grape industry. Because of the general interest in a fruit so abundantly grown, it seemed desirable to study the nutritive value of grapes and grape juices more extensively than had previously been done. Raisins have received the attention of several investigators (3, 12), who apparently agree that this product contains very little if any vitamin A, a small amount of vitamin B, and no vitamin C. However, up to the present time very few data have been published on the vitamin content of fresh grapes and their juices, and because of the value of such information the present experiments were planned to determine the amount of vitamins in two varieties of fresh grapes as well as in two brands of commercial grape juice.

Sultanina (Thompson Seedless) and Malaga (Vitis vinifera), European varieties, were the grapes selected for study. The Sultanina or Thompson Seedless is a raisin grape constituting about 90 per cent of the total crop of seedless grapes. In this country it is grown principally in California. The Malaga, a table grape, is also used for raisins and, together with the muscat, forms the chief source of the seed or seeded raisins.

In addition to the fresh grapes, two brands of commercial grape juice were analyzed for their vitamin content. The first, designated as commercial juice No. 1, was a mixture of juices approximately one-third from the Flame Tokay and two-thirds from the Zinfandel, European table and juice grapes, classified as Vitis vinifera. In the commercial process the juices after extraction from the fresh fruit are filtered and placed in cold storage until needed. Upon removal from cold storage, they are refiltered, sterilized through a machine at a temperature not to exceed 155° F., and bottled. The bottles of juice are then kept in a water bath held at 150° for 45 minutes. Sometimes a slight amount of tartaric acid is found necessary to bring the composition of the product to the standard formula used by the company. No other ingredients are added to the juice.

Commercial juice No. 2 was prepared from Concord grapes (Vitis labrusca), an American variety. In preparing this juice the fruit is washed, stemmed, and crushed, heated to about 135° F., and bottled. The juice is pasteurized and stored in 5-gallon glass vats.

1 Received for publication June 10, 1931; issued February, 1932. Presented before the division of medicinal chemistry of the American Chemical Society, at Indianapolis, Ind., Apr. 1, 1931.
2 Reference is made by number (italics) to Literature Cited, p. 69.
carboys for several weeks to a few months to permit settling; it is then siphoned into bottles and again pasteurized. Sugar is generally an added ingredient.

VITAMIN A

In the present study the vitamin A content of the grapes and grape juices was determined by the Sherman and Munsell method (14). The basal diet was irradiated to supply vitamin D. After the customary depletion period of from four to five weeks, certain groups of rats were fed daily six times per week weighed portions (1, 3, and 5 gm., respectively) of seeded Malaga grapes. Certain other groups received the Sultanina in the same amounts at the same intervals. The commercial juices were given in daily doses of 2, 3, and 5 c. c. for the same number of days each week. The results are shown in Figure 1 and Table 1.

Table 1.—Survival of rats receiving various quantities of grape juice as the sole source of vitamin A

<table>
<thead>
<tr>
<th>Test food</th>
<th>Daily portion, 6 times per week</th>
<th>Rats</th>
<th>Average weight of rats at 4 weeks of age</th>
<th>Average weight of rats at end of period</th>
<th>Average time of survival after period</th>
<th>Average total time of survival after 4 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C. c.</td>
<td>Number</td>
<td>Grams</td>
<td>Grams</td>
<td>Days</td>
<td>Days</td>
</tr>
<tr>
<td>Commercial grape juice No. 1*</td>
<td>2</td>
<td>6</td>
<td>45.8</td>
<td>95.2</td>
<td>33.6</td>
<td>21.7</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>8</td>
<td>45.9</td>
<td>99.0</td>
<td>33.9</td>
<td>23.3</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>8</td>
<td>45.4</td>
<td>95.0</td>
<td>33.5</td>
<td>27.2</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>7</td>
<td>46.1</td>
<td>99.3</td>
<td>34.3</td>
<td>30.3</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>8</td>
<td>56.0</td>
<td>108.2</td>
<td>35.8</td>
<td>25.6</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>8</td>
<td>55.5</td>
<td>101.2</td>
<td>35.6</td>
<td>19.5</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>8</td>
<td>50.2</td>
<td>98.9</td>
<td>35.9</td>
<td>23.6</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>8</td>
<td>52.9</td>
<td>105.8</td>
<td>35.8</td>
<td>16.3</td>
</tr>
</tbody>
</table>

* A mixture of juices, one-third from the Flame Tokay and two-thirds from the Zinfandel, which are European table and juice grapes classified as *Vitis vinifera*.

