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The acids of the cranberry

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TABLE OF CONTENTS

INTRODUCTION

A survey of literature regarding the chemical constitution of fruits reveals the incompleteness of data regarding their acid contents. In but few instances has a thorough study been carried out, and have all the acids been identified and determined quantitatively. The data are reported in terms of a single acid, usually the predominant one, and are obtained by simple titrations. In the case of the cranberry, the acid content is usually calculated and reported as citric.

A complete review of the literature was desired. Likewise it was decided to determine the actual amounts of citric, malic and benzoic acids, which were known to be present; and to attempt the isolation of quinic acid, which was believed to be present.

REVIEW OF LITERATURE

There are but few references to the American species of cranberry, Vaccinium macrocarpum, in scientific literature, but there are many regarding the European species, Vaccinium vitis idaea, or Preisselbeere; and Vaccinium oxycoccus, or Moosbeere. Since there is a fairly close botanical relationship among the three, and since both Preisselbeere and Moosbeere are usually translated as cranberry, all the literature referring to the acid contents of these species has been reviewed and is included in this report.

I. BENZOIC ACID

Benzoic acid was first reported to be present in Preisselbeeren by Oscar Loew (1). Mach and Portele (2) found 0.0665 to 0.0862 per cent benzoic acid in cranberry juice (Preisselbeere). They believed this to be of great value as a preservative. A. Kanger (3) reported 0.0676 per cent benzoic acid in Preisselbeeren. Mason (4) determined the benzoic acid content of American cranberries. He found 0.05 per cent in ripe berries and less in green berries. Lehman (5), working with Preisselbeeren, found 0.074 per cent of benzoic acid. Behre, Grosse and Schmidt (6) reported 0.045 to 0.112 per cent benzoic acid in cranberry juice (Preisselbeere). Nestler (7) described a method for the determination of benzoic acid in cranberries and reported it to be present in Vaccinium vitis idaea, Vaccinium oxycoccus, and Vaccinium macrocarpum. In a later article (8), he attributes the great resistance of cranberries to plant and animal parasites to their high benzoic acid content. Griebel (9) carried out a complete investigation of the benzoic acid content of Preisselbeeren, Moosbeeren, and cranberries. He found 0.054 to 0.144 per cent as free acid, 0.088 to 0.22 per cent combined as esters, and 0.021 to 0.061 per cent in the form of the glucoside, vacciniin. Polenske (10) reported 0.089 to 0.206 per cent benzoic acid in Preisselbeeren. Flanders (11) found cranberries to contain about 0.05 per cent benzoic acid. Blatherwick (12) found 0.05 per cent benzoic acid in his study of cranberries. Radin (13) reported

finding 0.06 per cent benzoic acid in cranberries. Rising (14) studied the Preisselbeere and reported the total benzoic acid to be 0.112 per cent, with only 0.036 per cent existing as the free acid. Nelson (15) reported finding 0.069 per cent benzoic acid in cranberries. Clague and Fellers (16), working in our own laboratory, found that the total benzoic acid content of twenty-four varieties of cranberries varied from 0.029 to 0.098 per cent with an average of 0.065 per cent. A comparison of the benzoic acid contents of the different varieties with their keeping qualities did not indicate any definite correlation.

II. OTHER ACIDS

While the data reported on the benzoic acid content of cranberries is quite satisfactory, that regarding the other acids and the quantities of each present is most unsatisfactory. There has existed some disagreement as to just what acids are present, and but two men have attempted any direct quantitative work on the individual acids. The other investigators merely reported total acidity as citric or malic, and obtained their results by simple titration.

Scheele (17) found citric acid in Preisselbeeren and Moosbeeren in 1785. Graeger (18) was the next to study the acid content of Preisselbeeren. He found them to be a good source of citric acid and reported that they contained malic acid also. Goessmann (19) who published the first work on the American species, stated that cranberries contained

both citric and malic acids. Ferdinand (20) reported that cranberries contained 1.41 per cent citric acid, but no malic, tartaric, or oxalic acids. Kossowitch (21) studied Moosbeeren and reported 2.44 to 2.8 per cent citric acid. He obtained his results by simple titration. He stated that the Moosbeeren contains no other acids. Formanek and Laxa (22) studied the composition of wine prepared from Preisselbeeren and reported 0.244 per cent tartaric acid, 0.33 per cent citric acid, and 0.253 per cent malic acid. Mach and Portele (2) found citric and malic acids in Preisselbeeren, as well as a volatile acid which they did not identify. They found 1.80 to 3.41 per cent non-volatile acid calculated as malic, and 0.325 per cent volatile acid calculated as acetic. They stated that oxalic, succinic, tartaric, and salicylic acids are not present. Stolle (23) reported finding glyoxylic acid in Moosbeeren. Aparin (24) repeated this study and reported that glyoxylic acid was not present, but that the acid was citric. Kunz and Adam (25) stated that citric acid was present in Preisselbeeren, but no malic acid. Windisch and Schmidt (26) reported from 1.37 to 2.10 per cent citric acid in Preisselbeere. Bigelow and Dunbar (27) included cranberries in their survey of the acid content of fruits and found both citric and malic acids present with citric acid predominating. Their article contains the first report of a quantititative determination of an individual acid other than benzoic in cranberries. They report 0.56 to 0.71 per cent malic acid present. The method

used has since been discarded as unreliable, so these results are probably inaccurate. The best work on cranberry acids was probably that of Nelson (15). He used the ester method and found both citric and malic acids present, the ratio being 80 per cent citric and 20 per cent malic. Lebedo and Lindquist (28) recently reported the acids of the Moosbeere to consist of one half citric and the rest quinic. Their work can hardly be termed quantitative, however, and it does not allow the separation and identification of all the acids present.

Kanger (3) found large quantities of quinic acid in the cranberry plant, but stated that none was present in the fruit. The first to obtain evidence of its presence in the fruit was Flanders (11). In his study of the metabolism of benzoic and hippuric acids, he carried out some feeding experiments using cranberries. The yield of hippuric acid in the urine was far in excess of the amount expected, considering the quantity of benzoic acid present. He believed the most likely source of this excess to be quinic acid. Although his attempts to isolate this substance from cranberries were unsuccessful, he did obtain positive qualitative tests for it. Calculating from the amount of hippuric acid obtained, he estimated quinic acid to be present in amounts up to 1.34 per cent. About ten years later, Blatherwick and Long (29) carried out a similar experiment and obtained similar results. They found that when large amounts of cranberries were eaten, large increases in both titrable and organic acid acidities of the

urine resulted. The increase was found to be due to large amounts of hippuric acid, and was too large to have been caused by the benzoic acid content of the fruit. They suggested that the hippuric acid was probably due to quinic acid, but carried out no tests to verify this. Lüdke (30) was the first to note the increase in hippuric acid excretion after the ingestion of quinic acid, and the results of other workers have verified his finding. Quick (31) agreed with Blatherwick and Long that the increased hippuric acid resulting from eating certain fruits and vegetables was due to quinic acid.

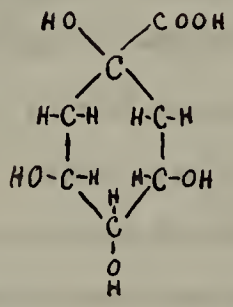
Kohman and Sanborn (32) stated that they had obtained quinic acid from cranberries in amounts up to 1 per cent. Morse (33) found that about one per cent acid calculated as quinic remained after the lead precipitate had been removed. Lebedo and Lindquist (28), as stated above, reported that the acids of Moosbeeren consist of one half quinic. They used the calcium precipitate to separate the acids, and only qualitative tests to identify the quinic acid. While their results give evidence of the presenee of quinic acid in Moosbeeren, they can hardly be termed quantitative. Fellers, Redmon, and Parrott (34) studied the effect of cranberries in the diet on urinary acidity and blood alkali reserve and obtained results similar to those of Flanders, and Blatherwick and Long. From their data, they estimated that cranberries contain from 0.5 to 0.9 per cent quinic acid, either as the free acid or in combined form. Jones (35), working in our own laboratory, was unsuccess-

ful in his attempts to obtain certain quinic derivatives starting with cranberries or cranberry extracts. With the evidence as stated above, it has been generally assumed that cranberries contain quinic acid either free or combined in amounts estimated at anywhere from 0.5 to 1.5 per cent.

OCCURRENCE AND PROPERTIES OF QUINIC ACID

Quinic acid was first isolated by Hofmann(36), an apothecary, as the calcium salt from cinchona bark. It is usually obtained as a residue in the preparation of quinine sulphate. Considerable work has been done in the occurrence of quinic acid and on its chemical and physical properties by German chemists, but practically none by Americans. It has been reported identified in meadow grass (1), in Vaccinium Myrtillus plants (37), in the plants and leaves of Vaccinium vitis idaea (3), in coffee beans (38), in Vaccinium Arctostaphylos (38), in beets, Beta vulgaris (38), in the fruit of Illicium verum (38), in the bark of the greenheart tree, Nectandra Rodioei (38), in tobacco, Nicotiana Tabacum (38), in the leaves of the black currant, Ribes Nigrum (38), in pine, Alburnum Pini (38), in Norway spruce, Picea Excelsa (38), and in cedar of Lebanon, Cedrus Libani (39). Usually it has not been actually isolated, but has been identified by qualitative tests. Gorter (40) believes that it is doubtful if quinic acid exists naturally in the free state, but rather that it exists in the form of chlorogenic acid, which on hydrolysis with alkali yields one molecule each of quinic and caffeic

acids. He found chlorogenic acid in 98 out of the 230 species of plants he investigated. Kiesel (38) believes quinic acid is an intermediate compound formed during the transformation from sugars to cyclic compounds. He formulates the reaction as follows: $C_6H_{12}O_6$ + HCOH = H_2O, $+C_7H_{12}O_6$ Fischer and his associates have carried out rather extensive investigations on quinic acid compounds. They have established its structure as follows (41):



From the above formula, it is apparent that quinic acid may exist in several isomeric forms. It is also apparent that a great number of compounds are possible since it has a carboxyl and four hydroxyl groups. A very great number of these compounds have been prepared and studied. A condensed list is to be found in Beilstein (42). By being heated in a closed tube with concentrated HI to 115° to 120° C., quinic acid is reduced to benzoic acid (43). Heating quinic acid with four parts $KMnO_4$ and one part H_2SO_4 yields hydroquinone (44). When an aqueous solution is boiled with PbO_2, hydroquinone is obtained (45). Heating quinic acid to 200-250° C. results

im the loss of water and the formation of the optically inactive quinide (46). Protocatechuic acid is formed by adding bromine water to a solution of quinic acid and evaporating on a water bath (47), and heating with fuming H_2SO_4 forms hydroquinone disulfonic acid with CO_2 splitting off (48).

Quinic acid (42) is a solid, crystallizing from water in white monoclinic prisms, melts at 161.6° C and is soluble in 2.5 parts of water, very slightly soluble in alcohol, and insoluble in ether. It is levo-rotatory, having a specific rotation of -44.03^0 at 20^0 in a 19.74 per cent aqueous solution. In a 5 per cent aqueous solution $[\alpha]_D^{25} = 42.1^0$ (49). Eykman (46) studied its dissociation and found its constant to be 2.77×10^{-4} at 14.1^0 C.

Loew (42) found that Schizomycetes fermented quinic acid, yielding protocatechuic acid if the fermentation was aerobic; and formic, acetic, and propionic acids if anaerobic. Emmerling and Abderhalden (50) found an organism, Micrococcus chinicus, which fermented quinic acid to protocatechuic. Butkewitsch (51, 52, 53) has shown that Citromyces, Penicillium, Mucor, and Asperigillus molds all use quinic acid as a source of carbon. He stated (54) that others had found beer yeasts incapable of fermenting quinic acid but that he found two species of wine yeasts which fermented quinic acid. He likewise found certain bacteria capable of growing with quinic acid as the sole carbon source. He found, however, that bacteria of the type of B. subtilis could not utilize quinic

acid. He mentioned the work of one W. W. Perwozwansky on the ability of a number of bacteria to utilize quinic acid as a carbon source, but gave no reference and no names of the bacteria studied. Butcher (55) reported that media containing quinic acid as the sole carbon source could be used for differentiating fecal and non-fecal strains of the colon-aerogenes group. The fecal strains were usually unable to utilize the quinic acid as a source of carbon.

