

COSHH ASSESSMENT

The Extraction of Vitamin C from Green Peppers

Assessment No.

Assessment Date

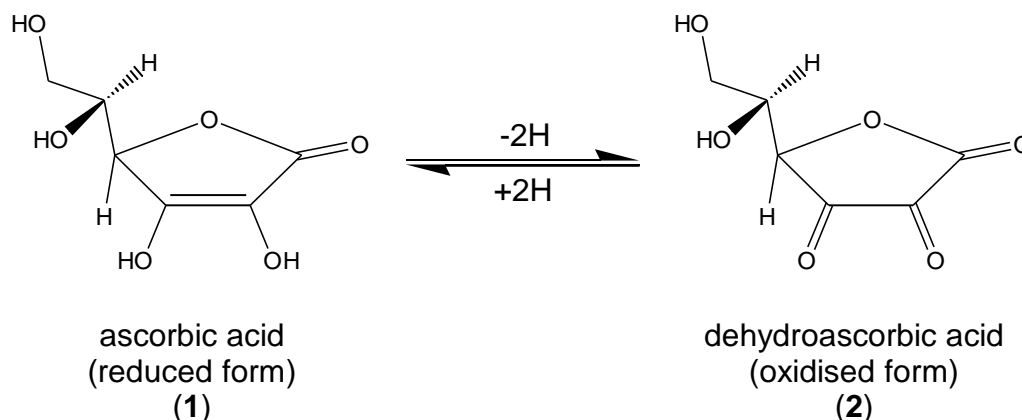
Chemicals – Hazardous in quantities used in experiment	
None	
Chemicals – Hazardous in other circumstances	
5% sulphuric acid	Irritating to the eyes and skin. Prolonged exposure may cause burns.
2,6-dichloro-phenolindophenol (0.25 g L ⁻¹ in water)	Irritant. Toxicology not fully investigated. May stain the skin
First aid for any of the above chemicals	
Eyes	Irrigate thoroughly with water for at least 10 minutes. OBTAIN MEDICAL ATTENTION.
Lungs	Remove from exposure, rest and keep warm. In severe cases OBTAIN MEDICAL ATTENTION.
Skin	Wash off thoroughly with water. Remove contaminated clothing and wash before re-use. In severe cases OBTAIN MEDICAL ATTENTION.
Mouth	Wash out mouth thoroughly with water and give plenty of water to drink. OBTAIN MEDICAL ATTENTION.

Determination of Vitamin C in Fruits and Vegetables

Introduction and Aims

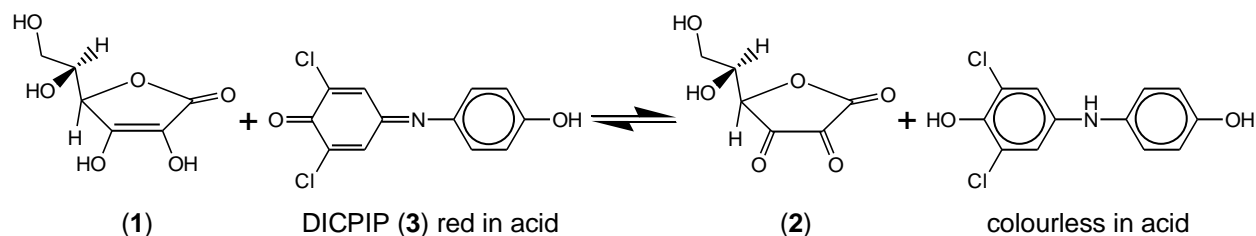
Vitamins are organic compounds required by living organisms in relatively small amounts to maintain normal health. Animals are unable to biosynthesise many vitamins and must have adequate amounts in the diet. Vitamin C (ascorbic acid) cannot be synthesised by humans, other primates and guinea-pigs and these species must ingest ascorbic acid in the diet. The U.K. Food Standards Agency recommends that adults require a daily intake of 40 mg of vitamin C (CMAFNP, 1991), although the amount required depends on gender and age amongst other factors. Since vitamin C is water soluble and cannot be stored in the body it needs to be taken daily.

It is believed that vitamin C is involved in several essential redox reactions in both plants and animals (Scheme 1).



