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CCXIV. THE LARGE SCALE PREPARATION OF ASCORBIC ACID FROM HUNGARIAN PEPPER (*CAPSICUM ANNUUM*)[1].

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Two main varieties of *Capsicum* are cultivated on the Hungarian plains: the smaller and drier "noble" type, used in dry powdered condition for spicing, and the fleshier type, which is eaten fresh. Both types have many sub-varieties. All the varieties tested seem to be equally suitable for the preparation of ascorbic acid. The juice of all varieties, titrated with acid iodine, uses 2–3 ml. 0·01 *N* iodine per ml., which reduction is mainly due to ascorbic acid (2–2·5 mg./ml.). On the whole "noble" pepper titrates somewhat higher than the fleshy varieties but gives less juice.

For the production of ascorbic acid only the fruit is used. The stem, core and seeds—forming about one-third of the total weight—are rejected, and the flesh is minced. Unripe, green pepper contains little ascorbic acid. The titre rises rapidly with ripening, when the green fruit turns first brown and then red. In the brown fruit the titre has almost reached maximum values. When the ripe, red fruits begin to shrink, dry or liquefy the titre falls again. Some varieties do not turn red, but become yellow on ripening. Their ascorbic acid content is equally high.

To every 450 litres of the pulp 22 kg. acid lead acetate are added in solution. This solution is prepared by dissolving 22 kg. plumbum aceticum in 9 litres water and adding 170 ml. 85 % formic acid. The pulp is thoroughly minced and pressed out on a fruit press. 450 litres of minced noble paprika leave about 100 kg. residue, 450 litres fleshy pepper leave 70 kg. residue. The juice, which leaves the press as a limpid fluid, can be stored for several days without loss in filled, stoppered bottles.

To every 10 litres of the juice are added 500 ml. of a lead acetate solution prepared by dissolving with the aid of heat 1 kg. plumbum aceticum in 1 litre of water. Ammonia is then added in a thin stream and stirred in till phenolphthalein, dropped on the surface, gives a faint colour, or bromothymol blue indicates a slightly alkaline reaction. The precipitate is separated quickly and sharply on suitable centrifuge. A Sharples supercentrifuge was used in our laboratory (big industrial type) with much success. 40 litres of juice yield on an average 5 kg. wet lead precipitate. This precipitate contains about half of the ascorbic acid originally present in the fruit. It can be stored for several days without loss of vitamin if air is excluded. This precipitate is well suited for the transport of the crude substance. The lead precipitate is then suspended in the smallest possible volume of water, strong hydrochloric acid is added to the well stirred mass till the fluid just begins to redden thymol blue. The $PbCl_2$ is separated on a centrifuge and washed with a little water. The aqueous

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fluids are united and concentrated under reduced pressure to a syrupy fluid' containing not more than 15–20 % water. It is very essential that this concentration should take place at a low temperature and in a short time. On heating the juice turns brown, and resinous matter appears which makes further work very difficult. We applied with much success a special vacuum-distilling apparatus, in which heating and distillation took place in a spiral tube. The time of heating of every part of the fluid was limited to the time necessary for the fluid to run through this spiral (1–2 minutes). Concentration was effected in two steps. Contact with copper should be avoided throughout the whole preparation.

The resulting light brown syrupy fluid contains 10 % ascorbic acid, calculated on the dry weight of the residue. In this form the vitamin is stable and can be stored in a cool place for weeks without loss of activity.

To this syrupy fluid an equal volume of anhydrous acetone is added and the mixture is strongly shaken mechanically for ten minutes. It is essential that the shaking should be very energetic and should lead to a good dispersion of the phases. The mixture is then allowed to settle for 15 minutes and the acetone phase poured off. The remaining oil is again shaken with an equal volume of acetone. After this second shaking the suspension usually does not separate, and it is necessary to effect a separation on a centrifuge. The acetone is again poured off and the residue treated twice more in the same manner. At the 3rd and 4th shakings the phases separate spontaneously. The 3rd and 4th acetone extracts may be used to extract the next batch of fluid.

The united acetone fluids contain the greater part of the vitamin, about one-fifth of the total vitamin remaining in the acetone-extracted residue. Part of this can be extracted by shaking the oil with 96 % ethyl alcohol. The extract is freed from alcohol by distillation, and the residue is then extracted with acetone or added to the next batch of syrup.

The acetone fluid contains 25 % vitamin calculated for dry weight. For its further treatment three different methods were applied with equal success. The yield in all the three is about equal, a quarter of the vitamin originally present in the plant being obtained in crystalline condition. The simplest method, given in the first place, yields good results if the extract to be treated has a relatively high vitamin content, almost 25 %. With weaker extracts the alkali method gives better results. The lead method involves the most labour.

Acetone method. The acetone extract is concentrated under reduced pressure and low temperature to a sticky syrup, which is treated on the shaking machine with dry acetone as described above. The greater part of the vitamin passes into the acetone phase, which contains 30–40 % vitamin calculated for dry weight.

The oily residue is dissolved in a little ethyl alcohol and again precipitated with excess of acetone, use being made of the shaking machine. This secondary acetone extract is concentrated and the resulting oil treated with pure acetone.