The physiological importance of quinic acid does not seem to be definitely established. Lücke (30), as was stated above, found that it was excreted as benzoic acid. Flanders (11) found that the excretion of quinic acid was slow and that after 48 hours but 86 per cent of the theoretical amount of hippuric acid had been excreted. Weiss (56) and others have reported that giving quinic acid decreases the formation of uric acid. This is believed to be due to the glycocoll which would normally be used in the formation of uric acid being diverted to form hippuric acid. Hupfer (57) and others have reported no decrease in uric acid excretion after the ingestion of quinic acid. In fact Weiss's theory is no longer accepted. In spite of this, good clinical results have been obtained in gout through the use of different quinic acid preparations. Among these are sidonal, originally piperazine quinate, but more recently quinide; and urol, quinate of urea. This situation is very peculiar. Either the observations of the various workers are open to criticism, or else chance has led to the use of drugs which for some un-

known reason are efficacious in gout.

DISCUSSION

The published analyses of cranberries, Preisselbeeren, and Moosbeeren indicate that the total acidity of these fruits calculated as malic or citric varies from slightly under two to nearly three per cent. They likewise indicate that this acidity is distributed definitely between at least three, and probably four, acids; benzoic, malic, citric, and quinic. The solubility of benzoic acid, and the fact that it is readily purified by sublimation, make for rather easy and accurate analyses of this material. Hence the published results on the benzoic acid content of cranberries, Preisselbeeren, and Moosbeeren are probably for the most part accurate, and may be accepted. It may be concluded that the total benzoic acid content of these three species of fruit is quite similar and varies according to the variety from slightly below 0.05 per cent to about 0.1 per cent.

The most carefully conducted investigations have indicated that the three fruits contain both citric and malic acids with citric predominating. Bigelow and Dunbar (27) reported 0.56 to 0.71 per cent malic acid using a method which has since been discarded. These results are probably of little value. Nelson (15) found the proportion of citric to malic to be approximately four to one. While he did not determine the exact amounts of each present, his work was of such character as to lead one to accept his results. If

there were no other non-volatile acids present, the ratio would be sufficient, but this does not seem to be the case. Lebedo and Lindquist (28) stated that half the acidity of the Moosbeere was due to citric acid. Their work was of the nature of an approximation, however. Hence it is apparent that the exact amounts of citric and malic acid in cranberries is not known, and should be determined.

The literature likewise reveals the probability of the presenee of quinic acid in cranberries. At least, Morse (33) found that the acids of the cranberry were not completely removed by precipitation with lead, and other investigators (11, 29, 34) found excessive hippuric acid excretion after cranberries had been eaten. Flanders (11) obtained qualitative evidence of the presence of quinic acid; Lebedo and Lindquist (28) found it to be present in Moosbeeren; and Kohman and Sanborn (32) claim to have isolated up to one per cent from cranberries. The qualitative results of Flanders, and Lebedo and Lindquist indicate, but do not definitely prove, its presence. There are reasons to doubt the results of Kohman and Sanborn. Repeated trials in our laboratory using the method they outline failed to give any results whatsoever. Moreover, they stated they were going to publish a method for the quantitative determination of quinic acid according to their scheme of isolation, and this has not been forthcoming in the four years which have elapsed. It is probable that further work indicated the impossibility of such

a method as they suggested. Hence, it becomes apparent that although it is known that there is at least one acid which is not precipitated as the lead salt, and there is qualitative and indirect evidence that this acid is quinic, no one has actually isolated this acid from cranberries and definitely proved it to be present.

PURPOSE OF THIS INVESTIGATION

The purpose of this investigation is to determine the amounts of citric and malic acids present in cranberries, and to isolate and study the properties of the quinic acid believed to be present.

EXPERIMENTAL WORK

I. DETERMINATION OF CITRIC AND MALIC ACIDS

After a study of the various methods suggested for the determination of citric and malic acids, it was decided to use those taken from the Journal of the Association of Official Agricultural Chemists.

The method used for the determination of citric acid was that of Hartmann and Hillig (58). This is based on the so-called Stahre reaction which is the formation of pentabromacetone from citric acid. The method is time-consuming and empirical, but the authors claim a recovery of from 95 to 105 per cent on fruit products, and very nearly perfect results when pure acid mixtures were used.

In order to test the method and become acquainted

with the technique, it was decided to carry through several determinations on synthetic acid and invert sugar mixtures. The citric, quinic, and benzoic acids were obtained from Eimer and Amend, and were the finest grades. The l-malic acid was obtained from Eastman Kodak Company. The results of these trials are summarized in table I.

TABLE I. ANALYSIS OF KNOWN SOLUTIONS FOR CITRIC ACID

Solution	Vol. of aliquot ml.	Wt. of citric acid in aliquot mg.	Wt. of citric acid recovered mg.	Per cent recovered	Average per cent	Factor
1	10	147	116.4	78.94		
2	10	74.7	59.5	79.65		
3	10	92	72.3	78.65	79.68	1.26
3	10	92	73.4	79.68		
3 Hydrolyzed with KOH	10	92	79.6			

After this, samples of immature and mature cranberries (Howes) were prepared and analysed. The immature cranberries (Howes) were obtained from the State Cranberry Experiment Station in Wareham just before coloration started, and kept solidly frozen at 15° C. until used. The mature berries were obtained from the same source and held in ordinary cold storage at 35° C.

In preparing the samples, three 100-gram portions of each lot of berries were taken. One of each was digested with distilled water until the berries were well disintegrated

then the mixture was washed into a 250 cc. volumetric flask and made up to volume. This mixture was then filtered with suction through duck, and 20 cc. portions of the filtrate were used for each analysis. Another portion of each was digested over night with 0.1 N H_2SO_4 at 60° C., an equivalent amount of KOH added, and then made to volume and filtered as the other was. To the third portion of each enough KOH solution was added to give an approximately 0.1 N KOH solution and this mixture digested at 60° C. over night. An equivalent amount of H_2SO_4 was added, and the solution treated as the others were. The above treatments were intended to hydrolyze any salts or esters of the acids which might have been present.

The results of the analyses are condensed in table 2.

TABLE II. CITRIC ACID CONTENT OF CRANBERRIES (HOWES)

Material	Equivalent of cranberries in aliquot g.	Wt. of citric acid obtained mg.	Per cent Citric Acid	Corrected per cent Citric Acid	Average per cent
Ripe berries					
1.Untreated	8	68.03	0.85	1.07	
"	8	69.90	0.87	1.09	
2.Acid Hydrolysis	8	68.03	0.85	1.07	1.07
"	8	67.10	0.84	1.06	
3.Basic hydrolysis	8	73.22	0.92	1.16	
"	8	72.90	0.91	1.15	
Immature berries					
1.Untreated	8	83.53	1.04	1.31	
"	8	83.48	1.04	1.31	
2.Acid hydrolysis	8	83.35	1.04	1.31	1.31
"	8	83.61	1.05	1.32	
3.Basic hydrolysis	8	89.55	1.12	1.41	
"	8	89.01	1.11	1.40	

In order to ascertain if the recoveries of citric acid were the same from cranberry solutions as from pure sugar and acid mixtures, citric acid was added in known amounts to one of the cranberry solutions. This was analyzed for citric acid and an untreated sample was analyzed at the same time in order

to duplicate conditions. The results are tabulated in table 3.

TABLE III. RECOVERY OF ADDED CITRIC ACID FROM CRANBERRY SOLUTIONS

Solution	Citric acid added mg.	Citric acid recovered mg.	Added citric recovered mg.	Per cent recovery
Immature untreated	0	83.90		
" "	49.7	123.6	39.7	79.9
" "	49.7	123.3	39.4	79.3
Pure solution	92	73.4	73.4	79.6

Malic acid was determined on the same samples using the official method of Hartmann and Hillig (59). This determination is even more time-consuming than that of citric acid, and is absolutely empirical. It depends on the rotation of the uranium compound of l-malic acid after the removal of interfering substances.

Trial determinations of known solutions indicated very good recovery. Two determinations carried out on solutions containing 40 mg. of l-malic acid yielded recoveries of 40.6 mg. and 40.2 mg.

The results of the malic acid determinations on the cranberry solutions are presented in table 4.

TABLE IV. THE L-MALIC ACID CONTENT OF CRANBERRIES (HOWES)

Material	Equivalent of cranberries in aliquot g.	L-Malic acid determined mg.	Per cent l-malic acid	Average per cent
Ripe berries				
1.Untreated	8	22.2	0.28	
"	8	21.1	0.26	
2.Acid hydrolysis	8	19.0	0.24	
"	8	19.9	0.25	0.257
3.Basic hydrolysis	8	19.7	0.25	
"	8	20.5	0.26	
Immature berries				
1.Untreated	8	20.5	0.26	
"	8	21.2	0.26	
2.Acid hydrolysis	8	20.8	0.26	
"	8	21.1	0.26	0.265
3.Basic hydrolysis	8	22.6	0.28	
"	8	21.4	0.27	

DISCUSSION OF RESULTS OF CITRIC AND L-MALIC ACID DETERMINATIONS

Hartmann and Hillig (58) reported recoveries of from 95 to 105 per cent by their method for the determination of citric acid. These results could not be duplicated when mixtures of cranberry acids were used. The determinations

required about twice the volume of ferrous sulphate mentioned by Hartmann and Hillig as usually being required to dissolve the precipitated MnO_2. It may be that some constituent of the cranberry causes interference. The fact that the same results were obtained with synthetic solutions indicates that it is either the quinic or benzoic acid.. The alcohol concentration of the solution during the lead precipitation is sufficiently high to cause their precipitation with the citric and malic acids at this stage. Time did not permit an investigation of this. It may be significant, however, that in the application of this method to the determination of citric acid in milk (60), Hartmann and Hillig found it necessary to introduce the factor 0.64 in the denominator of their equation. This would indicate only a 64 per cent recovery. Table (1) indicates a very consistent recovery of about 79 per cent of the citric acid in the pure solutions. Table (3) reveals the fact that a similar recovery was obtained in the case of the citric acid added to cranberry solutions. For these reasons, the author feels justified in considering that he obtained a like recovery of the citric acid naturally contained in cranberries.

From table (2) it is apparent that ripe cranberries contain 1.07 per cent citric acid, and immature berries 1.31 per cent. Since citric acid existing in the form of salts is not transformed into pentabromacetone by this method, it is apparent from a comparison of the results before and after hydrolysis with acid that it does not exist in this condition.

The higher results after hydrolysis with KOH are probably due to the destruction of part of the sugar rather than to the existence of part of the citric acid as an ester. At least, similarly treating a portion of the known solution gave similar results. The increase is practically the same in all three instances: the known solution, the ripe berries, and the green berries, i.e. about six milligrams.

In the case of the l-malic acid, the only difficulty encountered was in reading the polariscope. The solutions were quite yellow, due to the uranium, and hence the disc was very dim. However, six readings of each solution were made and averaged. The excellent recovery of l-malic acid from the known solution, and the close agreement of the results seem to indicate that they are dependable. From table (4) it is apparent that ripe cranberries contain 0.257 per cent l-malic, and green berries 0.265 per cent. The difference between these results is probably within the limits of accuracy of the method, and hence it may be concluded that both ripe and green cranberries contain close to 0.26 per cent l-malic acid. Since the method determines the l-malic existing in the form of salts, it would not be expected that any differences would result from acid hydrolysis. Likewise, from the results after hydrolysis with KOH, it is apparent that none of the acid exists in the form of esters.

It is interesting, if not significant, to note that in ripe cranberries the results obtained for the amounts of

the two acids present have a ratio of almost exactly 4 to 1, as was suggested by Nelson (15). In the case of immature berries, the ratio is practically 5 to 1.

For completeness, the benzoic acid contents of the two samples were determined. It was found to be 0.054 per cent for the ripe berries, and 0.017 per cent for the immature berries. These results agree with the findings of Mason (4) and with those of Clague and Fellers (16) who found the benzoic acid content of 20 varieties of cranberries varied from 0.029 to 0.098 with an average of 0.065 per cent.

