METHODS FOR THE DETERMINATION OF VITAMINS IN FOOD

Recommended by COST 91

Edited by

G. BRUBACHER, W. MÜLLER-MULOT

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D. A. T. SOUTHGATE

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F. Hoffmann-La Roche and Co. Ltd. Basle, Switzerland

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D. A. T. SOUTHGATE

Food Research Institute, Norwich, UK

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Preface

In the course of the project **COST 91***, on the Effects of Thermal Processing and Distribution on the Quality and Nutritive Value of Food, it became clear that approved methods were needed for vitamin determination in food. An expert group on vitamins met in March 1981 to set the requirements which these methods must meet. On the basis of these requirements, methods were selected for vitamin A, β-carotene, vitamin B_1 (thiamine), vitamin C and vitamin E. Unfortunately, for vitamins B_2 (riboflavin), B_6 and D only tentative methods could be chosen, since the methods available only partially fulfilled the requirements set by the expert group. For niacin and folic acid some references only could be given because none of the existing methods satisfied these requirements, and for vitamin B_{12}, vitamin K, pantothenic acid and biotin it was not considered possible to give even references.

All methods were carefully described in detail so that every laboratory worker could use them without being an expert in vitamin assay. In October 1983 an enlarged expert group on vitamins approved the compilation of methods and approached a publishing house with a view to publication. The editors wish to thank Dr Peter Zeuthen, the leader of the project COST 91, for his interest in their work, and Mr G. Vos who served as a secretary of the project and who was responsible for the Commission of European Communities which translated the text into English, German and French. The editors are especially obliged to the 'Bundesamt für Bildung und Wissenschaft' of the Swiss Federation for its financial support and to the company F. Hoffmann-La Roche and Co. Ltd, Basle, for placing its infrastructure at their disposal. They also are

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very grateful to Professor Seher, Münster, Federal Republic of Germany, who agreed that the method for determination of individual tocopherols in oils and fats developed by the German Society for Fat Science (DGF) (DGF 'Einheitsmethode' F-II 4) could be integrated in the manual.

We are indebted also to Elsevier Applied Science Publishers Ltd, London, for their generous offer to publish the English version of the manual and to allow the Commission of European Communities to distribute the German or French version between the collaborators of COST 91 and 91 bis.

The manual is intended to be used by laboratory workers and scientists involved in research work on food technology, nutritional surveys, establishing food composition tables and the quality control of food.

There are many gaps which should be closed in a second edition of the manual. For example, the missing data for precision and other characteristics should be elaborated, the methods should be compared with established methods and the limits of application should be investigated in collaborative trials. Unfortunately this work cannot be continued under the patronage of COST 91, but all those concerned with vitamin analysis are urged to arrange corresponding trials and to refer the results to the editors. It is hoped that the editors will find a new organisation to take over the patronage.

The editors hope that the present manual will be of help to many of their colleagues and wish to thank all those colleagues who have contributed to its completion.

G. BRUBACHER
W. MÜLLER-MULOT
D.A.T. SOUTHGATE

Members of the Expert Group on Vitamin Determination

Prof. Dr A. E. BENDER
2 Willow Vale
Fetcham, Leatherhead
Surrey KT22 9 TE
UK

Dr A. BLUMENTHAL
Institut für Ernährungsforschung
Seestrasse 72
CH-8803 Rüschlikon
Switzerland

Dr rer. nat. A. BOGNÁR
Bundesforschungsanstalt für Ernährung
Institut für Ernährungsökonomie und -soziologie
Garbenstrasse 13
D-7000 Stuttgart 70
Federal Republic of Germany

Dr F. BRAWAND
Schweizerisches Vitamininstitut
Vesalianum
Vesalgasse 1
CH-4051 Basel
Switzerland

Prof. Dr G. B. BRUBACHER
Department of Vitamin and Nutrition Research
F. Hoffmann-La Roche & Co. Ltd
Grenzacherstrasse 124
CH-4002 Basel
Switzerland

