THE PREPARATION OF EDIBLE WILD FRUIT AND PLANT SAMPLES FOR ANALYSIS AND SOME DIFFICULTIES ENCOUNTERED IN SUCH ANALYSES*

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The nutritive value of wild fruits and plants eaten mainly by Bantu and Bushmen in Southern Africa is at present being assessed by the National Nutrition Research Institute. Little is known about the chemical composition of these foods which play an important role in the diet of our indigenous population.


The preparation, however, of wild fruit and plant samples for analysis presents more difficulties than meet the eye. First of all, the perishable fruits and berries must be transported in such a manner that they arrive at the laboratory in as fresh a state as possible. A significant moisture loss must be prevented as all nutrient values are expressed on the basis of original water content.

Plastic bags, although they inhibit loss of moisture,
Moisture is often quite difficult to determine, and it seems almost impossible to obtain an absolute value. We use the hot-air oven, Brabender, or vacuum-oven methods for moisture determinations. Values obtained by these methods could be more accurately termed ‘drying loss’, though some foods, especially those with a higher sugar content, contain a certain proportion of water which is tightly bound and not easily removed by drying.

Drying at 105°C is suitable for most foodstuffs, but sugars may decompose at this temperature. As a result, products of high sugar content are dried in vacuo.

In view of the difficulty of determining absolute moisture contents it is essential that the freeze-dried samples be dried to the same degree as are the samples which are dried to determine the original moisture level. In order to achieve this, the same method of drying is employed for both the fresh and freeze-dried samples.

**Homogeneous Samples**

A prerequisite for all accurate analytical determinations is the preparation of a homogeneous sample. Doing this is relatively simple when dealing with a tuber or apple, for instance, but to prepare a homogeneous sample of a maroela or a berry presents considerable difficulty. The fleshy parts of these fruits are generally thin and juicy and have a fibrous layer tenaciously attached to the stone. Although the fruits are not large, one cannot use a whole fruit for a moisture determination as this would result in an erroneously low value. We use a very sharp knife to cut portions from the fruits which we then macerate in a small mortar and from which we immediately weigh out portions for the determination of total moisture content.

Since freeze-dried samples are hygroscopic and since the moisture of freeze-dried material is obviously important, one must often determine the moisture content several times, if samples for all determinations are not weighed out on the same day. One must also remember that for the determination of fibre, samples with a high fat content, such as the nuts of maroelas, mangetti, bao-bab, etc., must be defatted. In addition, the fibres must be present in a finely-divided form. Freeze-dried samples with a high sugar content are difficult to grind, and such samples must be cut into very small pieces, e.g. with a pair of scissors. Alternatively, a rather large sample is weighed out and a slurry is made in a Waring blender or Ultra Turrax by adding water. Portions of the slurry are then weighed and used for fibre determination; this of course entails another moisture determination.

The microbiological method is used for the determination of nicotinic acid and trouble is seldom encountered here, but microbiological methods are time-consuming and are used only when they are considered essential or highly desirable. For this reason we employ chemical methods for thiamin and riboflavin determination.

**Purification**

When assaying for thiamin, purification of the extract on a Decals column before oxidation of the thiamin to thiochrome may be omitted if the extract is not coloured or if blank readings for fluorescence are low. The Decals-purification step prolongs the analysis considerably and introduces an unknown factor into the determination, namely the question whether elution of
the thiamin from the column is complete or not. This is easy to determine where only one or two samples are concerned, but with a large number of samples it is not possible. In one sample we found that even purification on the Decalco column was inadequate and in the oxidation step with potassium ferricyanide solution a brown colour developed which passed into the iso-butanol phase.

We also found that some batches of Decalco, when activated according to the well-known procedure of boiling in 3% acetic acid, gave low thiamin recoveries. We then had to use another method of activating the Decalco.

Occasionally a sample which gives a clear extract, and which it therefore seems unnecessary to purify on the Decalco column, gives a higher blank reading than even the sample plus the added standard (we use 20% hydrochloric acid to destroy the thiocchrome). Under these circumstances purification on the column is again the answer.

The microbiological method for thiamin determination appears to be difficult, and, as a consequence, few laboratories employ this method.

The chemical method for riboflavin assay cannot be used for highly coloured extracts, either because it is impossible to obtain a result, or because the readings obtained are erroneous. Sometimes, even colourless extracts develop a brown colour during the permanganate oxidation step. Purification of the extract on a floridin-earth column is one answer to the problem, but it is not a very satisfactory solution for routine determinations. For such samples the microbiological method is the answer. The growth of Lactobacillus casei is, however, stimulated by fat which must, therefore, be removed by ether extraction. We use this method as a routine for all coloured extracts or for colourless extracts giving questionable results with the chemical method.

Due to the fact that carotene and ascorbic acid are so readily destroyed in the preparation of samples for analysis, we doubt whether it is possible to obtain absolute values for these two nutrients unless extreme precautions are taken in handling the samples before and during the weighing of an aliquot for analysis. Perhaps one must, for nutritional purposes, accept the inevitable lower results which are obtained by analysis. The fact that so many different methods are available for the determination of vitamin C stresses the difficulties which lie ahead for the analyst who has to determine this vitamin. However, some consolation lies in the fact that losses of nutrients also occur when the people who eat these fruits or plants peel or otherwise process them.

CONCLUSION

There are thus indeed many pitfalls in the path of the analytical food chemist and accurate results can be obtained only if a number of methods for assaying a particular nutrient in a foodstuff can be tried out and the best one used. However, this is seldom, if ever, possible, especially in the case of edible wild fruits and plants where the amount of sample available is often barely sufficient for carrying out one determination for each nutrient.

SUMMARY

Freeze-dried samples are used for analysis and the methods of preparation of samples for freeze-drying are discussed. Freeze-drying has obvious advantages but has also a few minor disadvantages. Difficulties encountered in the determination of some of the nutrients are discussed.