II. ISOLATION OF QUINIC ACID

A. By extraction

Since the first step in isolating a compound from any substance is the extraction of that compound, it is necessary to know something of its solubility. Morse (33), and Flanders (11), had found that the invert sugar content of cranberries was the principal interfering substance. If the sugar and acid could be separated by the use of a suitable solvent, the isolation would be greatly simplified. Although there is little difference in the empirical formulae, it seemed probable that the difference in structure might cause a significant difference in solubility if the proper solvent could be found. The results of the qualitative investigation using pure quinic acid and invert sugar are summarized in table (5).

TABLE V. COMPARATIVE SOLUBILITIES OF INVERT SUGAR AND QUINIC ACID

Solvent	Quinic Acid	Invert Sugar
Petroleum ether	Insoluble	Insoluble
Ligroin	"	"
Methyl alcohol (abs.)	Slightly soluble	Slightly soluble
Methyl alcohol & H_2O	Soluble	Soluble
Ethyl alcohol (abs.)	Very slightly soluble	Very slightly soluble
Ethyl alcohol & H_2O	Soluble	Soluble
Propyl alcohol	Very slightly soluble	Very slightly soluble
Isopropyl alcohol	" " "	" " "
Isobutyl alcohol	" " "	" " "
Butyl alcohol	" " "	" " "
Amyl alcohol	" " "	" " "
Ether	Nearly insoluble	Insoluble
Chloroform	" "	"
Chloroform (sat.with H_2O)	Slightly soluble	Slightly soluble
Chloroform & ethyl alcohol (various mixtures)	" "	" "
Carbon disulfide & carbon tetrachloride	Insoluble	Insoluble
Ethyl acetate	"	"
Amyl acetate	"	"
Benzene	"	"
Toluene	"	"
Xylene	"	"

Acetone	Insoluble	Very slightly soluble		
Glacial acetic acid	Very slightly soluble	"	"	"
75% acetic acid	Soluble		Soluble	

The close similarity of solubilities in all the solvents used is apparent from the above table. A slight difference was noted in the case of acetone. An attempted separation by acetone extraction using a Soxhlet continuous extraction apparatus was unsuccessful. Apparently the solubility of quinic acid in acetone is high enough to permit extraction with the sugars. Since the use of solvents is of no value for isolating quinic acid, no further work was attempted using solvents.

B. As a salt

The next series of experiments was carried out using metal salts of the acid. Salts are among the easiest compounds of organic acids to prepare, and are used at some point in most methods of their isolation and quantitative determination. However, the salt prepared must have solubility characteristics which permit its separation from the other substances present. This is quite readily accomplished in the case of citric and malic acids, but this is not true of quinic acid. Even in pure solutions, most quinic acid salts are crystallized only with difficulty; and in the solutions with which it is necessary to work in the attempts at isolation from the cranberry, the invert sugar present makes crystalli-

zation practically impossible. When the concentration where the salt would normally crystallize is reached, the sugar concentration is so high that a very viscous syrup results. Moreover, it was found that the concentration of the salt is always less than that expected.

Clem (61) reported that manganese quinate is soluble one part in 200 parts of water; and cadmium quinate, one part in 230 parts of water. He prepared the salts by boiling the acid with the oxides of the metals. The author found that they were not prepared readily by this method, but could be prepared from barium quinate, using the sulphates of the metals. However, the salts were found to be fairly soluble in water and of no value in the isolation of quinic acid.

The ordinary copper salt, $Cu\ (C_7H_{11}O_6)_2 + 2H_2O$, is very soluble (42), but Liebig (62) is reported to have prepared a copper salt, $CuC_7H_{10}O_6 + 2H_2O$, which is soluble only one part in 1150-1200 parts water. According to Hesse (64) the latter was formed in a solution containing $Ba\ (C_7H_{11}O_6)_2$, $Ba\ (OH)_2$, and $CuSO_4$. At this time, attempts to prepare this salt were unsuccessful, although in later work it was prepared and found to be very useful. The difficulty was probably due to improper concentrations of alkali and to the use of too much heat. A green solution was obtained, but no crystals, and heating resulted in decomposition. Moreover, Hesse's reference was indefinite and gave formulae as follows: $C_{28}\ H_{10}\ Cu_2\ O_{22} +\ 2\ Cu\ O\ +\ 8\ HO$, $C_{14}\ H_{11}\ Cu\ O_{12} + Cu\ HO_2 +$

H_2O_2, and $C_{14}\ H_{10}\ Cu_2\ O_{12}$. These formulae led to the belief that he had probably been working with impure materials and had obtained a salt of the impurity rather than of the quinic acid.

The ammonium, barium, cobalt, iron, lead, magnesium nickel, silver, sodium, strontium, and zinc salts are all very soluble (42). Gundelach (63) found a commercial preparation of calcium quinate to be a double salt composed of equal moles of calcium quinate and calcium acetate. He prepared such a compound by mixing equivalent amounts of the two salts and allowing crystallization to take place. His analyses indicated that the crystals had the following composition:

$$\left.\begin{matrix} C_7\ H_{11}\ O_6 \\ C_2\ H_3\ O_2 \end{matrix}\right\rangle Ca + H_2O$$

This compound was very soluble in water but insoluble in alcohol.

Kohman and Sanborn (32) state that much use was made of the above compound. Repeated trials by their method on cranberry extracts have failed to yield any quinic acid. Even when pure acid solutions were used, the results were unsatisfactory. It seems improbable that a compound requiring such carefully balanced concentrations of its components could be consistently formed in such conglomerate solutions as one has to deal with in work with fruit extracts.

The method used by Zwenger (37) depended on the

insolubility of the calcium salt of quinic acid in strong alcohol solutions. An attempt was made to isolate quinic acid according to his method. About two kilograms of ripe cranberries were boiled with water until completely disintegrated, a large excess of $CaCO_3$ added, and the mixture boiled and filtered. The filtrate was evaporated to about 100 ml., and 500 ml. of 95 per cent alcohol added. A gummy mass was thrown down, The precipitate was dissolved in hot water, made slightly acid with acetic acid, lead acetate was added in slight excess, and the solution allowed to stand. The filtrate from this was decomposed with H_2S and the precipitate filtered off. The filtrate was evaporated to small volume, decolorized with norite, and then evaporated to dryness. Less than a gram of material was obtained, and this was apparently very impure. This material was dissolved in water and again precipitated with alcohol. This time 0.3 grams of a pure white material were obtained. This was taken up with water, and oxalic acid added just to the point of complete precipitation. After filtering, the mixture was evaporated. A very sticky brownish residue was obtained, which defied all attempts at crystallization. This material did give a positive test for hydro-quinone after having been boiled with PbO_2 in aqueous solution. Flanders (11) used this method to test cranberries for the presence of quinic acid. His results were approximately the same as those reported here.

The above method was used to obtain a quantity of

the crude material in order to subject it to further attempts at purification. About two grams were obtained. This was dissolved in water, $CaCO_3$ added in excess, the mixture filtered, the filtrate evaporated to small volume, and four volumes of 95% alcohol added. The residue was still very gummy. It was dissolved in hot water and lead acetate added, but no precipitate was obtained. The lead was removed by H_2S, and the mixture filtered. The filtrate was evaporated to about 50 ml. and boiled with norite to further decolorize it. The last traces of color seemed to be impossible to remove. Oxalic acid was added just to the point of complete precipitation and the mixture filtered. The filtrate was evaporated to dryness. All that remained was about 0.2 grams of a gummy yellowish mass which could not be decolorized by washing with alcohol, ether, petroleum ether, or benzene, and which could not be made to crystallize. The residue still gave hydroquinone an oxidation, so it was believed to contain some quinic acid. The extremely small recovery seems unexplainable at present.

Tanret (39) isolated quinic acid from the cedar of Lebanon according to the following scheme: 1. Repeated extracting with boiling 70 per cent alcohol; 2. Precipitating with lead subacetate and filtering; 3. Decomposing the filtrate with H_2SO_4, filtering, and evaporating to small volume; 4. Removing acetic acid by washing with large volumes of ether; 5. Neutralizing with $Ba(OH)_2$ and evaporating to a

syrup; 6. Washing out the sugars with boiling 70 per cent alcohol; 7. Dissolving in water and again adding lead subacetate and filtering; 8. Decomposing the filtrate with H_2S and filtering; 9. Evaporating to dryness; 10. Taking up residue in boiling 95 per cent alcohol and evaporating; 11. Taking up residue in a small volume of water and allowing crystallization to take place. Successive trials of this method using as much as 6 kilograms of cranberries failed to yield more than a trace of material which seemed to give a positive test for hydroquinone after oxidation with PbO_2.

Kiesel's article (38) was not immediately available but the abstract appeared complete and was followed, using two kilograms of cranberries. The dried material was extracted with benzene, air-dried, extracted three times with hot water, and pressed. Lead acetate was added to the water extract and the precipitate filtered off. The filtrate was evaporated to about one-half its original volume and lead acetate added to complete precipitation, and the mixture filtered. The filtrate was decomposed with H_2SO_4 and filtered. This filtrate was evaporated to a surup in a vacuum desiccator, and then alternately extracted with hot and cold 80 per cent alcohol. A very gummy residue was left, from which, according to Kiesel, quinic acid should crystallize on standing a short time. No crystals were obtained, but again obtained positive qualitative tests for quinic acid. Changing the material back to the lead or calcium salt, or keeping it as

the free acid and washing with solvents, resulted in no further purification and merely caused a gradual loss of the material. The final residue was not an acid and gave no hydroquinone on oxidation.

Considering that quinic acid is indicated in amounts exceeding one per cent in cranberries, it would seem that one should be able to obtain it by the above methods. It is true that in fruits we have to deal with large amounts of sugars, but the residues obtained which gave positive tests for quinic acid did not reduce Benedict's solution. Hence, sugars were not involved in the final steps of purification.

C. As an organic derivative

Since no separation had been accomplished by use of solvents or metal salts, it was decided to attempt the preparation of organic derivatives of quinic acid. The difficulty of such a procedure was fully appreciated, yet it seemed that there might be a possibility of a separation by this method. Jones (35) worked on cranberry extracts and had been unsuccessful in his attempts to prepare or detect in his final product, benzoic acid, protocatechuic acid, or hydroquinone-disulfonic acid, although he did obtain some positive tests for quinone and hydroquinone after oxidation. He used the methods which had been found to produce these derivatives from solutions of the pure acid by former investigators (43, 47, 48, and 44, 45 respectively). Since it was desired to isolate the acid in order to determine

whether it was identical with that obtained from other sources, none of these compounds or procedures were of value.

Nelson (15) has been very successful in his work on fruit acids, using the esters. His work on cranberry acids was carried out according to this scheme, but unfortunately he worked with only the lead precipitate which Morse (33) has shown does not contain all the acids present.

The ethyl ester was the first compound of quinic acid prepared. This compound was prepared by Hesse (64) from the silver salt and ethyl iodide. He found it to be a viscous yellow liquid soluble in water and ethyl alcohol, and slightly soluble in ether. It was decided to prepare the compound from the pure acid in order to study it further.

This compound was very easily prepared according to the method of esterification used by Holland and Buckley (65). Five grams of pure dry quinic acid were refluxed in 200 ml. of absolute ethyl alcohol containing 2 per cent H_2SO_4 which had been heated to 225° C. The quinic acid went into solution rapidly and the esterification was completed within one hour. The H_2SO_4 was removed by adding dry $BaCO_3$. The alcohol was removed by distillation under reduced pressure. Approximately 90 per cent of the theoretical yield was obtained. The ester was a viscous straw-colored liquid soluble in water and alcohol, but only slightly soluble in ether.

Ester mixtures are usually readily separated by

distillation; hence the boiling point of ethyl quinate was studied. At atmospheric pressure it decomposed without boiling. The pressure was reduced successively until a pressure of but three mm. of mercury was obtained, but even then the material decomposed without boiling.

Since it was apparently impossible to separate the ester from other materials by the usual method it was decided that the high solubility in alcohol would suffice for its separation from the sugars at least, and since ethyl malate and ethyl citrate are highly soluble in ether, while ethyl quinate is only slightly soluble in ether, a separation of these compounds could be effected.

