

## THE PREPARATION AND PROPERTIES OF VITAMIN C CONCENTRATES FROM LEMON JUICE.\*

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The preparation and properties of vitamin C concentrates have been recently studied by several investigators. Zilva and coworkers (1) have succeeded in obtaining a concentrated material from lemon juice and have made important contributions relative to the molecular weight of the vitamin and its stability toward oxidation. Scotti-Foglieni (2) prepared a concentrate and subjected it to distillation *in vacuo*. Solubility studies have been carried out by Vedder and Lawson (3) and by Hart, Steenbock, and Lepkovsky (4).

In the present investigation an active concentrate from lemon juice was subjected to esterification and distillation, but no evidence was found to indicate any distillation of the vitamin. Fractional precipitation of the active material was then carried out, lead salts, as indicated by Zilva's work, and organic solvents being used.

A vitamin C concentrate prepared according to the method of Zilva (1927) from a liter of lemon juice was dried for an hour at 50° *in vacuo*, partially dissolved in 15 cc. of absolute ethyl alcohol, then saturated with dry hydrogen chloride, and allowed to stand at room temperature for 15 hours. The esterified product was distilled *in vacuo* at 50° and then up to 250°. The lowest pressure reached in the 50° distillation was 0.2 mm. The lowest pressure reached in the 250° distillation was 0.03 mm. The distillation was continued at 50° for 90 minutes, the receiver changed, and the temperature slowly raised through a period of 150 minutes to 250° at which it was held for 30 minutes. Distillates and residues ob-

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tained at both temperatures were fed to guinea pigs, with the results shown in Table I. The procedure followed for measuring the activity of the fractions was essentially that described by Sherman and coworkers (5).

It is evident from Table I that the distillates obtained at both temperatures were inactive, and that the residue from the 250° distillation was practically inactive. The residue from the 50° distillation had practically the same activity as the original lemon juice.

TABLE I.  
*Vitamin C Concentrates Subjected to Distillation after Esterification*  
(Test Period 56 Days).

Lemon juice equivalent fed daily.	No. of animals.	Average scurvy score.*	Average gain on test.	Average survival on test.
			gm.	days
0.....	2	19	-93	35
1.5 cc. lemon juice.....	2	0	+155	56
3 " " " .....	2	0	+173	56
1.5 " residue from 50° distillation.....	3	3	+146	56
3 " " " 50° " .....	3	0	+173	33
35 " distillate " 50° " .....	3	18	-107	33
10 " " " 250° " .....	3	11	-65	35
5 " " " 250° " .....	3	20	-80	43

\*The highest possible score is 24.

#### *Extraction of Lemon Juice with Butyl Alcohol.*

The observation that butyl alcohol extracted the yellow color and wax from lemon juice led to an experiment to determine whether vitamin C was extracted by this means. During the decitration and precipitation procedure it had been found that the yellow color was parallel with the active phase. 100 cc. quantities of juice were extracted three times with 20, 10, and 10 cc. portions of butyl alcohol. The material from the butyl alcohol extracts after evaporation and the lemon juice fraction were fed to guinea pigs in quantities of 1 and 2 cc. of lemon juice equivalents. The results of the feeding experiments are given in Table II. The butyl alcohol extraction failed to remove an appreciable amount of vitamin C

from the water phase but did remove approximately 0.33 mg. per cc. of fatty material, thus proving to be a valuable step in the concentration of the active material.

*A New Method of Preparing Vitamin C Concentrates from Lemon Juice.*

Zilva's method (1924, 1927) of preparing vitamin C concentrates from lemon juice involves long and tedious evaporations and leaves magnesium acetate and ammonium acetate in the final product. The average total solids found in the final fraction were 1.1 mg. per cc. of lemon juice equivalent. Since the acetates are soluble in all of the solvents in which vitamin C is known to be soluble, they are hard to separate from the final product by means of solvents.

TABLE II.  
*Extraction of Lemon Juice with Butyl Alcohol (Test Period 48 Days).*

Lemon juice equivalent fed daily.	No. of animals.	Average scurvy score.*	Average gain on test.	Average survival on test.
			gm.	days
1 cc. lemon juice.....	3	3	+121	48
1 " water fraction.....	4	3	+116	48
2 " " " .....	5	0	+143	48
1 " alcohol " .....	5	19	-96	31
2 " " " .....	5	18	-52	33
0.....	2	16	-100	28

\*The highest possible score is 24.

