Chromatography and Mass Spectrometry in Nutrition Science and food Safety

Proceedings of the International Symposium
1983

Edited by
Alberto Frigerio and Hubert Milon

ANALYTICAL CHEMISTRY SYMPOSIA SERIES – volume 21

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Proceedings of the International Symposium on Chromatography and Mass Spectrometry in Nutrition Science and Food Safety, Montreux, June 19–22, 1983

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ELSEVIER
Amsterdam – Oxford – New York – Tokyo 1984

ELSEVIER SCIENCE PUBLISHERS B.V.
Molenwerf 1,
P.O. Box 211, 1000 AE Amsterdam, The Netherlands

Distributors for the United States and Canada:

ELSEVIER SCIENCE PUBLISHING COMPANY INC.
52, Vanderbilt Avenue
New York, NY 10017

Library of Congress Cataloging in Publication Data

International Symposium on Chromatography and Mass Spectrometry in Nutrition Science and Food Safety (1983 : Montreux, Switzerland)
Chromatography and mass spectrometry in nutrition science and food safety.

(Analytical chemistry symposia series ; v. 21)
Includes index.
1. Food--Analysis--Congresses. 2. Chromatographic analysis--Congresses. 3. Mass spectrometry--Congresses. I. Frigerio, Alberto. II. Milon, Hubert. III. Title. IV. Series.
TX541.I574 1983 664'.07 84-5963
ISBN 0-444-42339-7

ISBN 0-444-42339-7 (Vol. 21)
ISBN 0-444-41786-9 (Series)

Printed in The Netherlands

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PREFACE

The papers published in this volume were presented at the "International Symposium on Chromatography and Mass Spectrometry in Nutrition Science and Food Safety" held in Montreux, Switzerland, in June 1983.

The aim of this Symposium was to bring together analysts and scientists working in different fields of nutrition and to promote fruitful discussions with the hope of mutual benefit and improvement.

By publishing these papers, many of them being reviews on the state of the art, we hope that they will also benefit readers who could not attend the meeting.

We wish to express our gratitute to all those who contributed financially and hence permitted this Symposium to be held.

Milan and La Tour-de-Peilz
January 1984.

A. Frigerio and H. Milon

C O N T E N T S

FOOD SAFETY

STABLE ISOTOPES

NUTRITION AND DISEASE

A. Frigerio and H. Milon (Editors),
Chromatography and Mass Spectrometry in Nutrition Science and Food Safety

CHROMATOGRAPHY AND MASS SPECTROMETRY IN NUTRITION SCIENCE AND FOOD SAFETY

INTRODUCTION

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It is generally recognized that the advancement of sciences is conditioned by the progress in methodology.

Looking at the historical development of different methods, it is interesting to observe that quite often three separate phases can be distinguished.

The initial discovery is made more or less fortuitously by a scientist who tackles a particular problem and is not interested in the generalisation of the application of the new principle.

In the second phase which may be much later, the method is taken up by people often working in other fields and systematically developed and applied to the solution of problems in different domains. I would call this second phase the proliferation phase in which there is an outburst of papers in a great many disciplines with a tendency to overrate the value and precision of the new method.

In the third phase, the consolidation phase, the number of papers tends to diminish but they improve in quality, are more critical and finally the fields of applications of the new method are well established.

With the acceleration of the scientific development, these three phases draw nearer and nearer and their distinction may become more difficult.

It is interesting to note that the basic concept for both chromatography and mass spectrometry were laid down at approximately the same period of time namely the beginning of this century; for chromatography, by the Russian botanist Tswett in the years 1903 to 1910 and for mass spectrometry, by Wien in 1898 and Thompson in 1912.

Whereas chromatography was then relatively dormant for about two decades, mass spectrometry developed steadily in the physical sciences but for both methods, it took about 30 years - that means up to the early 40's - until they were introduced on a significant scale in the chemical sciences. From then on, they have become indispensable tools, not only in chemistry but also in biology, pharmacology, medicine and, last but not least, in nutrition and food science.