A water extract of cranberries was prepared and evaporated to dryness on the steam bath. The residue was allowed to stand 48 hours in the steam oven, and then for several days in a vacuum dessicator over H_2SO_4. This material was then transferred to a flask, and absolute alcohol containing two per cent of the heated H_2SO_4 was added at the rate of 100 ml. for each 100 grams of cranberries taken. This was refluxed on a water bath over night. The liquid was decanted into a dry flask, dry $BaCO_3$ added, and the solution filtered through a dry filter. The alcohol was removed by distillation under reduced pressure. The residue was a black tar which defied all methods of fractionation.

It was decided that a separation might be possible if the material was treated to remove the lead precipitate.

Another sample of cranberries was taken and the water extract obtained. An equal volume of alcohol was added to precipitate the pectin, and the mixture was filtered. Basic lead acetate was added to the filtrate until no more precipitate formed. The filtrate from this was decomposed with H_2S, and filtered. The clear filtrate was evaporated to small volume and extracted with ether to remove the acetic acid; then it was evaporated to dryness on the steam bath, dried in the steam oven, and finally dried in the vacuum dessicator. The dried material was transferred to a flask, and absolute ethyl alcohol containing two per cent heated H_2SO_4 was added at the rate of 50 ml. per 100 grams of cranberries used. This mixture was refluxed for four hours and then treated as the others had been. A residue resembling ethyl quinate in consistency was obtained, but it was very dark in color. It was found to consist mainly of sugar, most of which had caramelized. No solvent was found which would effect a separation of any material resembling ethyl quinate. It is known that fructose is moderately soluble in absolute ethyl alcohol, and that sugars form compounds of the ether type with alcohol. It is quite possible also the ethyl quinate helps dissolve the sugars, or that the sugar compounds are readily soluble in alcohol. It was concluded that the ester method was useless for the isolation of quinic acid from cranberries.

Since neither of the two most easily prepared compounds involving the carboxyl group, metal salts and esters,

seemed to be useful in isolating the pure acid, it was decided to make a survey of possible compounds which depended on the presence of the hydroxyl groups. Since the monosaccharides contain five such groups as compared to four in the quinic acid molecule, it is to be expected that similar compounds would be formed. The literature indicates a solubility difference between tetra-acetyl quinate and penta-acetyl compounds of the sugars. The tetra-acetyl quinate was stated to be slightly soluble in hot water, while the acetylated sugars were practically insoluble.

Tetra-acetyl quinate was prepared from pure quinic acid according to the procedure of Erwig and Koenigs (66), and the acetylation of the sugars was accomplished according to the method used by the same authors (67). The solubility of the tetra-acetyl quinate in hot water was found to be very slight. Moreover, unless conditions are absolutely correct, tri-acetyl quinate is formed by the method used and has the same solubility as the acetylated sugars. In view of the extremely slight solubility difference of the compounds and the difficulty in preparing them using cranberry extract as the source of materials, it was concluded that a separation in this manner was highly improbable.

None of the other simple organic derivatives offered as much encouragement as the above; hence it was decided not to continue the work on such compounds.

D. By the use of microorganisms

Since the attempts to separate quinic acid from sugars by chemical means had failed to yield any crystallizable material, it was decided to attempt to remove the sugars by fermentation.

Butkewitsch (51, 52, 53, and 54) has found that while molds of the aspergillus, citromyces, mucor, and penicillium genera, and wine yeasts, as well as certain types of bacteria ferment quinic acid, some bacteria do not. Butcher (55) found that bacteria of the colon group were unable to utilize quinic acid, and Butkewitsch (54) stated that another worker had found that beer yeasts did not ferment quinic acid.

The first fermentation attempted was one using a freshly isolated strain of E. coli. Since bacteria require a media of nearly neutral reaction, calcium carbonate was added to a sample of cranberry juice until no further effervescence resulted. The mixture was filtered and the filtrate diluted to give a sugar concentration of about 0.5 per cent. It was next sterilized under pressure, and then heavily innoculated with the bacteria. Even after four weeks' incubation, the sugar had not been completely removed. At that concentration it should have been removed in a few days. Microscopic examination revealed but few organisms in the material.

This would seem to indicate that the cranberry

juice was not a satisfactory medium for bacterial growth. Of course, in undiluted juice the benzoic acid content is sufficiently high to prevent good growth, but at the dilution used this material should exert practically no inhibiting action. The acid content had not been sufficiently increased to cause cessation of growth.

In order to retain the solution in a proper condition for the future isolation of quinic acid, it was necessary to avoid adding materials which could not be subsequently removed with ease. This could not be accomplished if the solution were to be supplemented with materials to promote bacterial growth. Hence it was decided to attempt the removal of sugars by yeast fermentation. Of course yeasts are much more efficient than bacteria in sugar fermentation, but there seems to be some question as to whether or not yeasts attack quinic acid, so they were not used at first. It was decided that since sugars are fermented first, if the process was controlled and stopped as soon as the sugars disappeared, the quinic acid probably would be left intact. Also since yeasts grow over a wide range of pH, the neutralization would not be so important.

A sample of cranberry juice was prepared as in the case of the bacterial fermentation and inoculated with the Lister strain of Saccharomyees cerevisiae. Although a froth developed and the solution showed the presence of alcohol, the sugars were not entirely removed after two

weeks' incubation.

Another sample of cranberry juice was prepared. This time the benzoic acid was removed by steam distillation and a small amount of ammonium acid phosphate was added. After dilution and sterilization, the solution was heavily inoculated with the yeast. After five weeks, the sugars were still incompletely fermented. Since it was undesirable to add other materials to encourage growth, this method for removing sugars was discontinued.

E. As the copper salt.

Since all the customary methods used in isolation of naturally occurring compounds were apparently of no use in the isolation of quinic acid from cranberries, it was decided to attempt again the formation of the copper salt prepared by Liebig (62). In previous attempts, although the solution became green, nothing would crystallize out, and on heating the copper was largely reduced. Since the compound is not a common type and had been mentioned by no one since Hesse, the author was inclined to doubt the possibility of preparing such a salt and discontinued his efforts without too thorough a study. He fully expected to be able to isolate the quinic acid by some of the other methods. The failure of the other methods led him to retrace his previous work. However, by adding a solution of $CuSO_4$ to a solution of barium quinate just to complete precipitation of the $BaSO_4$, a salt was formed which was transformed to a fairly insoluble compound by heating to

boiling. Even this was reduced on continued heating. Although this salt was not prepared as Hesse stated, apparently it was the one prepared by Liebig, $CuC_7H_{10}O_6 + 2H_2O$, or the solubility given for the other copper salt $Cu(C_7H_{11}O_6)_2 + 2H_2O$ in Beilstein (42) was incorrect. It was decided to attempt the formation of a similar compound using a cranberry extract.

Five hundred grams of cranberries were taken and extracted three times with boiling water, and pressed. The pectin was precipitated by adding an equal volume of 95 per cent alcohol. This mixture was filtered through cotton, and basic lead acetate was added to the filtrate until no further precipitate formed. The mixture was then centrifuged and the clear liquid collected. The lead was removed from this with H_2S and filtration. The filtrate was boiled vigorously and allowed to go to small volume repeatedly in order to remove the acetic acid. Finally, it was washed with large quantities of ether to remove the acetic acid remaining. The solution was then decolorized by boiling with norite, filtered, and evaporated, to about 20 ml. This was exactly neutralized with $Ba(OH)_2$, using phenolphthalein as an indicator and four volumes of 95 per cent alcohol were added. The gummy residue was taken up in a small volume of hot water, and reprecipitated by the addition of four volumes of alcohol. The residue was rinsed with 80 per cent alcohol. A 10 per cent solution of $CuSO_4$ was added just to the point where no more $BaSO_4$ was precipitated

and the precipitate removed by filtration. The solution was heated to boiling, then allowed to evaporate at room temperature in a vacuum desiccator. A small yield of light green crystals was obtained which under a microscope looked exactly like the crystals obtained from the pure quinic acid. The salt decomposed without melting so it was necessary to obtain a larger quantity before further study could be made.

The above procedure was followed using three kilograms of cranberries. After three crystallizations 0.9 of a gram of pure crystals were obtained. During the last recrystallization, portions of the solution were analyzed for their copper content. From the results obtained, it was calculated that one part of the compound was soluble in approximately 900 parts of water. This was low enough to permit its separation in the presence of sugars, hence the alcohol precipitation as the barium salt was eliminated. Better yields were obtained since only a fraction of the barium salt is precipitated by 76 per cent alcohol solution.

Six kilograms of cranberries were taken for the next isolation and 2.5 grams of the impure salt were obtained. After two recrystallizations, 1.87 grams of crystals were left. In order to obtain the acid in the free condition, 1.50

grams of these pure crystals were taken and dissolved in hot water. Hydrogen sulfide was bubbled through until the salt was completely decomposed. The copper sulfide was filtered off and the solution was evaporated to small volume. Several volumes of alcohol were added, and then ether just until turbidity started. This solution was set aside and allowed to evaporate at room temperature. Seven-tenths of a gram of the material were obtained.

The material was a white, finely crystalline solid, and was strongly acid. Its equivalent weight was determined by titration with 0.1N KOH, using phenolphthalein as an indicator. Its specific rotation and melting point were determined, and qualitative tests were made for hydroquinone after oxidation with PbO_2. The copper content of the purified copper salt was determined electrolytically.

A quantity of immature berries, 1.5 kilograms, was carried through the same procedure. Approximately 0.7 of a gram of the crude copper salt were obtained. From 0.35 of a gram of this, 0.22 of a gram of the free acid were obtained by decomposition with H_2S. The same tests were carried out as in the case of the ripe berries.

In table 6 the data are tabulated and compared with parallel results obtained from pure quinic acid.

Table VI. COMPARISON OF MATERIAL ISOLATED FROM CRANBERRIES WITH QUINIC ACID

Material	Description	Melting point °C.	$[\alpha]_D^{25}$	Equivalent weight	Oxidation to hydroquinone	Per cent copper in salt
Quinic acid from ripe berries	white crystalline	159	-42.95	191.8	+	21.94
From green berries	white crystalline	164	-43.41	193.5	+	21.82
Pure quinic acid (42)	white crystalline	161.6	-42.10	192	+	22.04

A study of the solubility of barium quinate indicated that 3.23 grams were soluble in one liter of 70 per cent alcohol, while barium malate and citrate were insoluble in this concentration of alcohol. It was decided to modify further the method of isolation used to see if better yields of quinic acid could be obtained. The method developed was found to give greater yields of quinic acid than any other tried.

Six hundred grams of cranberries were taken and the water extract prepared by boiling and pressing. This was exactly neutralized with $Ba(OH)_2$ solution, and then made up to two liters. After standing two hours with frequent shaking the mass was filtered. One liter of the filtrate was evaporated to 300 ml. and made up to 1200 ml. with 95 per cent alcohol. After a thorough mixing, the solution was filtered through fluted filters which were kept covered to prevent evaporation of the alcohol. The precipitate con-

tained the pectin and barium salts of malic and citric acids. The filter was washed with 400 ml. of 70 per cent alcohol, and the washings added to the filtrate. This solution was evaporated nearly to dryness, and then made up to about 300 ml. and decolorized by boiling with norite. The barium was precipitated by adding $CuSO_4$ solution to the hot liquid. The $BaSO_4$ was removed by filtration and the filtrate seeded with a few crystals of the copper salt obtained in previous work. Crystallization was allowed to take place as the solution was evaporated at slightly above room temperature (about 45° C.). The crystals were filtered with suction, washed with cold water, alcohol, and finally with ether, and dried in the steam oven. A yield of one gram of crude crystals was obtained. These contained 21.60 per cent copper which corresponds to a purity of 98 per cent.

The mother liquor was noted to be strongly acid to litmus. This seemed to indicate that in the transformation from the barium salt to the copper salt one of the molecules of the acid bound to the barium was liberated as the free acid, and the two valences of the Cu were satisfied in some manner by one molecule of the acid. This solution was titrated with a solution of $Ba(OH)_2$, and two ml. less than an equal volume of the base was added in excess. A slight precipitate formed and was removed by filtration. An excess of $CuSO_4$ was added to the filtrate and the $BaSO_4$ removed by filtration. The fil-

trate was acid to litmus, and on standing, crystals of the copper salt formed. These were filtered, washed, and dried as before. The yield was 1.5 grams, bringing the total from the 300 grams of cranberries up to 2.5 grams. This weight of the copper salt is equivalent to about 1.6 grams of quinic acid.