*• 1 rat lived out the full experimental period.

*• Juice prepared from Concord grapes, *V. labrusca*.

Sherman (12) reported the presence of vitamin A both in grapes and in grape juice, but the variety of grapes was not indicated. He stated that an ounce of grapes contained 16 to 22 units of vitamin, i. e., 0.57 to 0.7 unit per gram. If a unit of vitamin is present in that amount of the test food necessary to produce a gain of 25 gm. in eight weeks, then 1.75 to 1.43 gm. of the fruit were necessary to produce this unit gain. In the present study, in order to obtain a gain of 25 gm. in eight weeks, it was necessary to feed approximately 5 gm. of grapes in the cases of both Sultanina and Malaga. These results indicate that Malaga and Sultanina grapes are less rich in vitamin A than the grapes studied by Sherman. Nevertheless, the growth response induced by these varieties shows the presence of a small but measurable quantity of vitamin A.

Neither of the commercial juices tested showed any indication of the presence of vitamin A. With but one exception the animals died long before the termination of the experiment and showed no better growth than the negative controls. (Table 1.) Upon autopsy the gross anatomical changes due to a deficiency of vitamin A were found to be as severe in the test animals as in the controls.
Recorded data furnish but scanty information regarding the vitamin B content either of grapes or of their juices. Osborne and Mendel in 1920 (10) reported that when 10 c. c. of commercial grape juice was fed daily it was found to contain some "water soluble B" but in amounts insufficient for the normal growth of rats. Sherman (12) reported the occurrence of 7 to 9 units of vitamin B per ounce of grapes. Both of these investigators, however, were considering the vitamin B complex before cognizance had been taken of the antipellagric factor. There appears to be no recent data regarding the vitamin B content of these products.

The vitamin B (antineuritic) tests were carried out according to a method worked out in this laboratory, similar to that outlined by Chase (1). Rats, 28 days old, were placed on diet 107 G, which furnished, with the exception of the antineuritic vitamin, all factors necessary for normal growth and apparent well-being of the animals. This diet had the following composition: Vitamin B-free casein, 18 per cent; starch, 58 per cent; yeast (autoclaved four hours at 20 pounds pressure), 10 per cent; Osborne and Mendel salt mixture, 4 per cent; butterfat, 8 per cent; and cod-liver oil, 2 per cent. All of the animals were kept on this vitamin B-free diet for two weeks, a period of time judged from former observations in this laboratory to be sufficient to deplete the animals of their store of the antineuritic vitamin.

At the end of this depletion period the rats were given weighed or measured portions of the material to be tested. Both varieties of grapes were readily consumed, but a temporary difficulty was expen-
rienced in getting some of the animals to take the grape juice. The Malaga grapes, after the removal of the seeds, were fed in portions of 0.5, 1, 2, 3, 5, and 6 gm., respectively, while the Sultanina grapes were given in amounts of 3, 5, and 6 gm. Each of the two samples of juice were fed daily from small glass containers in 2, 3, and 5 c. c. portions. The results are summarized in Figures 2 and 3.

Figure 2 shows that 5 and 6 gm. of fruit in the case of both Malaga and Sultanina grapes induced approximately the same gain in weight, indicating that the maximum effect of the grapes as a source of vitamin B had been reached. A maximum average gain in weight of 17.5 gm. resulted from feeding both 5 and 6 gm. of Malagas, while 5 gm. of Sultanina produced an average gain of 18.8 gm. and 6 gm. portions of this same grape gave 21.6 gm. gain in weight. These rates of growth of the test animals indicate that the two varieties of grapes are fair sources of vitamin B.

From Figure 2 it may also be observed that the growth curves for these animals show a maximum point between the sixth and seventh week of the test, after which there is a loss in weight to the end of the period. It was difficult to obtain as good grapes at the end of the season as had been fed through the major part of the experiment, but this consistent drop in weight in all of the animals can not be explained satisfactorily on the basis of poor-quality grapes, since only a comparatively few animals received the inferior product. It is entirely possible that another factor necessary for normal growth was absent from the diet, and upon the depletion of the reserve store of this factor in the animal body the growth curves began to show a decline.