The acetone extracts are united and concentrated *in vacuo* to a smaller volume. To this concentrated acetone extract 1 ml. of *n*-butyl alcohol is added for every g. of vitamin present. The rest of the acetone is then distilled off under reduced pressure. The vitamin is thus left dissolved in butyl alcohol as a sticky syrup, which on cooling soon begins to crystallise. Crystallisation is allowed to proceed for 2–3 days at 0°.

Alkali method. To the acetone extract cold saturated aqueous NaOH solution is added in small portions with very energetic mechanical shaking. The Na salt of the vitamin separates in the form of a dark brown liquid phase. The addition of alkali is continued till 1 ml. of the supernatant fluid does not require more than

0·6 ml. of 0·01 *N* iodine in acid solution, starch being used as indicator. Excess of alkali is to be avoided.

The acetone is syphoned off and the phase containing the vitamin is tested with phenolphthalein. If it does not colour the indicator, NaOH is added till the reaction is alkaline. Then an equal volume of 96 % ethyl alcohol is added to the fluid, and the mixture is shaken up and allowed to separate. The alcohol is poured off and the residue shaken out once more with the same solvent. The alcohol takes out only relatively little vitamin, using up no more than 1 ml. iodine per ml. on titration.

A further quantity of alcohol is added and then strong hydrochloric acid in small portions till the fluid just begins to change the colour of thymol blue; thus the total Na is neutralised. More alcohol is then added until five parts of alcohol are present for every part of HCl used. The fluid is set aside for the night at $-20°$, the NaCl being allowed to crystallise. Next day the NaCl crystals are separated on a Büchner funnel and washed with alcohol.

The alcoholic filtrate is concentrated under reduced pressure to a syrup, and to this acetone is added in small portions. The first quantities of acetone mix with the fluid, but the excess produces a bulky oily precipitate, which is repeatedly shaken out with acetone. The acetone extracts are united, the acetone partly distilled off under reduced pressure, *n*-butyl alcohol added as above, distillation continued and crystallisation effected.

Acid lead method. Lead acetate is melted and heated to boiling. Then to every kg. of lead acetate 1 litre of 96 % ethyl alcohol is added. This hot solution is added in a thin stream to the above acetone extract with vigorous stirring. The vitamin separates in form of a resinous mass. Lead acetate is added, till the titre of the fluid does not fall on further addition of the reagent. The final titre of the fluid is about 0·6 ml. 0·01 *N* iodine per ml. For every litre of acetone extract 300–400 ml. of lead acetate solution are used.

The resinous precipitate is separated and decomposed in a mortar by strong HCl, the acid being added in small portions, as fast as it is used up, till the fluid just begins to redden thymol blue faintly but permanently. The $PbCl_2$ is separated, the precipitate washed with a little water on the centrifuge, and the watery extracts are concentrated *in vacuo* to an oil, which is extracted with acetone as above and crystallised from *n*-butyl alcohol.

Crystallisation, etc. The crystals of ascorbic acid can be separated on a Büchner funnel, but this process is usually very tedious owing to the viscosity of the mother-liquor. It is preferable to separate the crystals on the centrifuge, pour off the mother-liquor and then suspend the crystals in a small volume of acetone, to which enough methyl alcohol is added to prevent the formation of a precipitate (mostly 8 : 2). This suspension is filtered on a Büchner funnel and washed by the same alcohol-acetone mixture. The crystals are again suspended and washed with acetone, to which the necessary quantity of methyl alcohol is added. In the later washings less methyl alcohol is needed.

It has been found that the mother-liquor of any crystallisation always contains 20 % of vitamin, calculated for dry weight. If the crystallisation is induced in a fluid containing 30 % vitamin (for dry weight), only one-third of the vitamin comes out in crystals. From a fluid with 40 % vitamin, one-half of the ascorbic acid crystallises. It is very advisable to control the vitamin content and relative purity of the fluids at every step of the preparation.

The mother-liquor from the crystallisation can be fractionated once more by concentrating it to a sticky oil and extracting this with acetone on the shaking machine. Still better results are obtained by treating the mother-liquor with

NaOH and repeating the whole procedure described as the alkali method. This second fractionation yields about half as much crystalline vitamin as the first crystallisation. Further attempts to recover more of the vitamin after the second crystallisation were unsuccessful. The loss of vitamin in the mother-liquors is quite substantial and it is felt that improvements on this point are necessary and possible.

In dry recrystallised form the vitamin is stable even at room temperature.

Recrystallisation was effected in the following way. Methanol and dioxan were mixed in the proportion of 4 : 1 and in this mixture a saturated solution of vitamin was prepared by boiling. For every kg. of the vitamin about 6 litres of fluid are needed. The liquid was then concentrated under reduced pressure till strong crystallisation and bumping made further concentration difficult. The vitamin, originally yellow, now separated in the form of colourless crystals with the correct M.P. The suspension was set aside for the night in the ice-chest and filtered on a Büchner funnel, the mother-liquor being concentrated further and again allowed to crystallise.

3 kg. of pure ascorbic acid were prepared in our laboratory by the above methods.

No patents were taken out for the process.