The method was entirely satisfactory. Further studies on isolation methods for quinic acid were discontinued at this time.

DISCUSSION OF THE ISOLATION AND IDENTIFICATION OF QUINIC ACID

Although the use of solvents, metal salts according to the methods of other investigators, organic derivatives, and microorganisms were all without success in the attempts made to isolate quinic acid from cranberries, a method was finally developed which resulted in the isolation of slightly over 0.5 per cent quinic acid in the form of one of its copper salts. The physical properties of the free acid obtained from this salt by decomposition with H_2S reveal that it is identical with pure quinic acid as obtained from other sources. The poor yields obtained when the citric and malic acids were removed by precipitation with lead were probably due to the carrying down of the quinic acid in the heavy precipitate. This method of isolation is not quantitative since its purpose is the isolation of the pure acid at the sacrifice of a quantitative recovery. It is significant, however,

that a yield corresponding to over 0.5 per cent of quinic acid in cranberries (Howes) was obtained.

The copper salt prepared was probably that first reported by Liebig and referred to by Hesse (64). The formula Hesse gives for the compound at 100° C., $C_{14}H_{10}Cu_2O_{12}$, is the same as the one accepted now, $CuC_7H_{10}O_6$, since water at that time was considered to be HO; and hence everything except the hydrogen has the subscript doubled. Kiesel's (38) brief study of the salt indicated that the crystals contained two molecules of water of crystallization.

The theoretical copper content of the salt $CuC_7H_{10}O_6 + 2H_2O$ is 22.04 per cent. The copper contents of samples prepared from cranberries as determined electrolytically were 21.94 and 21.82 per cent for the ripe and green fruit respectively. The solubility at room temperature, 23°C., was determined in two solutions from which the salt was crystallizing and which had been standing two weeks. Twenty-five ml. portions were taken from each solution and the copper content determined electrolytically. A calculation of the solubility based on these values indicates that one part $CuC_7H_{10}O_6 + 2H_2O$ is soluble in about 900 parts of water at 23°C.

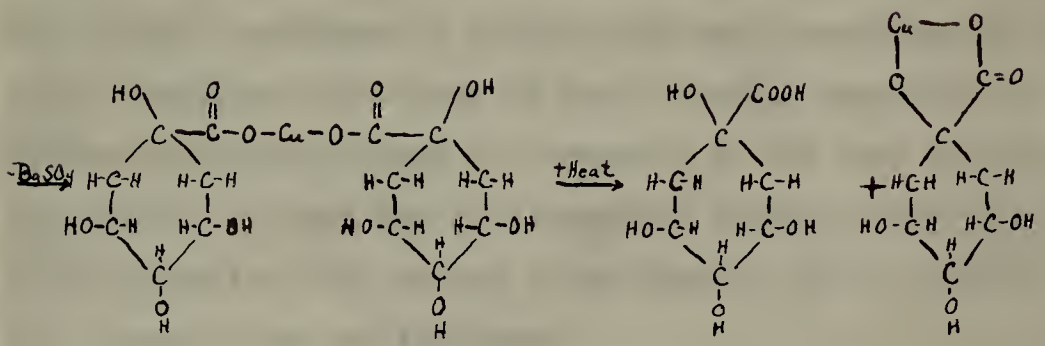
The fact that a single molecule of monobasic acid, such as quinic acid, can unite with copper satisfying both valences is interesting. None of the standard organic chemistry textbooks mention this or refer to similar compounds, and the references in the literature are very old and give rather fanciful formulae and obscure directions for the

preparation of such compounds. Hence, it was not surprising when attempts to form the compound $CuC_7H_{10}O_6 + 2H_2O$ were unsuccessful and were discontinued at first in the belief that such salts could not be prepared. It has since been found that lactic acid does form similar compounds with copper and even with sodium under proper conditions (68). Lactic acid was for some time considered to be a dibasic acid for this reason.

Although the method which was successful did involve the use of $Ba(OH)_2$, it was found that all $Ba(OH)_2$ must be removed before the solution was heated. This is not in agreement with Hesse's directions (64) since he states that the salt is formed in a solution containing $Ba(OH)_2$, $Ba(C_7H_{11}O_6)_2$, and $CuSO_4$. When his directions were followed, each attempt resulted in the decomposition of the desired compound. When a slight excess of $CuSO_4$ was present, little or no decomposition resulted. Kiesel (38) formed the salt $CuC_7H_{10}O_6 + 2H_2O$ by boiling his solution of free quinic acid with $CuCO_3$, but had no other acids present. The copper salts of malic and citric acid are too insoluble to permit the use of his method for the isolation of pure quinic acid from cranberries.

The reactions involved in the formation of $CuC_7H_{10}O_6 + 2H_2O$ are probably as follows:

$$Ba(OH)_2 + 2\,C_7H_{12}O_6 \xrightarrow{-H_2O} \begin{matrix} C_7H_{11}O_6 \\ C_7H_{11}O_6 \end{matrix}\!\!>Ba + CuSO_4$$



The proposed structural formula given for the copper salt is based on the fact that lactic acid, $CH_3 \div CH(OH) \div COOH$, forms a similar compound, and that the hydroxyl is the only one which can be involved and yield the more stable five-membered-ring compound. Hence this is a compound wherein one valence of the copper combines with the carboxyl group as a salt, while the other combines with a hydroxyl group as an alcoholate,

The similarity of the chemical and physical properties of quinic acid to those of compounds of the aliphatic series rather than to those of compounds of the aromatic series was evidenced throughout this investigation, and was responsible for many of the difficulties encountered

as well as for the ultimate success. The impossibility of separating the quinic acid from sugars through differences in solubilities was rather surprising considering the type of compounds involved. The metal salts of quinic acid have properties similar to those of monobasic aliphatic acids. The organic compounds of quinic acid have properties practically identical with those of corresponding compounds of glucose and fructose and are prepared by the same procedures. And finally, it was the similarity of quinic to lactic acid in its reaction with copper which finally led to success in the attempts for its isolation.

It is believed that by the use of the method developed, quinic acid may be isolated from material containing benzoic, citric, and malic acids, and sugars, where the sugar content is not too high. It is necessary that the volume of the solution during the precipitation of the barium salts of the other acids by alcohol be large enough to hold the barium quinate in solution, that there be no excess barium hydroxide present when the solution is heated, and that the heating be brief and followed by immediate cooling.

The advantages of the copper compound employed over any other compound of quinic acid are that it has a comparatively low solubility and crystallizes readily, whereas most of the others tend to form syrups and go to dryness without crystallizing.

Conclusions

1. A complete survey of the scientific literature regarding cranberries, Preisselbeeren, and Moosbeeren has been made, revealing the following facts:

   a. The benzoic acid content of these fruits varies from slightly under 0.05 per cent to slightly over 0.1 per cent, depending on the variety.

   b. Malic and citric acids are present in undetermined amounts, the ratio being four parts of citric to one of malic.

   c. The presence of quinic acid has been indicated by various indirect or qualitative methods.

2. The citric acid content of ripe cranberries (Howes) was found to be 1.07 per cent, of immature cranberries (Howes) 1.31 per cent.

3. The malic acid content was found to be practically the same for both ripe and immature cranberries (Howes); i.e. 0.26 per cent.

4. Attempts to isolate quinic acid from cranberries according to methods used by investigators of other materials, as well as attempts to isolate it by the use of solvents, by the formation of organic derivatives, or by the fermentation of sugars by microorganisms, were unsuccessful.

5. The results reported by Kohman and Sanborn (32) on the isolation of quinic acid from cranberries by means of the

double calcium salt of quinic and acetic acids could not be duplicated.

6. A method has been developed for the isolation of quinic acid from materials containing benzoic, citric, and malic acids, and sugars. The method is not quantitative. The method is as follows: 1. A water extract of the material is prepared by boiling and pressing; 2. This is exactly neutralized with $Ba(OH)_2$ solution; 3. After standing, this mixture is filtered and the filtrate evaporated to small volume; 4. The solution is then diluted with 95 per cent ethyl alcohol until the alcohol concentration reaches 70 per cent; 5. After thorough mixing, the mass is filtered using covered filters to prevent evaporation of the alcohol; 6. The alcohol is removed from the filtrate by evaporating nearly to dryness; 7. The resulting solution is diluted with water and decolorized by boiling with norite; 8. The barium is precipitated by adding a slight excess of $CuSO_4$ solution to the hot liquid; 9. The mixture is heated to boiling and then filtered; 10. Crystallization is allowed to take place at slightly above room temperature (45°C); 11. The crystals are purified by recrystallization. A greater yield may be obtained if the mother liquor is titrated with $Ba(OH)_2$ and slightly less than an equal volume added in excess, and then an excess of $CuSO_4$ solution added.

'. By use of this method, 0.5 per cent of quinic acid was obtained from cranberries.

8. The physical and chemical properties of the quinic acid isolated agree with those given in Beilstein.

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Effect of Manufacturing and Preserving Processes on the Vitamins of Cranberries

By Paul D. Isham and Carl R. Fellers

Cranberries are an important crop in Massachusetts. Nearly all of them are processed in some way before they are consumed. This study was undertaken to determine the effect of various methods of processing on their nutritive value.

MASSACHUSETTS STATE COLLEGE
AMHERST, MASS.

EFFECT OF MANUFACTURING AND PRESERVING PROCESSES ON THE VITAMINS OF CRANBERRIES[1]

By Paul D. Isham, Research Fellow, and Carl R. Fellers, Research Professor of Horticultural Manufactures

PURPOSE

A comprehensive investigation of the vitamin content of the American cultivated cranberry, *Vaccinium macrocarpon*, was undertaken to include several varieties, seasons, storage conditions, and manufactured products such as evaporated, frozen, and canned cranberries. This is the first of a series of studies planned to investigate the effect of manufacturing methods on the nutritive value of fruits and vegetables. Only limited data are available on this general subject. Results obtained in the present investigation show the necessity of actually determining the changes which may occur in a fruit during storage, processing, and preservation.

INTRODUCTION

Since cranberries are a very important crop in Massachusetts, data relative to their vitamin content and the effect of storage, home cooking, and commercial manufacturing processes on these vitamins are very desirable. Approximately 300,000 cases or 10,000,000 pounds of cranberry sauce are produced each year. Since a pound of cranberries makes about 2.6 pounds of sauce, the cranberries used for commercial canned sauce manufacture represent close to 40,000 barrels. However, most of the fruit is consumed as sauce prepared in the home. The per capita consumption of cranberries in the United States is 0.54 pound. Cranberries are harvested in September and October and may be found on the market until late winter.

Because of confusion in nomenclature in published articles on the cranberry, and to clarify several literature citations in this paper, a very brief discussion of the botanical relationships of the cranberry is given. The common, large, cultivated American cranberry so widely consumed in this country is known as *Vaccinium macrocarpon*. European references to cranberries generally refer to other species. Most important of these is *V. Vitis-Idaea*, also known as the mountain or rock cranberry (U.S.), cowberry, foxberry (Gt. Britain, Canada), Preisselbeere (Germany), tyttebaer (Norway), lingon berry (Sweden), Kroesa (Denmark), and partridge berry (Newfoundland). This species grows on upland and rocky places rather than in swamps. The other species, *V. Oxycoccus*, is known as the moss, bog, or swamp cranberry, and also as the small or speckled cranberry. The fruit and vine of this species are smaller than either *V. macrocarpon* or *V. Vitis-Idaea*.