The failure of commercial juice No. 1, in 2, 3, and 5 c. c. daily portions, to induce growth indicates the absence of any measurable quantity of vitamin B in this grape juice. (Fig. 3.) On the other hand,
Figure 3 shows that commercial juice No. 2 contains the antineuritic vitamin. The amount is only minimal, however, since a daily portion of 5 c. c. of this juice induced a total gain in weight of only 3 to 4 gm. during the entire test period.

VITAMIN C

The vitamin C content of grapes and grape juices appears to have been more extensively studied than that of any of the other vitamins. Chick and Rhodes (2) found the juice of grapes to be about one-tenth as rich in the antiscorbutic vitamin as oranges. Givens and Macy (5) found no antiscorbutic properties in dehydrated grape juice which was 14 to 20 months old at the time of testing. According to Merjanian (9), grapes contain vitamin C, the amount varying with the kind of grapes and their freshness. Taking orange juice as 100 for a standard of comparison, Sherman (12) reported that grapes and grape juice have a potency of 4 to 5.

The vitamin C tests on commercial grape juice No. 1 were carried out after the method of Sherman, LaMer, and Campbell (13), the 90-day test period being used. The basal diet designated as 12 D was a modification of that used by Sherman and had the following composition: Equal parts mixture of bran and oats, 57 per cent; table salt, 1 per cent; butterfat, 9 per cent; heated skim-milk powder, 30 per cent; cod-liver oil, 1 per cent; and yeast, 2 per cent. Six, eight, and ten cubic centimeters of the commercial juice No. 1 were fed from a graduated pipette to groups of guinea pigs every day except Sunday during the test period. Table 2 shows that the survival period of the test animals on this brand of juice was no longer than that of the negative controls and upon autopsy they showed just as severe symptoms of scurvy. Such evidence indicates that commercial grape juice No. 1 contains no vitamin C.
TABLE 2.—Survival of guinea pigs receiving various quantities of commercial grape juice No. 1 (juice of Flame Tokay and Zinjandel grapes, Vitis vinifera) as the sole source of vitamin C

<table>
<thead>
<tr>
<th>Daily dose, 6 times per week</th>
<th>Guinea pig No.</th>
<th>Weight at beginning</th>
<th>Maximum weight</th>
<th>Weight at end</th>
<th>Survival</th>
<th>Severity of scurvy symptoms at autopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 c.c.</td>
<td>245 F</td>
<td>338 Grams</td>
<td>371 Grams</td>
<td>202 Days</td>
<td>Moderate to severe.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>254 F</td>
<td>320 Grams</td>
<td>316 Grams</td>
<td>200 Days</td>
<td>Do.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>265 F</td>
<td>323 Grams</td>
<td>350 Grams</td>
<td>222 Days</td>
<td>Moderate.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>272 F</td>
<td>327 Grams</td>
<td>336 Grams</td>
<td>216 Days</td>
<td>Do.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>251 F</td>
<td>322 Grams</td>
<td>384 Grams</td>
<td>210 Days</td>
<td>Severe.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>252 F</td>
<td>325 Grams</td>
<td>335 Grams</td>
<td>206 Days</td>
<td>Do.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>257 F</td>
<td>323 Grams</td>
<td>363 Grams</td>
<td>195 Days</td>
<td>Do.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>261 F</td>
<td>328 Grams</td>
<td>337 Grams</td>
<td>214 Days</td>
<td>Moderate to severe.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>274 F</td>
<td>319 Grams</td>
<td>319 Grams</td>
<td>198 Days</td>
<td>Do.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>256 F</td>
<td>336 Grams</td>
<td>401 Grams</td>
<td>213 Days</td>
<td>Moderate to severe.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>261 F</td>
<td>329 Grams</td>
<td>349 Grams</td>
<td>182 Days</td>
<td>Do.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>269 F</td>
<td>324 Grams</td>
<td>341 Grams</td>
<td>166 Days</td>
<td>Do.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>273 F</td>
<td>310 Grams</td>
<td>310 Grams</td>
<td>242 Days</td>
<td>Do.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>162 F</td>
<td>351 Grams</td>
<td>427 Grams</td>
<td>247 Days</td>
<td>Moderate to severe.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>261 F</td>
<td>327 Grams</td>
<td>347 Grams</td>
<td>222 Days</td>
<td>Do.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>118 M</td>
<td>344 Grams</td>
<td>344 Grams</td>
<td>245 Days</td>
<td>Do.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>122 M</td>
<td>467 Grams</td>
<td>467 Grams</td>
<td>267 Days</td>
<td>Moderate to severe.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>132 M</td>
<td>327 Grams</td>
<td>379 Grams</td>
<td>203 Days</td>
<td>Do.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>113 M</td>
<td>300 Grams</td>
<td>390 Grams</td>
<td>202 Days</td>
<td>Do.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>170 M</td>
<td>402 Grams</td>
<td>450 Grams</td>
<td>295 Days</td>
<td>Do.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>177 M</td>
<td>367 Grams</td>
<td>367 Grams</td>
<td>223 Days</td>
<td>Do.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>182 M</td>
<td>361 Grams</td>
<td>413 Grams</td>
<td>254 Days</td>
<td>Do.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>258 M</td>
<td>351 Grams</td>
<td>425 Grams</td>
<td>244 Days</td>
<td>Do.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>270 M</td>
<td>316 Grams</td>
<td>522 Grams</td>
<td>217 Days</td>
<td>Do.</td>
<td></td>
</tr>
</tbody>
</table>