To avoid these difficulties the following procedure was adopted. A slightly less than theoretical amount of basic lead carbonate, 10 to 12 gm. depending upon the acidity of the lemons, was added to lemon juice and stirred slowly until the evolution of carbon dioxide ceased (2 to 3 hours). The crystalline precipitate was filtered off after cooling and the filtrate fermented with Fleischmann's yeast. The fermentation was practically complete after 24 hours, and took place a little more rapidly if a few drops of 20 per cent phosphoric acid were added and the solution centrifuged before the yeast was added. After fermentation was complete, the yeast was centrifuged out and 17 cc. of a saturated solution of neutral lead acetate were added to each 100 cc. of decitrated lemon juice. A white, inactive precipitate resulted which was removed by centrifuging. The

filtrate containing excess lead acetate was brought to a pH of 7.2 to 7.4 (by use of either phenol red as indicator or a quinhydrone electrode), with dilute ammonia and the yellow precipitate containing most of the activity removed by centrifuging. This was dissolved in dilute acetic acid, made up to one-half the original volume with water, and the precipitation with ammonia repeated. The second active precipitate was treated with an excess of dilute hydrochloric acid and after all of the yellow precipitate had been converted over to white lead chloride, the mixture was extracted three times with 10 cc. portions of butyl alcohol. The lead chloride was precipitated from the water phase by the addition of

TABLE III.

*New Method for Preparation of Vitamin C Concentrates from Lemon Juice (Test Period 56 Days).*

Lemon juice equivalent fed daily.	No. of animals.	Average scurvy score.*	Average gain on test.	Average survival on test.
			<i>gm.</i>	<i>days</i>
1 cc. lemon juice.....	4	3	+100	56
1 " active precipitate, delead- ed with HCl and alcohol.....	5	5	+106	56
1 cc. after precipitation of solid with acetone..	4	5	+36	56
2 " absolute acetone extract.....	3	3	+28	56
2 " solid not extracted by absolute acetone...	5	19	-107	27
0.....	5	19	-112	29

\*The highest possible score is 24.

sufficient ethyl alcohol to bring the concentration up to 90 per cent alcohol. After the removal of the lead chloride, the filtrate was evaporated to dryness and then made up to one-fifth the volume of lemon juice represented with water containing a trace of acetic acid. These preparations were made at least once a week and were stored in an ice box in small flasks filled with carbon dioxide.

The results of feeding this preparation to guinea pigs are given in Table III. It will be observed that the procedure involved very little loss in the antiscorbutic value of the preparation.

Since a large amount of the total solids in the preparation as obtained by the above method was ammonium chloride, it was

thought that this could be largely removed by the addition of acetone to a concentrated water solution of the preparation. The results in Table III show that most of the vitamin remained in solution in the acetone phase. The total solids after this step were reduced to 0.5 to 1 mg. per cc. of lemon juice equivalent.

Although it has been reported that vitamin C is insoluble in absolute acetone, it was thought that it might be soluble from a more concentrated material than desiccated lemon juice. The material from the acetone precipitation was carefully dried *in vacuo* and the dried material extracted with 50 cc. of absolute acetone (dried over sodium amalgam) for 24 hours. The results are given in Table III. It might be mentioned that this experiment was carried out with lemons which were very low in antiscorbutic value. This unfortunate occurrence somewhat obscured the results of this experiment, but the vitamin was definitely soluble in absolute acetone, as the guinea pigs that survived were gaining rapidly at the end of the test, when better lemons had been obtained.

Analysis of the absolute acetone extract gave the following results:

Total solids.....	0.38-0.6 mg. per cc. lemon juice.
“ N.....	3.46 per cent of total solids.
Ammonia N.....	1.9 “ “ “ “ “
Reducing sugars as dextrose...19.0	“ “ “ “ “

The final active material gave a faint carbylamine test for amino nitrogen, reduced potassium permanganate and ammoniacal silver nitrate quickly, and gave a faint coloration with ferric chloride. Bromine water produced a slight cloudiness. The phthalic anhydride and Liebermann reactions for phenols were too faint to be considered positive.

Further extraction of the dried acetone-soluble material with absolute ethyl ether, in which the vitamin is practically insoluble, reduced the total active solids to 0.28 mg. per cc. of lemon juice.

#### SUMMARY.

A new procedure for concentrating vitamin C from lemon juice is described.

Treatment of the vitamin C concentrate with ethyl alcohol and anhydrous hydrogen chloride, followed by distillation *in vacuo*, gave no evidence of esterification or distillation of the antiscorbutic factor. There was very little loss in activity of the preparation after it stood 15 hours in a saturated alcoholic solution of hydrogen chloride at 25–50°.

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