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[1] Part of a thesis submitted by the senior author to the faculty of the Graduate School, Massachusetts State College, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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HISTORICAL

No thorough investigation of the vitamin content of cranberries has been reported. In fact, no work has been reported on vitamins A,B,D, and G. MacLeod and Booher (22) reported the American cranberry as being a poor source of vitamin C. However, this conclusion is open to criticism, first, because the cranberries had been in storage for seven or more months and, as will be demonstrated in this paper, had undoubtedly lost much of their original vitamin C content; and second, because the experimental animals did not consume their full portion of fruit plus basal ration. Naeslund (28) investigated the vitamin C content of *V. Vitis-Idaea* in Norway and found them to be weakly antiscorbutic. He estimated the protective portion of fruit or freshly expressed juice to be more than 50 grams, with no difference in potency between the whole berry and its expressed juice. Using the same fruit, Laland (21) was unsuccessful in his attempts to isolate narcotine, believed by Rygh and his coworkers (31,32,33) to be the precursor of vitamin C. However, Dalmer and Moll (9) and Brüggemann (6) were unable to verify Rygh's results; and recently Svirbely and Szent-Györgyi (40), and also King and Waugh (18) have proved beyond question that vitamin C is closely related to or identical with hexuronic acid.[2]

After the completion of the vitamin C studies reported in this paper, a recent Russian investigation on *V. Oxycoccus* came to our attention. Bogoliubova (3) found that 2.5 grams of these wild cranberries, after storage for three months near the freezing point, fully protected guinea pigs from scurvy during a seven weeks' test period. However, cranberries which had lain under snow all winter or which had been alternately frozen and thawed, showed no protective action. Similarly, dried or fermented cranberries lost their vitamin C potency. She believes that cranberries are an important antiscorbutic in all northern countries and states that the value of cranberries for the treatment of scurvy in Russia was known as early as 1848. Mention is made that sailing vessels used cranberry juice as an antiscorbutic many years ago.

Bogoliubova also noted the absence of xerophthalmia in her guinea pigs, and since the oat diet was deficient in vitamin A, concluded that cranberries contained some of this vitamin. However, no data were presented.

The resistance of vitamin C to heat and oxidation is materially increased by high acidity, and the cranberry is characterized by a high acidity which averages about 2.3 per cent (calculated as citric acid). According to Nelson (29), the fixed acids are 88 per cent citric and 10 per cent malic. Mason (24) detected benzoic acid in the cranberry; this finding was verified by Griebel (17), and also in this laboratory where it was found that the benzoic acid content of 25 varieties ranged from 0.027 to 0.098 per cent. It is probable that the good keeping qualities of fresh and preserved cranberries are in part due to the presence of free or combined benzoic acid. The presence of quinic acid in cranberries was presumed by Blatherwick and Long (2) and by Quick (30). Kohman and Sanborn (20), Morse (27), and Fellers, Redmon and Parrott (15), have gathered further positive evidence of the presence of 0.38 to 1.03 per cent of quinic acid in cranberries. Unlike benzoic acid, quinic acid has no significant antiseptic properties against microorganisms.

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[2]Recent investigations by Hahn (Ztschr. Untersuch. Lebensmtl. 61:369-411, 545-611, 1931) show that raw or cooked mountain cranberries are very poor sources of vitamin C.

The red pigment of the *V. Vitis-Idaea* cranberry has been proved by Willstätter and Mallison (41) to be the anthocyan idaein, hence is unrelated to vitamin A. By using Willstätter's technic, Morse (26) obtained 2.1 grams of idaein from 22 kilograms of American Early Black cranberries.

Of possible significance in vitamin C retention is the gas content of cranberries. The oxygen content varies from practically zero to 14 per cent depending upon the freshness of the fruit and storage conditions. A considerable percentage of carbon dioxide is always present in the tissues and voids of the fruit.

SOURCE AND PREPARATION OF SAMPLES

Fresh and Frozen Cranberries

In general the fruit was obtained from the Massachusetts Cranberry Station at East Wareham in 1930, 1931, and 1932, though samples were also furnished by the A. D. Makepeace Company, Wareham, the United Cape Cod Cranberry Company, South Hanson, and the American Cranberry Exchange, New York, N. Y.

Cranberries were harvested in September and October and held in cold storage at 40° F. in 50-pound, one-half barrel, ventilated boxes. In general, the fresh fruit and products were assayed for vitamin C within four months from the harvesting date, which corresponds to the active commercial marketing season. Early Black, Howes, and Perry Red varieties were used. The first two are the most important commercial varieties in Massachusetts and represent early and late maturing varieties. In order to determine the effect of storage upon vitamin C content, fresh cranberries from the same lot were stored at 40°, 15°, 0°, and -15° F. Thus the effect of freezing was also ascertained. The lots held at 0° and -15° F. were frozen at -30° F., in small waxed-paper cartons holding one-half pound of cranberries, by the Birdseye Multiplate Freezer. All frozen cranberries were not defrosted until just before feeding. The frozen samples included whole, sliced and sweetened, and crushed and sweetened berries, and a strained product. The samples held at 15° and 40° were in the bulk, 50-pound boxes.

The freezing of cranberries as a means of preservation has been carried on in this department since 1927 and has been practiced by the canners for several years as a means of extending the canning season. Quick-freezing of whole cranberries for the retail trade has been conducted on a limited scale since 1931.

Dehydrated Cranberries

Commercially dehydrated whole cranberries, usually called "evaporated" by by the trade, were examined for vitamin activity. These berries were pricked 20 to 30 times with steel needles to allow evaporation of moisture and dehydrated for 8 to 12 hours at temperatures of 120° to 150° F. in forced-draught dryers. The moisture content is normally less than 5 per cent. One hundred pounds of fresh fruit yield about 10 pounds of dehydrated cranberries.

By grinding this product a fine red powder was obtained which served as the basis of the tests for vitamins A, B, D, and G.

Cranberry film is a dehydrated cranberry product prepared by boiling fresh cranberries in a small amount of water for two to three minutes, hot pulping, and immediately drying in a thin film on a rotating steam-heated drum dryer. The film resembles crepe paper. One hundred pounds of fresh cranberries yield about 12.3 pounds of film. The film can be used in the preparation of cranberry sauce.

Film samples were pulped and dried in air, and in nitrogen gas. The film was prepared for feeding by mixing with water in definite concentrations. In this way, desired amounts were accurately administered by pipette.

Cranberry Juice

Cranberry juice or cocktail has recently become of some commercial importance. The juice possesses a very deep red color and a characteristic flavor. It is particularly suitable for use as a blender in punches and similar beverages.

Cranberry juice was prepared by two methods and is designated "cold-pressed" and "heat-extracted." The cold-pressed juice was prepared by grinding the fresh cranberries in a food chopper and expressing the juice by means of a large hand press. The yield of juice averaged 6 gallons per barrel (100 pounds) of cranberries.

The heat-extracted juice was prepared by boiling 100 pounds of cranberries with 6.5 gallons of water for 8 to 10 minutes. The pulpy mass was cooled to room temperature and pressed. The yield was about 8 gallons per barrel of fruit. The sweetened juice was prepared by adding sufficient sugar to yield a soluble solids content of 50 per cent. Both fresh and pasteurized raw pressed juices were examined for vitamin C. Pasteurization was effected by a heat treatment of 20 minutes at 160° F. of the juice sealed in small tin cans or one-half pint bottles. One series of samples in the bottles was sealed under a partial vacuum of 25 inches of mercury.

Cranberry juice cocktail was prepared according to the recipe recommended by the American Cranberry Exchange (1). One quart of cranberries was boiled with one quart of water until soft, then strained through a cheese-cloth, the juice brought to boiling, two-thirds cup of sugar added, and the boiling continued for two minutes. This juice was filled into sterile jars, sealed, and immediately cooled.

Commercially, cranberry juice cocktail is prepared by grinding the fruit, cooking with water until soft, adding a pulp filter aid, centrifuging, making up filtrate to a volume representing two parts water to one of fruit, adding sugar to 15° Brix, heating to 180° F., and immediately filling into sterile glass containers and sealing. Frozen cranberries are preferred to fresh in the manufacture of this product.

Cranberry Sauce

Cranberries are normally consumed in the form of a cooked sweetened sauce, either strained or unstrained. The sugar content of the sauce varies from 41 to 48 per cent.

Whole-fruit, unstrained cranberry sauce was prepared according to the widely used "Ten Minute" Cranberry Sauce (1) recipe of the American Cranberry Exchange. The recipe calls for 1 pound of cranberries, 2 cups of water, and $1\frac{1}{2}$ to 2 cups of sugar. The sugar and water are boiled together for 5 minutes, then the cranberries are added and boiled without stirring until the skins pop open. This usually requires about 5 minutes. This material was sealed hot in small jars and immediately cooled. Investigations on canned foods (10, 11) have shown that practically no deterioration of the vitamin C content occurs in the can even over a period of several years. All sauce was kept in the refrigerator at 33° F., and no jar was used for feeding after being open 24 hours. The surface of the material in the opened jars was kept covered with paraffin. Just prior to feeding, the sauce was pressed through a fine screen to allow its being administered by

pipette. This process is the one referred to as cold pulping or cold straining in this paper.

It was brought to our attention that cranberry sauce is often prepared in the home by cooking cranberries in water and then adding the sugar. Whole-fruit sauce was therefore prepared in this manner and treated as was the "Ten Minute" cranberry sauce.

Commercially prepared whole-fruit cranberry sauce was also examined for its vitamin C content. This material was manufactured by a method practically identical to that specified for "Ten Minute" cranberry sauce.

The strained cranberry sauce was prepared in several ways. In each case, however, the berries were pulped while at boiling or near boiling temperatures without any special precautions to prevent oxidation. Strained "Ten Minute" cranberry sauce was prepared and immediately sealed in small jars. Other strained sauce was prepared in small amounts according to commercial practice. The cranberries were boiled for two or three minutes in water, pulped while hot to remove seeds and skins, the sugar added, and the mixture concentrated in a jelly kettle to a jelly test (216° to 218° F. or 43 per cent soluble solids by refractometer). The hot liquid sauce was filled into cans and sealed without further heat treatment. As an average, 1 pound of cranberries will yield 2.6 pounds of sauce. Several brands of commercially packed strained sauce were likewise assayed.

Cranberry Jelly and Candy Filling

Cranberry jelly was prepared according to the recipe of the American Cranberry Exchange (1). This calls for 8 pounds of cranberries, 12 cups of water, and 2½ pounds of sugar. The cranberries are cooked in the water until soft, and the juice strained off through a jelly bag. The juice is measured and heated to boiling, 1 cup of sugar added for each 2 cups of juice and stirred in until dissolved, and the whole boiled briskly for 5 minutes. It is then poured into glasses and covered with paraffin. Small glasses were used, and a new one taken for each feeding.

The cranberry content of cranberry candy as such was too low to allow feeding in amounts sufficient to afford protection to the animals. Therefore, the cranberry material as it is mixed into the candy was obtained and used for feeding tests. This material was nothing other than finely chopped cranberries. It was stored, sealed in large jars, in the refrigerator at 33° F. Just prior to feeding, a suitable quantity was pressed through a fine screen while still cold, and this material used for feeding.

EXPERIMENTAL RESULTS

Vitamin C

The Sherman, LaMer, and Campbell (34) method was used, with the exception that the amounts fed were proportioned to the weight of the guinea pigs; i. e., a 400-gram animal was fed 4/3 as much as one weighing 300 grams. The animals were all young and healthy, weighed between 280 and 325 grams, and were housed in individual cages. The basal ration consisted of 58 per cent equal parts of rolled oats and wheat bran, 30 per cent of vitamin C-free baked milk powder, 10 per cent butter fat, and 1 per cent each of cod-liver oil and salt. This basal ration and water were kept before the animals at all times.

Normally three guinea pigs were used at each feeding level. At the end of the feeding period all animals were chloroformed and carefully examined for lesions of

scurvy. Negative controls died in from 26 to 36 days with an average Sherman scurvy score of 19.

Because of the dislike of guinea pigs for cranberries, it was necessary in all the vitamin C assays to express the juice and finely divided pulp from fresh, frozen, and soaked evaporated cranberries, and to force-feed the animals by pipette. All samples were freshly prepared daily immediately before feeding. In cooked and canned cranberries, the pulp freed from skins was likewise fed by pipette. In order to be assured that each animal received its full share, weighings were made before feeding and several times during the feeding process until the required weight was reached.

The results obtained on vitamin C are presented in Tables 1 to 5 and Figures 1 to 5. In general, these data are largely self-explanatory and require only brief discussion. Only certain representative results are shown in the curves, but all the data are summarized in the tables.