* The 9 males were controls carried with other experiments in this laboratory.

Tests on the commercial juice No. 2 and the fresh grapes were conducted according to the method of Höjer (7, 8), who determines the degree of scurvy by a study of the pathological condition of the teeth. Attention is focused especially on the microscopical examination of a cross section of the root of the incisor tooth of the guinea pig. Höjer claims that this method is more sensitive than the one used by Sherman. A comparative study of these methods has been made by Eddy (4) and Goettsch and Key (6). Following Höjer's technic, they found that twice the amount of test food is required to afford complete protection when the criterion depends on a histopathological examination of the teeth, than is apparently necessary when judgment is based on the gross external and internal anatomical changes. Höjer has shown that differential changes in tooth structure begin to take place between the tenth and fourteenth day of the test and are materially sharpened as the experiment continues.

In the present study an 18-day test period was adopted. During this time 10 and 12 c. c. portions of grape juice were fed to guinea pigs weighing between 300 and 350 gm. Because earlier evidence had indicated that there was little if any vitamin C present in grape juice it was considered unnecessary to feed this test food in smaller quantities. Twelve cubic centimeters was the maximum daily amount which the guinea pigs would take. Seven animals were used. Seeded Malaga grapes were given in 2, 5, 10, and 15 gm. portions to 16 animals, while Sultanina grapes in the same quantities were fed to 12. On the eighteenth day the animals were killed, the incisor teeth removed, and sections prepared.

Figure 4, A, shows the structure of a normal tooth taken from one of the control animals that had received an adequate supply of vitamin C from a daily allowance of cabbage. Figure 4, B, portrays the con-
dition of the tooth of one of the negative control animals fed only the basal diet with no antiscorbutic vitamin for 18 days. From Figure 4, A, it may be observed that the normal tooth possesses a wide band of evenly stained dentine inside of which is a narrow layer of uncalcified predentine, and then a row of very tall parallel columnar odontoblasts surrounding the normal pulp. An inadequate supply
of vitamin C causes the layer of dentine to become narrower, the predentine becomes calcified, and the odontoblastic layer of cells loses its soldierlike formation, while the cells themselves become shorter and gradually work their way into the pulp cavity to function as osteoblasts, the bone-forming instead of the dentine-forming cells. Consequently the scurbutic condition portrayed in the tooth structure in Figure 4, B, can be readily recognized even before the guinea pig develops outward symptoms of scurvy.

All of the teeth taken from the guinea pigs receiving commercial grape juice No. 2 showed marked pathological conditions analogous to those portrayed in Figure 4, B. Thus, it is concluded that this juice contains no appreciable amount of vitamin C.