In the latter field, which is the main subject of the present symposium, the application of chromatography preceeds that of mass spectrometry by about 20 years. Indeed, the chromatographic separation of amino acids, first by paper chromatography in the early 40's and then by ion exchange separation in the early 50's, had a tremendous impact on the progress of the nutrition sciences, a fact that was recognized by the Nobel Prize for the inventors of the method, Dr. Stein and Dr. Moore. This is a good example of the crucial importance of new methods for the development of a particular science.

Mass spectrometry has not yet made a decisive impact on nutrition but is already well established in food science. The reason for the delay of mass spectrometry in comparison to chromatography in this field is relatively simple. Foods are extremely complexed mixtures of up to thousands of substances and, therefore, the development of the methods of separation had to preceed logically identification by mass spectrometry. In the analysis of food, the real breakthrough came with a combination of mass spectrometry with gas chromatographic separation methods, first as independent techniques, then in linked gas chromatography/mass spectrometry systems. However, the full complexity of a mixture like, for instance, coffee aroma can only be revealed through capillary GC coupled with fast-scanning mass spectrometry developed in the 70's. More than 500 compounds have thus been identified in the volatiles of roast coffee. This is quite an achievement from the methodological point of view but shows at the same time the limitations of analytical chemistry since we are unable to interprete these results in terms of the subjectively perceived coffee aroma without introducing physiological methods, such as sensory evaluation. The discrepancy in the development stage reached by the analytical methods in comparison to the sensory evaluation is partly responsible for a certain disappointment of many scientists working in the field of food flavours, although an important contribution of mass spectrometry in this field has been to show that the chemistry of volatiles is not as complicated as it first seemed and that the different flavour compounds can be ordered in a relatively limited number of classes.

As regards the solids, which constitute the bulk of our food - the proteins, carbohydrates, lipids, vitamins, steroids, etc. - the problems facing the mass spectrometrist are in the main the same as those in other biological or biochemical areas of application. Most routine analyses in these fields are carried out by the different chromatographic methods - with an enormous impact of HPLC during the last years - whilst mass-spectrometry is used for identification or confirmation of the identity of a compound, for the the assignement of substances on chromatograms, for the study of biological reaction mechanisms, for structure analysis (protein sequencing), etc. However, more and more mass spectrometry is also applied for direct quantitative analysis of compounds in foods, especially residues.

With this key word we approach another field of interest of this Congress, namely food safety.

Many substances both of natural and synthetic origin find use in the production, preservation and preparation of foods. These include herbicides, pesticides, fungicides, anti-oxidants, sweeteners, coulouring agents, technological aids, etc. These substances, either intact or as metabolites or derivatives, can be present in food materials, usually in trace amounts and a combination of chromatographic with mass-spectrometric methods offers a convenient tool for their qualitative and quantitative control. Here again, the progress has been so rapid that the classic toxicological evaluation as well as the legislation are left far behind. Obviously a so-called zero level of a toxic substance in a food becomes an ambiguous term since the detection threshold is constantly lowered. Although we know that it is the dose which determines whether a substance is toxic or not, the possibility to detect now minute traces of any foreign residue in foods has led the general public to believe that there is poison everywhere in our daily food ! I may quote here a well known toxicologist, Prof. Truhaut, who said : "our main problem today in food safety evaluation is not the toxicity of the compounds but the analytical chemist". This was, of course, a joke but it contains some truth as the gap between the scientific specialist and the people who must apply the new knowledge in day to day practice is widening !

A field in which mass spectrometry will have an increasing impact in the future is certainly human nutrition, especially for metabolic studies. Indeed, the use of stable isotopes such as C^{13} and N^{15} opens the way for dynamic studies in vivo of the intermediary metabolism and turnover of proteins, fats and carbohydrates. Several papers on this subject will be presented at this symposium.

A related field is that of disease. The detection of inborn errors of metabolism in the neonate is nowadays best achieved by GS-MS acid profiling in the urine. Two-dimensional, high resolution electrophoresis allows the separation and detection of about 2000 proteins in tissues and body fluids and can be envisaged as a diagnostic tool in metabolic disorders. Stable isotopes are already used to evaluate dietetic therapy in diseases such as obesity.