Prof. Dr F. FIDANZA
Istituto di Scienza dell' Alimentazione
Università degli Studi di Perugia
Casella Postale 333
I-06100 Perugia
Italy

Dr U. MANZ
F. Hoffmann-La Roche & Co. Ltd
Grenzacherstrasse 124
CH-4002 Basel
Switzerland

Dr W. MÜLLER-MULOT
Bürgelerweg 15
D-7889 Grenzach-Wyhlen 1
Federal Republic of Germany

Dr P. SHEFFELDT
Institut für Ernährungsforschung
Seestrasse 72
CH-8803 Rüschlikon
Switzerland

Dr W. SCHÜEP
F. Hoffmann-La Roche & Co. Ltd
Grenzacherstrasse 124
CH-4002 Basel
Switzerland

Mr D. SCUFFAM
Laboratory of the Government Chemist
Department of Industry
Cornwall House
Stamford Street
London SE1 9NQ
UK

Dr D. A. T. SOUTHGATE
Food Research Institute
Agricultural and Food Research Council
Colney Lane
Norwich NR4 7UA,
UK

Dr S. SREBRNIK
Institut d'Hygiène et d'Epidémiologie
rue Juliette Wytsman 14
B-1050 Bruxelles
Belgium

Dr J.-P. VUILLEUMIER
F. Hoffmann-La Roche & Co. Ltd
Grenzacherstrasse 124
CH-4002 Basel
Switzerland

Prof. Dr P. WALTER
Schweizerisches Vitamininstitut
Vesalianum
Vesalgassse 1
CH-4051 Basel
Switzerland

Dr C. E. WEST
Co-ordinator of the Eurofoods Project
Department of Human Nutrition
Agricultural University
De Dreijen 12
6703 BC Wageningen
The Netherlands

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PART I

INTRODUCTION

1

Introduction

The present manual on methods for determination of vitamins in food has been written mainly for practical purposes. It consists of a compilation of methods, which have been used successfully in the hands of experienced experts, and which have been chosen according to certain criteria given below. Unfortunately information on all criteria was not available for any of the methods. Therefore the methods were chosen by a consensus of the expert group where this information was missing. The lack of information is noted in the descriptions of the methods. It is hoped that in a second edition these gaps will be closed.

The description of the method consists of the following sections.

1. Purpose and Scope
2. Definition

These two sections should be noted carefully, since many misunderstandings have occurred in the past when these fundamental considerations have been disregarded. Some criteria on these two points used in choosing the method will be discussed later.

3. Brief description of the method (principle of the method)

The description gives a short overview of the method and allows the reader to decide whether a method may be performed with the available equipment in a certain laboratory or not.

4. Chemicals
5. Apparatus and Accessories

In sections 4 and 5 all chemicals and apparatus which are used for performing the actual determination are enumerated. This means that the description of the procedure refers only to these reagents and equipment. It does not mean that other similar chemicals and equipment cannot be used, but that the laboratory workers may need to modify the method accordingly.

It was decided not to use the complexity of equipment nor the availability of chemicals as selection criteria. But it is open to discussion as to whether or not methods which can be performed with less sophisticated equipment and chemicals should be included in a second edition.

6. Sample, sampling and preparation of the sample for the laboratory

This is one of the most critical stages in food analyses. Originally it was the intention to choose only methods for which enough information was available on these matters. Unfortunately there was little or no information available. It was therefore decided not to include this as a criterion for selection, but to include a discussion on these points in this chapter.

7. Procedure

This section is the major part of the description of each method. Every step is described in detail so that every competent laboratory worker should be able to perform the determination without special knowledge of vitamin assay techniques.

8. Evaluation

This part contains the formula for calculating the result with the aid of the measured values and the criteria by which the results should be judged. These are also the main criteria by which the selection of the methods were made—namely precision, accuracy, sensitivity, specificity and robustness. A discussion of these terms is given below.

9. Analysis report
10. References.

Where some critical points are discussed, notes are inserted in the text, a comment on how to prepare the report is made and some actual references related to the method in question are given.