The sections prepared from the teeth of animals fed 2-gm. and 5-gm. supplemental portions of either Malaga or Sultanina grapes showed that neither variety in these quantities prevented the occurrence of severe pathological changes in the teeth. While there was a slight protection in the teeth of guinea pigs fed 10 gm. of grapes, still this quantity furnished far too little vitamin C to give a normal tooth structure. Figure 5, A and B, representative of the teeth of those animals receiving 15 gm. daily of Malaga and Sultanina grapes, respectively, shows that even this quantity of the fruit was insufficient to afford border-line protection. Of the two varieties of fresh grapes studied, Sultanina contained the greater amount of vitamin C (fig. 5, B); 15 gm. of this fruit offered approximately the same protection as 2 c. c. of orange juice. (Fig. 6.)
VITAMIN G (B₂)

No report of any kind has been found that gives the vitamin G content of grapes or grape juice.

A method for the determination of vitamin G was worked out in this laboratory, and is similar in some respects to that used by Sandels (11). For a period of two weeks, 28-day-old rats were given the basal diet alone in order to deplete them of any vitamin G that might
be stored in their bodies. Vitamin B was supplied in the form of an 80 per cent by weight alcoholic extract of white corn which up to the present time has been found in this laboratory to be the most satisfactory source of vitamin B free from grossly interfering amounts of vitamin G. The basal diet consisted of the following ingredients: Purified casein, 18 per cent; Osborne and Mendel salts, 4 per cent; butterfat, 8 per cent; cod-liver oil, 2 per cent; starch, $68 - X$ per cent; and corn extract, $X$ per cent. Sultanina grapes were fed to the different groups of animals in 0.5, 1, 2, 3, and 5 gm. daily portions six times per week; the Malagas were given in the same manner in 2, 4, and 6 gm. amounts, while each grape juice was fed in 2, 3, and 5 c. c. allotments. The experiment covered a period of 10 weeks, but no significant changes occurred during the ninth and tenth weeks, and therefore only 8 weeks of the test are portrayed in the curves showing the rate of growth of the animals. (Fig. 7.)

The animals that received an insufficient amount of vitamin G first showed woolliness of fur and then a thinning of the hair, especially on the sides of the head. About the sixth week of the test a brownish-red discharge appeared caked along the inner sides of the forelegs and paws, and considerable of this material appeared on the nose and whiskers of the animals. Although it strongly resembled blood, there was no sign of broken skin or bleeding on the legs directly under the deposit, and it was not possible to obtain a positive blood reaction when the benzidine test was used. Upon autopsy, the contents of the stomach and intestines appeared very similar to this discharged material. Very often lesions appeared on the side of the head out from the eyes and the corners of the mouth. The animals seemed very nervous and spent considerable time rubbing their heads. Priapism was very commonly found.

$X$ represents the amount of extract, evaporated on cornstarch, obtained through the extraction of 90 gm. of corn.
Sultanina grapes in 5-gm. daily portions induced an average gain of 13.7 gm. for the entire eight weeks and therefore contain a small but significant amount of vitamin G. The animals receiving the Malagas did not make sufficient growth, as shown in Figure 7, to indicate the presence of any of this vitamin. From the same figure it may be seen that neither juice contains any vitamin G.

SUMMARY

Fresh Sultanina (Thompson Seedless) and Malaga grapes (Vitis vinifera) and two brands of commercial grape juice (No. 1, a mixture of juices from Flame Tokay and Zinfandel varieties, V. vinifera, and No. 2, the juice from Concord grapes, V. labrusca) were tested for their vitamin A, B (B1), C, and G (B2) content. The results showed that:

Both varieties of grapes contained a small but measurable amount of vitamin A. There was no evidence of this vitamin in either juice.

Vitamin B (antineuritic) was present in fair amounts in both kinds of fresh grapes tested and in small quantity in the commercial juice designated as No. 2. Commercial juice No. 1 did not contain vitamin B in a measurable quantity.

Fifteen grams of fresh grapes fed daily were found to contain insufficient amounts of vitamin C to protect guinea pigs from scurvy as determined by the Höjer method. This quantity of Sultanina grapes furnished approximately the same protection as 2 c. c. of orange juice and contained more of the antiscorbutic vitamin than the Malaga grapes. There was no indication of vitamin C, as determined by the Sherman method, in commercial juice No. 1. Tests made by the Höjer method indicated the absence of antiscorbutic vitamin in commercial juice No. 2.

Sultanina grapes appeared to contain a minimal amount of vitamin G, while Malaga grapes and both juices were lacking in this vitamin.

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