TABLE 1. — VITAMIN C CONTENT OF RAW CRANBERRIES

Variety	Year	Storage period	Number of guinea pigs	Amount fed daily	Gain or loss in weight average	Survival period average	Scurvy score average
		Months		*Grams*	*Grams*	*Days*	
Early Black	1930	2 to 5	5	10	+153	90	0
	1930	2 to 5	5	5	+134	90	0
	1930	2 to 5	2	3	+199	90	2
	1931	1 to 4	3	4	+165	90	1
	1931	7 to 8	3	10	−111	29	9
	1931	7 to 8	3	5	−112	31	12
	1932	3 to 6	3	4.5	+183	90	0, 0, 3
	1932	3 to 6	3	3.5	+30	90	4
Howes	1930	2 to 5	3	4	+123	90	0
	1930	2 to 5	3	2	+4.5	90	7
	1930	2 to 5	3	1	−58	42	12
	1930	9 to 11	3	10	+176	90	2
	1931	4 to 7	2	5	+130	90	6
	1931	4 to 7	2	3	−35	87	13
Perry Red*	1932	3 to 5	3	4.5	+290	60	0
	1932	3 to 5	3	3.5	+169	60	0, 0, 1

*Supply exhausted at 60 days.

Fresh and Frozen Cranberries

Several significant deductions can be drawn from Table 1 and Figure 1. Fresh cranberries of Early Black, Howes, and Perry Red varieties are very good sources of vitamin C, the minimum protective portion being close to 4 grams. There appears to be but little variation in vitamin C content due to differences in season or variety. For example, Early Black and Perry Red varieties differ greatly botanically, yet show similar vitamin C activity. The cranberry thus contains one-third to one-half as much vitamin C as the orange or tomato and is comparable with such foods as blueberries (14), pineapple (25), peaches (19), and some varieties of apples (12, 5).

Figure 1

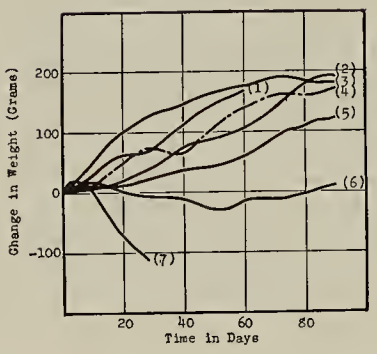

Figure 2

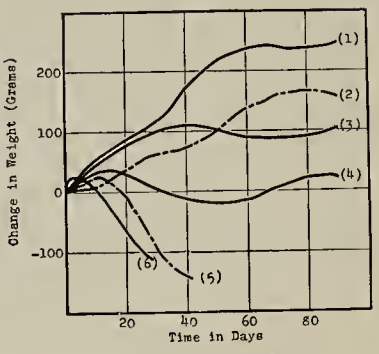

Figure 1. — Influence of Variety and Season on the Vitamin C Content of Cranberries

	Variety	Year	Grams fed daily	Scurvy score
1	Perry Red	1932	3.5	0
2	Early Black	1931	4	1
3	Early Black	1932	4.5	1
4	Early Black	1930	3	2
5	Howes	1930	4	0
6	Howes	1930	2	7
7	Basal Ration only			19

Figure 2. — Effect of Storage Temperature on the Vitamin C Content of Cranberries

	Storage		Grams fed daily	Scurvy score
	Months	°F.		
1	7 to 9	15	5	0
2	2 to 4	40	4	1
3	7 to 9	0	5	0
4	9 to 11	15	10	12
5	7 to 8	40	10	9
6	Basal Ration only			19

From a study of Tables 1 and 2, it is evident that cranberries gradually lose vitamin C during storage at 40° F. There was little loss in Early Blacks or Howes stored under 3 months. After 4 to 6 months the loss was approximately 20 per cent; after 7 to 10 months, the loss became still greater — possibly 60 to 70 per cent in some cases. Howes keep better than Early Blacks, and this better keeping quality is reflected in better retention of vitamin C in storage. These results serve to explain why MacLeod and Booher (22) found little or no vitamin C in cranberries. Their samples had been in storage for 7 to 11 months, and the highest level fed was only 5 grams — an amount entirely insufficient to protect animals from scurvy after so long storage.

TABLE 2. — EFFECT OF STORAGE TEMPERATURE ON THE VITAMIN C CONTENT OF EARLY BLACK CRANBERRIES

Description of sample	Storage period	Storage temperature	Number of guinea pigs	Amount fed daily	Gain or loss in weight average	Survival period average	Scurvy score average
	Months	°F.		*Grams*	*Grams*	*Days*	
Whole fruit	2 to 4	40	3	4	+165	90	1
	7 to 8	40	3	10	−111	29	9
	7 to 8	40	3	5	−112	31	12
	9 to 11	15*	3	10	+22	90	12
	7 to 9	0	2	10	+122	90	0
	7 to 9	0	2	5	+102	90	0
	7 to 9	−15	2	10	+213	90	0
	7 to 9	−15	2	5	+246	90	0
†Crushed	7 to 9	0	2	10	+106	90	0
	7 to 9	0	2	5	+27	90	3
‡Sliced	7 to 9	0	2	10	+512	90	0
	7 to 9	0	2	5	+61	90	4.5

*Known to have been thawed and refrozen at least once.
†Frozen with added sugar — 10 g. = 7.5 g. cranberries; 5 g. = 3.8 g. cranberries.
‡Frozen with added sugar — 10 g. = 6.7 g. cranberries; 5 g. = 3.3 g. cranberries.

It is apparent from Table 2 and Figure 2 that the retention of vitamin C during storage is dependent on the storage temperature. Cranberries stored at −15° and 0° F. suffered no significant loss. Unfortunately, the cranberries in storage at 15° F. were allowed to thaw and then refreeze at least once during the storage period of 9 to 10 months. This probably explains the excessive loss of vitamin C at this temperature. However, even these cranberries retained at least twice as much vitamin C as those stored at 40° F. for the same period. The crushed and sliced cranberries to which sugar was added retained all their original vitamin C during storage at 0° F. Solidly frozen cranberries, therefore, retain fully their original antiscorbutic properties.

Dehydrated Cranberries

The vitamin C content of dehydrated cranberries, shown in Table 3 and Figure 3, varies according to the method of manufacture. Evaporated cranberries contained practically no vitamin C, while the cranberry film [3] prepared in an at-

[3] The name of the manufacturer will be furnished upon request.

mosphere of nitrogen suffered but a slight decrease in antiscorbutic activity. The dried film itself was fully protective at the estimated low level of 0.7 gram, equivalent to about 8.4 grams of fresh cranberries. This represents a loss of about 35 per cent. Film manufactured by the same process with no effort to exclude oxygen lost 65 to 70 per cent of the vitamin C.

TABLE 3. — VITAMIN C IN DEHYDRATED CRANBERRIES

	Number of guinea pigs	Amount fed daily	Fresh fruit equivalent	Gain or loss in weight average	Survival period average	Scurvy score average
		Grams	*Grams*	*Grams*	*Days*	
Evaporated cranberries	3	0.83	8.3	−110	31	13
	3	.42	4.2	−88	31	16
Cranberry film (pulped and dehydrated in air).....	2	2	24	+164	90	0
	2	1	12	+3	90	4
Cranberry film (pulped and dehydrated in nitrogen)...	3	1.5	18	+118	30*	
	3	1.0	12	+333	90	0
	3	.5	6	+119	90	3

*Discontinued.

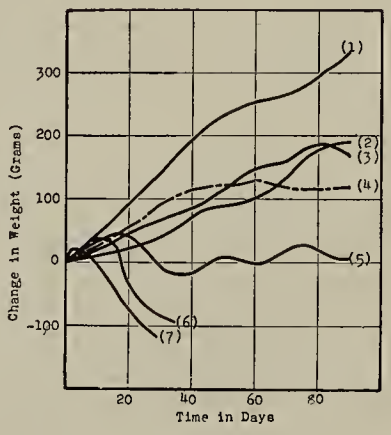

Figure 3. — Vitamin C Content of Dehydrated Cranberries

	Product tested	Grams fed daily	Fresh fruit equivalent *Grams*	Scurvy score
1	Cranberry film prepared under nitrogen	1	5 4	0
2	Raw cranberries	4	4	0
3	Cranberry film prepared under air	2	10.8	0
4	Cranberry film prepared under nitrogen	.5	2.7	3
5	Cranberry film prepared under air	1	5.4	4
6	Evaporated cranberries	.83	8.3	13
7	Basal Ration only			19

Cranberry Juice

Cold-pressed cranberry juice was equal to the fruit in vitamin C content and when boiled in an open vessel for two minutes showed no loss. However, when either this or heat-extracted juice was bottled and pasteurized, there was very little retention of the vitamin C. This was true regardless of whether the juices were sweetened or unsweetened, or sealed in the bottles with or without vacuum. The two cranberry-juice cocktails which were examined were manufactured by heat extraction methods and likewise showed almost complete loss of vitamin C. A cocktail was prepared from cold-pressed juice and pasteurized, but this was likewise devoid of any appreciable antiscorbutic property.

TABLE 4. — VITAMIN C IN TREATED CRANBERRY JUICE AND COCKTAIL

Method of preparation	Number of guinea pigs	Amount fed daily	Fresh fruit equivalent	Gain or loss in weight average	Survival period average	Scurvy score average
		Grams	*Grams*	*Grams*	*Days*	
Cold-pressed juice						
Boiled 2 minutes	3	8	8	+191	90	0
	3	4	4	+192	90	0
	3	4*	4	+188	90	0
Pasteurized 20 minutes at 160°F	3	6	6	−113	37	13
	5	4	4	−102	38	15
	3	3	3	−73	35	16
Sweetened and pasteurized	5	8	4	−169	40	10
Vacuum sealed and pasteurized	3	6	6	−140	36	12
Heat-extracted juice						
Pasteurized	3	6	6	−102	37	16
	3	3	3	−98	34	16
Vacuum sealed and pasteurized	3	6	6	−118	19	9
Cranberry juice cocktail						
Exchange's recipe	3	10	3.4	−165	46	15
	3	5	1.7	−162	33	15
Exchange's recipe, modified:						
Hot pressed	2	10	3.4	−134	33	15
Cold pressed	2	10	3.4	−80	32	18
Commercial	3	20	6.9	−91	40	15
	3	15	5.2	−131	26	12
	5	10	3.4	−117	34	14

*Plus 5 g. sugar.

These numerous examinations show that both cold-pressed and heat-extracted cranberry juices when bottled, sealed, and pasteurized retain very little vitamin C. It is possible that methods designed to avoid oxidation would be of value.

Cranberry Sauce

Since the greater proportion of cranberries are consumed in the form of cranberry sauce, a more extensive investigation of this product was carried out. The data are presented in Table 5 and Figure 5.

TABLE 5. — VITAMIN C CONTENT OF CRANBERRY SAUCE

Method of Preparation	Number of guinea pigs	Amount fed daily	Fresh fruit equivalent	Gain or loss in weight average	Survival period average	Scurvy score average
		Grams	*Grams*	*Grams*	*Days*	
Whole-fruit "Ten Minute" sauce	3	14*	8	+279	90	0
	4	7*	4	+82	90	2
	4	20	7	+42	90	2?
	7	10	3.5	+90	90	5
Whole-fruit sauce (sugar added last)	2	10	3.5	−99	90	3
Hot-strained "Ten Minute" sauce	3	8*	4.5	−49	52	9
	3	20	7	+39	83	10
	3	15	5.3	−92	33	8
	3	8	2.8	−134	38	10
Hot-strained sauce	5	20	7	−70	25	11
	5	10	3.5	−164	38	18
	5	8	2.8	−62	57	17
M. S. C. canned strained sauce	5	26	9.1	−146	31	16
	5	13	4.6	−148	31	15
Commercial strained sauce 1	2	20	7	−50	29	12
	2	10	3.5	−61	33	13
Commercial whole-fruit sauce 1 (1930)	3	20	7	−58	38	14
	3	10	3.5	−23	35	16
Commercial whole-fruit sauce 1 (1931)	3	12	4.2	−60	65	5
Commercial strained sauce 1, vacuum packed	2	20	7	−58	50	17
	2	10	3.5	−52	52	15
Commercial strained sauce 2	2	20	7	−73	37	17
	2	10	3.5	−48	33	16
Strained sauce, frozen and stored 9 months at 0°F.	2	12	9.6	−16	70	9
	2	6	4.8	−128	62	14
Jelly (Exchange's recipe)	3	12	6	−156	40	12
Cranberry candy filling	2	10	10	−80	36	16
	2	6	6	−157	35	14
	2	5	5	−103	47	18
	2	3	3	−122	36	15

*Made without sugar.