In addition to the description of well-established methods (vitamin A, β-carotene, vitamin B_1 (thiamine), vitamins C and E), tentative methods are also similarly described (vitamins B_2, B_6 and D) which have been tested with only a few food items or which are not sufficiently sensitive to cover the whole concentration range. For folic acid and niacin only references are given, since it was felt that time is not ripe to include detailed description of methods for these two vitamins. Finally there are

neither methods nor references for determination of vitamin B_{12}, biotin, pantothenic acid and vitamin K. It is hoped that in the next edition vitamin B_{12} will also be included.

PURPOSE, SCOPE AND DEFINITION

Vitamin determinations in foods are carried out for many purposes, e.g. in food technology it is desirable to know the fate of vitamins during processing, and in nutritional surveys the vitamin content of meals at the point of consumption frequently has to be measured. The vitamin content of food has also to be determined for establishing food composition tables or for legal purposes in connection with nutritional labelling. All these purposes have different criteria for selecting the most appropriate method. The main purpose of the present book is to give food technologists an instrument for investigating the fate of vitamins during processing.

In the vitamin assay of foodstuffs it must be kept in mind that the term 'vitamin' is a physiological one rather than a chemical one, expressing a certain physiological activity, which is related to the chemical substances which are responsible for this activity. In this connection the following two points have to be considered.

(a) Vitamers

Vitamin activity may be due to a group of different chemical compounds (vitamers). These chemical compounds can be divided into two classes: (i) compounds which can be easily converted by simple chemical or biochemical reactions into the active form, e.g. vitamin A palmitate may be saponified to retinol, or dehydroascorbic acid may be reduced to ascorbic acid; (ii) compounds which cannot be inter-converted by simple means, e.g. α-, β-, γ- and δ-tocopherol, or α- and β-carotene. In Figs. 1–9 the most common compounds which are found naturally in foods or which are added during processing, are given for vitamins A, B_1, B_2, niacin, and vitamins B_6, C, D, E and folic acid.

A comprehensive method would allow determination of each chemical compound separately. In this way the fate of each compound during storage and food processing could be followed. Unfortunately, no practicable comprehensive methods exist. Only in special cases, usually in connection with research on biological problems, has such an approach been realised by developing special analytical procedures. For practical

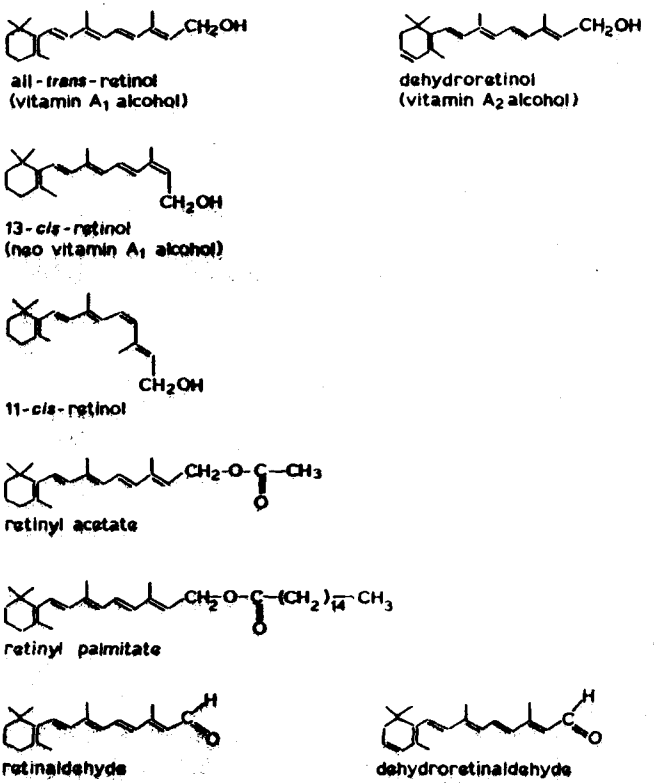
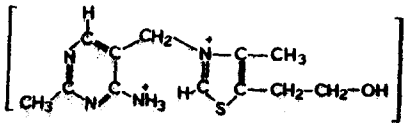
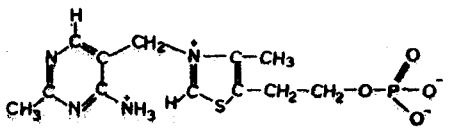


Fig. 1. Most common compounds with vitamin A activity (vitamers of the vitamin A group).