Scheme 1: Redox Interconversion of Vitamin C

Vitamin C in body fluids (*e.g.* serum, plasma and urine), fruit juices and fresh fruits can be determined by making use of its redox properties. The vitamin, which is present in tissues almost entirely in the reduced form (1), is extracted from samples into 5% sulphuric acid. It is then oxidised to dehydroascorbic acid (2) by titration with 2,6-dichlorophenolindophenol (3; DICPIP), which is itself reduced. DICPIP is red in acid solution but when it is reduced (*i.e.* by the ascorbic acid) it is colourless. Thus, when all of the vitamin C has been oxidised by addition of DICPIP, addition of a slight excess of DICPIP produces a red/pink solution.



Scheme 2: Redox Reaction Between Vitamin C and 2,6-Dichlorophenolindophenol

1. Preamble

You are provided with a standard aqueous solution of ascorbic acid (accurately approximately 0.5 g L^{-1}), an aqueous solution of DICPIP (approximately 0.25 g L^{-1}), dilute sulphuric acid (5%; w/v) and some green peppers (a cultivar of *Capsicum annum*).

Since the concentration of the DICPIP solution is not known precisely, it needs to be standardised by titration against the solution of ascorbic acid. It is then possible to calculate the amount of vitamin C (in grams) that is oxidised per unit volume (mL) of DICPIP solution. This “factor” is particular to the batch of DICPIP solution (and will vary from day to day) and it should not be thought of as a concentration. The factor may then be used to calculate the amount of vitamin C in the raw and cooked tissue.

2. Standardisation of the DICPIP Solution

Add 5 cm^3 of standard ascorbic acid solution to a 100 cm^3 conical flask, followed by 5 cm^3 of sulphuric acid (5%; w/v). Titrate the mixture rapidly with the solution of DICPIP until it is distinctly rose-pink for at least 15-20 seconds. Repeat the titration until concordant results are obtained. Titrate a control, containing 5 cm^3 of water instead of ascorbic acid solution, in a similar manner. This is referred to as a blank determination and should be subtracted from all other titres.

3. Determination of Vitamin C in Raw and Cooked Tissue

(a) Quantify the vitamin C in the raw tissue.

Take 10 g of tissue, weighed to the nearest 0.1 g, from the edible portion of a pepper. Cut the material into small pieces and transfer it to a mortar. Add about 2 g of sand and 25 cm^3 of sulphuric acid (5%; w/v) and grind the mixture carefully with a pestle until no obvious lumps of tissue persist (*N.B.* the skin of the pepper usually remains intact and take care not to lose any extract by splashing due to too vigorous grinding). Decant the extract carefully into a 50 cm^3 centrifuge tube, wash the mortar with a further 5 cm^3 portion of sulphuric acid (5%; w/v) and add the washings to the extract in the centrifuge tube. Remove the solid matter from the combined extract and washings by centrifugation (*N.B.* the correct method to use will be demonstrated to you but the centrifuge heads **must** be balanced before the centrifuge is operated). Decant the supernatant quantitatively into a 50 cm^3 volumetric flask and add sulphuric acid (5%; w/v) to bring the volume to 50 cm^3 . Ensure the diluted extract in the volumetric flask is well mixed.

Pipette 10 cm^3 of the diluted extract into a clean 100 cm^3 conical flask and titrate it rapidly with the DICPIP solution as described in above. Repeat the titration with further 10 cm^3 aliquots of the diluted extract until concordant results are obtained.

- (b) Cook the pepper in one of three ways and quantify the vitamin C in the cooked tissue.

Baking

Place about 10 g of pepper, weighed to the nearest 0.1 g, in a small beaker and bake in an oven at 180-200°C for 15 minutes (time to nearest 30 seconds). Extract the vitamin C from the cooked tissue, and quantify it, as described above

Boiling

Wrap a sample of pepper (10 ± 0.1 g) in cheesecloth so that a bag is formed. Close the mouth of the bag with a test tube holder and suspend it in boiling water for 20 minutes. Extract the vitamin C from the cooked tissue, and quantify it, as described above.

If time permits, quantify the amount of vitamin C in the water remaining after the pepper has been cooked. The water will need to be diluted to an appropriate known volume; however, the precise dilution will depend on the amount of water remaining after cooking. Take 10 cm³ of the diluted water and titrate it rapidly with the DICPIP solution as before. The amount of DICPIP required may be quite small so perform a rough titration first.

Microwaving

Place a sample of pepper (10 ± 0.1 g) in a small beaker and microwave for one minute. Extract the vitamin C from the cooked tissue, and quantify it, as described above.

4. Data analysis

In each case, calculate the Vitamin C content of the fruit in g per 100 g of tissue and express the vitamin C content of the cooked tissue as a % of the uncooked control.

Collate all the data for the raw and cooked samples of pepper. What conclusions can be drawn?

References and Resources

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