I am convinced that in the realm of medicine and biology, the judicious combination of the advanced chromatographic methods - including high resolution electrophoresis - with mass-spectrometry will open a new area for diagnosis and research.

I do not want to prolong this introduction, since you are all eager to know what's new in your scientific discipline.

I am especially happy, first, that this symposium could be organized in Montreux jointly by the Italian Group for mass-spectrometry in Biochemistry and Medicine and the Nestlé Research Department and second that Nutrition and Food Science are this time in the center of interest. This demonstrates that at last nutrition has emerged as a major science in the world today, a science that is not only interested in the scientific tools as such, but has as its primary goal the well-being of man and freedom of disease in affluent as well as in underpriviledged societies.

FOOD SCIENCE

A. Frigerio and H. Milon (Editors),
Chromatography and Mass Spectrometry in Nutrition Science and Food Safety

APPLICATION OF QUANTITATIVE GC/MS TO THE STUDY OF AMINO ACID RACEMIZATION

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ABSTRACT

A procedure was developed for the accurate determination of amino acid racemization in processed proteins. The separation of D and L enantiomers was achieved by capillary GC using a chiral stationary phase. The method was designed to differentiate D-amino acids formed during the acid hydrolysis of the protein from those originally present in the sample. To this effect, the hydrolysis was carried out in deuterated hydrochloric acid and GC separation was combined with selected ion monitoring mass spectrometry (SIM). Taking advantage of the facilities provided by a calculator-controlled bench top GC-MS, it was possible to cover most protein amino acids in a couple of GC runs. In contrast to common practice in SIM, a fairly large number of ions (15 to 20) had to be monitored during each run. Problems arising from this situation are discussed here.

Applications of this methodology included a survey of amino acid racemization in different model systems: severely heated protein, stored or heated milk powder, alkali-treated protein. A more detailed investigation of the kinetics of amino acid inversion has also been initiated. The inversion rate constants of the various amino acids were found to be for a good part governed by the electron-withdrawing capacity of the amino acid side chain. Environmental factors related to protein structure also appeared to have a significant influence in the process of racemization.

INTRODUCTION

Proteins are the most reactive of the major food components, and during processing, they can react with most other food constituents. These reactions influence both the sensory properties of the food and the nutritional quality of the proteins (ref.1). Racemization of amino acid residues is another phenomenon which can influence protein quality. At higher racemization levels, a significant decrease of protein nutritional value is generally observed (ref.2). Therefore, there has been some concern about the extent of amino acid inversion induced by the various technological treatments used in the food industry. Amino acid racemization is known to occur most readily after alkaline treatments (ref.3-5), but also to a lesser extent in acid conditions (ref.6-8) and during roasting (ref.9).

In the past, studies of amino acid racemization have been constrained by the lack of suitable analytical means. Data could only be obtained for a limited number of amino acids. More recently, investigation in this field was given a new impetus by the development of a thermally stable optically active

phase for capillary gas chromatography (ref.10). It permitted the resolution of all protein amino acid enantiomers in a single run, as shown in Fig. 1.