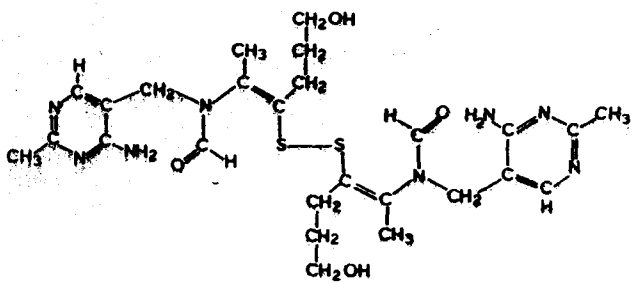
purposes a more pragmatic approach has to be adopted. Methods are selected, where the main interconvertible vitamers are converted to the same vitamer in the course of the analysis. For example, in the method recommended for vitamin A, retinyl palmitate and retinyl acetate are converted to retinol and the result of the analysis is given as weight units of retinol per 100 g. It is not possible to derive from this result the original quantity of retinol, retinyl palmitate and retinyl acetate. If one is following the fate of vitamin A during storage and processing, and at the end of the experiment a lower value is found for retinol than at the



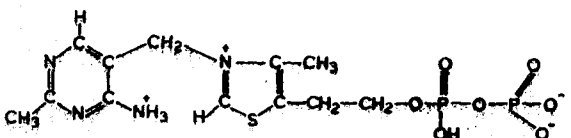
thiamine chloride hydrochloride



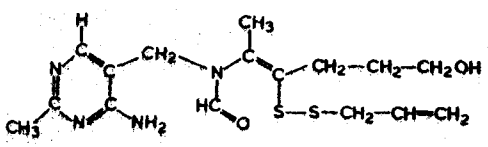
thiamine monophosphate



thiamine disulphide

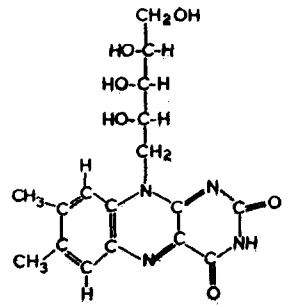


thiamine diphosphate, thiamine pyrophosphate (TPP)

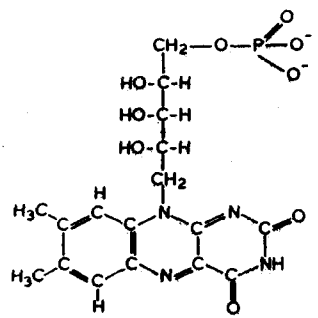


allyl thiamine disulphide

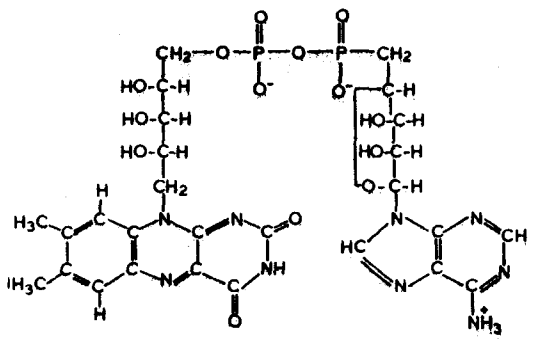
Fig. 2. Most common compounds with vitamin B_1 activity (vitamers of the vitamin B_1 group).



riboflavin



riboflavin 5'-phosphate,
flavin mononucleotide (FMN)



riboflavin adenine dinucleotide,
flavin adenine dinucleotide (FAD)

Fig. 3. Most common compounds with vitamin B_2 activity (vitamers of the vitamin B_2 group).