Figure 4

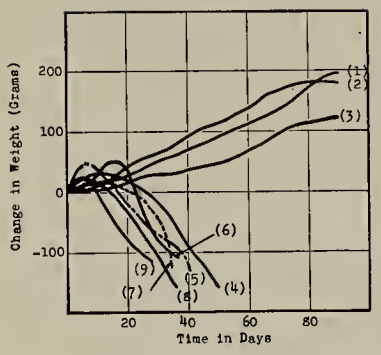

Figure 5

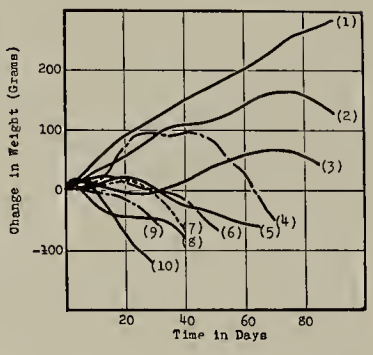

Figure 4. — Vitamin C in Cranberry Juice Prepared by Different Methods

	Method of preparation	Grams fed daily	Fresh fruit equivalent *Grams*	Scurvy score
1	Cold-pressed, boiled 2 minutes	4		0
2	Cold-pressed, boiled 2 minutes, plus 5 grams sugar	4		0
3	Cold-pressed, untreated	4		0
4	Cranberry juice cocktail (1)	10	3.4	15
5	Cold-pressed, vacuum packed, pasteurized	6		12
6	Heat-extracted and pressed, pasteurized	6		16
7	Cold-pressed, pasteurized	6		13
8	Cranberry juice cocktail (2)	10	3.4	14
9	Basal Ration only			19

Figure 5. — Vitamin C Content of Whole-Fruit and Strained Cranberry Sauce

	Method of preparing sauce	Grams fed daily	Fresh fruit equivalent *Grams*	Scurvy score
1	Whole-fruit sauce without sugar	14	8	0
2	Whole-fruit "Ten Minute" sauce	10	3.5	5
3	Hot-strained "Ten Minute" sauce	20	7	10
4	Strained sauce, frozen and stored at 0°F.	12	9.6	9
5	Commercial whole-fruit sauce (1)	12	4.2	5
6	Commerical strained sauce (1), vacuum packed	20	7	17
7	Commercial strained sauce (2)	20	7	17
8	Fresh, hot-strained sauce	20	7	11
9	Commercial strained sauce (1)	20	7	12
10	Basal Ration only			19

Whole-fruit cranberry sauce prepared in small amounts according to much used recipes (1,23) was found to retain approximately 80 per cent of the vitamin C of the cranberries. In order to make it more palatable to the animals, whole-fruit sauce was prepared without sugar, and the same percentage loss occurred. The time of adding sugar did not alter the results. Commercially packed whole-fruit sauce contained little vitamin C. Samples of two years' packs were assayed, one showing complete destruction and the other a 30 to 40 per cent retention.

Hot-strained cranberry sauce (1) retained a small part of the original vitamin C of the cranberries — possibly 20 per cent. However, five samples of strained sauce prepared by commercial methods retained only traces of vitamin C. Three of these samples were obtained from leading cranberry-sauce manufacturers; the other two were prepared in this laboratory.

One sample of strained sauce was prepared by cooking the cranberries to soften them, cooling, pulping, adding the sugar, and packing in one-pound waxed-paper cartons. The cartons were frozen at −30° F. and stored at 0° F. This sample retained only 10 to 15 per cent of the original vitamin C content of the cranberries.

Summarizing, whole-fruit sauce retains fully three-fourths and strained sauce less than one-fourth of the vitamin C of the fruit. Pulping has a decidedly harmful effect upon the antiscorbutic property of cranberries.

Cranberry Jelly and Candy Filling

Table 5 shows that true cranberry jelly is practically devoid of vitamin C, the experimental animals living but little longer than the controls. The guinea pigs very much disliked this sticky sweet product and they were force-fed only with difficulty.

Cranberry candy filling, consisting mainly of finely chopped cranberries packed in glass jars without heat treatment, showed only very slight antiscorbutic activity. If the fresh berries were chopped and used immediately for candy filler, no doubt a much higher percentage of the vitamin C would be retained.

Vitamin A

The technic developed by Sherman and Burtis (37) and Sherman and Munsell (36) was used in this investigation. Litters of young rats, 28 days old, were placed on a vitamin A-free diet consisting of vitamin-free casein 18, salt mixture 4, irradiated dried brewers' yeast 10, sodium chloride 1, and cornstarch 67 parts. When the body stores of vitamin A were depleted, the rats were placed in individual cages and fed the vitamin A-free diet supplemented by finely ground dehydrated cranberries which were substituted for an equal weight of starch in the diet. One rat from each litter was continued on the vitamin A-free diet as a negative control. Accurate records were kept of the quantity of food consumed and of the weight of the animals. On death or at the termination of the experimental period of 42 days, the rats were autopsied and carefully examined for lesions characteristic of vitamin A deficiency. The data are presented in Table 6.

It is apparent that the quantity of cranberry required to produce unit growth of 3 grams per week is probably slightly over 0.5 gram daily or 8 per cent of dried cranberry in the diet. This would place the vitamin A content of fresh cranberries at about 0.2 unit per gram. These deductions do not allow for possible vitamin A losses during the drying process; hence it is possible that raw cranberries may be richer in vitamin A than is indicated in the dried product. Recent findings (16)

indicate that losses do occur during drying and storage. Fruits comparable to cranberries in vitamin A content are apples, orange juice, and peaches (38).

TABLE 6.— VITAMIN A IN CRANBERRIES

Dried cranberry in diet	Amount of diet consumed daily	Amount of dried cranberry daily	Fresh fruit equivalent	Survival period average	Gain or loss in weight average	Remarks
Per Cent	*Grams*	*Grams*	*Grams*	*Days*	*Grams*	
10	7	0.7	7.0	42	+25.1	Complete protection
5	6.5	.33	3.3	42	+5.8	Very slight xerophthalmia in one rat
2.5	6.5	.16	1.6	40	−.4	Slight xerophthalmia—pus in one rat
1	6	.06	.6	38	−8.8	Xerophthalmia — pus in three rats
0	6	0	0	22	−26	Severe xerophthalmia and copious pus

Vitamin B (B_1)

In the vitamin B investigations the method suggested by Sherman and Spohn (35) and modified by Chase (7) was employed. Healthy young rats, 28 days old, were placed in metal cages with raised wire-screen floors and fed a vitamin B-free diet consisting of vitamin-free casein 18, salt mixture 4, butter fat 8, cod-liver oil 2, autoclaved yeast 15, and starch 53 parts. The animals continued to gain for about two weeks. When growth ceased, their diet was supplemented by dried cranberry powder which was incorporated in the diet in definite proportion, replacing an equal weight of starch. Accurate records were kept of the weights of the animals and the amount of food consumed. The data are presented in Table 7.

TABLE 7. — VITAMIN B (B_1) IN CRANBERRIES

Dried cranberry in diet	Amount of diet consumed daily	Amount of dried cranberry daily	Fresh fruit equivalent	Survival period average	Loss in weight average	Remarks
Per cent	*Grams*	*Grams*	*Grams*	*Days*	*Grams*	
15	8	1.2	12.0	16	−20.5	No better than controls
5	7	.35	3.5	18	−14.7	No better than controls
0	8	0	0	15	−18.9	Definitely neuritic

From these data, it is apparent that the material used contained no vitamin B. Even when cranberry powder comprised 15 per cent of the diet, it did not benefit the animals. Here, of course, even more than with vitamin A, losses might have occurred during the drying. But if a significant amount of vitamin B had been present in the raw cranberries, an amount of the dried material equivalent to 12 grams of fresh cranberries daily should have given at least some protection.

Vitamin G (B_2)

Vitamin G assays were conducted according to the method developed by Bourquin (4). Normal young rats, 28 days old, were placed in metal cages with raised wire-screen floors and fed the vitamin G-free diet consisting of purified casein 18, salt mixture 4, butter fat 8, cod-liver oil 2, and cornstarch 68 parts. The cornstarch carried the alcoholic extract from 50 grams of whole wheat for each 100 grams of diet. The animals were continued on this diet until they ceased growing. At this time the diet was supplemented with dried cranberry which was incorporated

in the diet, replacing an equal weight of starch. Accurate records were kept of the weight of the animals and of the food consumed. The data are presented in Table 8.

TABLE 8. — VITAMIN G (B_2) IN CRANBERRIES

Dried cranberry in diet	Amount of diet consumed daily	Amount of dried cranberry daily	Fresh fruit equivalent	Survival period average	Gain or loss in weight average	Remarks
Per cent	*Grams*	*Grams*	*Grams*	*Days*	*Grams*	
25	8	2.0	20.0	42	+0.3	No better than controls
15	8	1.2	12.0	42	+ .8	No better than controls
0	8	0	0	42	−1.3	Negative controls

These data indicate that cranberries contain very little, if any, vitamin G. Dried cranberry equivalent to 20 grams of fresh cranberry daily caused no significant increase in weight over the controls. Here again some destruction of the vitamin may have occurred during the drying process, but considering the large amount fed, there can be no more than a slight trace present in fresh cranberries.

Vitamin D

The method of testing for vitamin D was that developed by Steenbock and Black (39). Young rats were weaned when they weighed between 45 and 50 grams at about 24 days of age. They were placed on the Steenbock No. 2965 ration consisting of yellow corn 76, wheat gluten 20, calcium carbonate 3, and sodium chloride 1 part. The animals developed rickets in from 28 to 40 days. At this time dried cranberry was added at the rate of 15 and 25 per cent of the diet. This was fed to the animals for a ten-day period, at the end of which X-ray photographs were taken, then the animals chloroformed and line tests carried out. Negative controls were carried to indicate that spontaneous healing had not occurred, and positive controls which received cod-liver oil, in order to allow comparisons.

Animals on both amounts of cranberry showed no improvement over the negative controls. Hence, it was concluded that cranberries contain no significant amounts of vitamin D.

SUMMARY

The American cultivated cranberry, *V. macrocarpon*, is a very good source of vitamin C, the minimum daily protective amount for guinea pigs being 3.5 to 4.5 grams. Varietal differences were slight.

There was a gradual loss of vitamin C in cold storage. However, the loss was only slight during the active marketing season. After four to six months the loss was approximately 20 per cent, and after seven to ten months 60 to 70 per cent. Good keeping quality seemed to be associated with good vitamin C retention.

Inasmuch as vitamin C in cranberries, apples, and possibly other fruits decreases during storage of the fruit, this point must be considered in making vitamin C assays.

Freezing did not injure the antiscorbutic properties of whole, sliced, crushed, or sweetened cranberries. Temperature of freezing and temperature and length of storage had little effect. There was some evidence, however, that thawing and subsequent freezing lowered the vitamin C content.

Evaporated whole cranberries were very deficient in vitamin C. Dehydrated cranberry pulp (film) when prepared in an atmosphere of nitrogen gave full protection from scurvy at the 0.7 gram level, equivalent to a retention of 65 per cent.

Fresh or boiled cold-pressed cranberry juice with or without added sugar was nearly equal to the fruit in vitamin C content. However, when either cold-pressed or heat-extracted juice was bottled and pasteurized, most of the vitamin C was lost. Vacuum sealing of the bottles did not aid in retaining this vitamin.

Whole-fruit sweetened cranberry sauce prepared by several formulas retained 75 to 80 per cent of the fruit's vitamin C. On the other hand strained sauce usually retained less than 20 per cent. The pulping or straining process had a decidedly deleterious effect upon the antiscorbutic vitamin.

When clear cranberry juice was made into jelly, the latter contained no significant amounts of vitamin C. Similarly, cranberry candy filling was very low in vitamin C.

Dehydrated cranberries contained 2 units per gram of vitamin A. Assuming that no loss occurred during dehydration, fresh cranberries would thus contain at least 0.2 unit per gram.

No significant amounts of vitamins B (antineuritic), D (antirachitic), and G (antipellagric) were found in cranberries.

In conclusion it is clear that handling and manufacturing methods may greatly alter the nutritive value of cranberries and cranberry products.

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