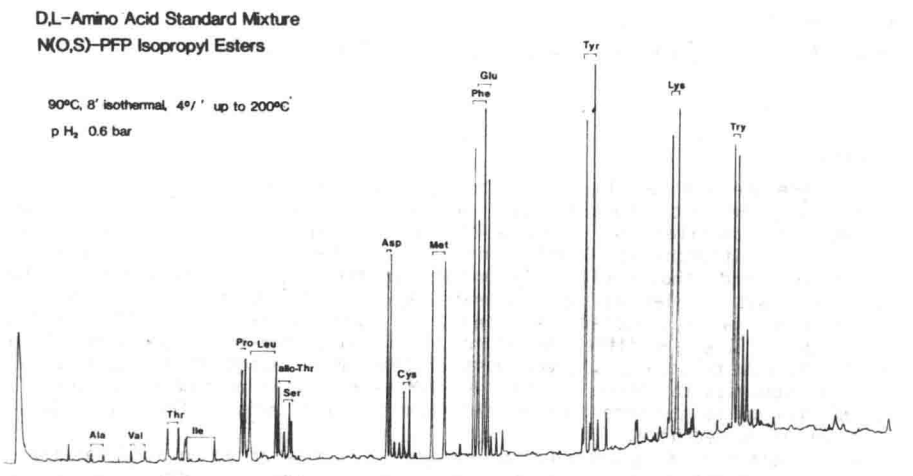

Fig. 1. Standard mixture of D and L amino acid N-pentafluoropropionyl isopropyl esters analysed on a 25 m x 0.2 mm i.d. fused silica capillary column coated with OS 6411 (C.G.C. Analytic, D-7406 Mössingen, FRG).

As it turned out, however, the accurate determination of racemization in protein or food sample could not be achieved on the basis of GC separation alone. The main reason lay in the necessity of submitting the protein to acid hydrolysis prior to the analysis. During this step, amino acid inversion would occur to some undetermined extent, resulting in a systematic overestimation of the amounts of D-isomers actually present in the sample. The kind of error to which this could lead is illustrated in Fig. 2 by the example of D-methionine formation in alkali-treated α-lactalbumin. Only the cumulated racemization could be determined by GC alone.

Other problems were also encountered: Despite the fact that all pairs of enantiomers could be resolved, it was often difficult to achieve a complete separation of the different amino acids (ref.11). Furthermore, at low racemization levels, the identification and integration of D-amino acid peaks was complicated by the presence of secondary products formed during the derivatization step.

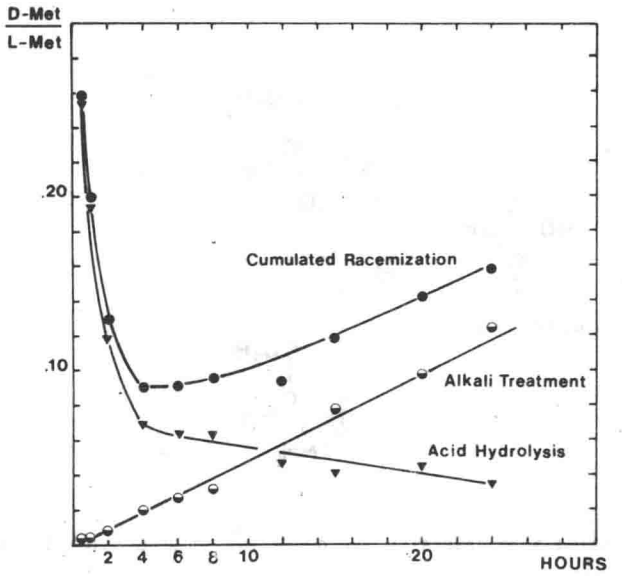

Fig. 2. Methionine racemization in α-lactalbumin heated at 83°C in pH 9.0 aqueous solution. The duration of the treatment is reported on the horizontal axis; each sample was hydrolyzed for 24 hours at 110°C in 6N DCl. ◒ D-Methionine formed in the protein by the alkaline treatment, ▼ D-methionine formed during acid hydrolysis, ● sum of the two contribution to D-methionine formation.

METHODOLOGY

The solution to these different problems was found in combining GC separation with selected ion monitoring (SIM) mass spectrometry. First, SIM provided the means for discriminating unresolved amino acids. More important yet, this combination permitted to develop a methodology whereby the two contributions to amino acid racemization could be distinguished and evaluated in a single measurement (ref.12). The key step of the procedure consisted in performing the hydrolysis of the protein sample in deuterated hydrocholric acid. By this expedient, a molecule of amino acid undergoing inversion during the hydrolysis would automatically incorporate a deuterium atom. The corresponding mechanism is detailed in Fig. 3.

Taking advantage of this automatic labelling, D-isomers formed during the hydrolysis could be distinguished by mass spectrometry from those which were present in the sample before the hydrolysis (Fig. 4). This again is illustrated by the example of methionine in α-lactalbumin. The characteristic fragment ion selected for methionine appears at m/e 337 and its labelled