FOOD ANALYSIS

Principles and Techniques

Volume 4

Separation Techniques

DIETER W. GRUENWEDEL
JOHN R. WHITAKER

FOOD ANALYSIS

Principles and Techniques

Volume 4

Separation Techniques

Edited by

DIETER W. GRUENWEDEI
JOHN R. WHITAKER

Department of Food Science and Technology
College of Agricultural and Environmental Sciences
University of California
Davis, California

MARCEL DEKKER, INC. New York and Basel

Library of Congress Cataloging in Publication Data
(Revised for vol. 4)
Main entry under title:

Food analysis : principles and techniques.

Contents: v. 1. Physical characterization -- -- v. 3. Biological techniques.
Includes bibliographies and indexes.
1. Food--Analysis--Collected works. I. Gruenwedel, Dieter W., [date]. II. Whitaker, John R.
TX541.F64 1984 664'.07 84-4284
ISBN 0-8247-7573-2

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Current printing (last digit):
10 9 8 7 6 5 4 3 2 1

PRINTED IN THE UNITED STATES OF AMERICA

FOOD ANALYSIS

FOOD ANALYSIS

Principles and Techniques

A Treatise in Eight Volumes

Edited by

Dieter W. Gruenwedel

John R. Whitaker

Department of Food Science and Technology

College of Agricultural and Environmental Sciences

University of California

Davis, California

Volume 1: Physical Characterization

Volume 2: Physicochemical Techniques

Volume 3: Biological Techniques

Volume 4: Separation Techniques

Volume 5: Proximate Analysis

Volume 6: Physical Techniques

Volume 7: Spectroscopy

Volume 8: Electroanalytical Techniques

Introduction to the Treatise

Food Analysis: Principles and Techniques was conceived to meet the need for an up-to-date detailed treatment of the methods of modern food analysis. Each chapter, written by an expert in the area, is carefully reviewed to provide as much uniformity of style as possible, yet to allow for individual creativity and the requirements of specific topics. Each chapter addresses first the underlying principles of an area in sufficient detail that those not initially familiar with the method can develop sufficient knowledge of the area to utilize the principles and techniques required. Each chapter then contains numerous examples of the application of the principles in the analysis of compounds of interest in foods.

The treatise is not a handbook of methods of analysis, and the reader should not expect to find a detailed description of the step-by-step procedures to be used in an analysis. The treatise also is not intended as a primary textbook for the instruction of undergraduate students in the principles and techniques of food analysis, although it will prove valuable as a reference source for these students. Rather, the treatise is intended for use by graduate students and by all scientists involved in the analysis of biological materials.

Food analysis has grown by leaps and bounds in recent years, in part due to better instrumentation, but also in part because it has recognized the need to understand and to apply the modern principles of analytical techniques—developed in large part by the physicists, the chemists, and the biochemists—to problems associated with

quality assurance, product development, and food safety. In this treatise, we have deliberately sought out authors who are at the cutting edge of their area of science, who understand what the major advances have been, how they can be applied to analysis related to food products, and what the future needs are in analytical techniques.

It is a truism that we all benefit from the availability and application of proper analytical techniques, whether we be scientist or consumer of food products. The need to know whether a particular compound is present, in what quantity, and any adverse effect it may elicit is an ever-present question that must depend for its answer on the most modern analytical techniques available and applied by those familiar with the advantages, limitations, and statistical significance of methods. Determination of the presence of a compound is often required at the picogram and fentogram levels.

Food analysis is a very broad and rapidly developing science. It is impossible to cover every conceivable method, even in a treatise of eight volumes as planned for *Food Analysis: Principles and Techniques*. Yet, it seemed to us that there are a number of areas of food analysis that must be incorporated and that the potential must be provided for additional volumes when emerging analytical techniques have reached a sufficient level of development.

The eight volumes will deal with the following topics:

Volume 1. Physical Characterization
Volume 2. Physicochemical Techniques
Volume 3. Biological Techniques
Volume 4. Separation Techniques
Volume 5. Proximate Analysis
Volume 6. Physical Techniques
Volume 7. Spectroscopy
Volume 8. Electroanalytical Techniques

The continuous encouragement, advice, and help of Dr. Maurits Dekker and the dedicated assistance of the staff of Marcel Dekker have provided the climate that made this treatise possible.

It is the authors of each of the chapters who have earned our undying gratitude for their dedication to the principles of excellence, for their desire to educate others in the principles and techniques of food analysis and thereby contribute to the quality and adequacy of the food supply, and in particular for their patience with the editors as this project evolved.

We welcome constructive suggestions from the users of the treatise. We hope that it will make a major contribution to the continued improvement in the application of modern analytical techniques in food analysis.

Dieter W. Gruenwedel
John R. Whitaker

Preface

The chapters of Volume 4 of *Food Analysis: Principles and Techniques* describe and discuss in detail a number of techniques used in the separation of food components. Separation is often the first essential step in food analysis. Although the classes of food components are limited in number - comprising categories such as proteins, carbohydrates, lipids, nucleic acids, vitamins, pigments, minerals, flavors, food additives - their individual members are in the thousands if not millions. Without employment of separation techniques, their analytical determination would be extremely difficult, in fact impossible. Separation techniques are therefore indispensable in the quantitation, structural analysis, elucidation of in vivo modes of action, or determination of the physical properties of food components.

Food components (or compounds) may be separated on the basis of their relative volatilities. One technique, utilizing differences in volatilities, is distillation. Separation can be achieved via regular distillation under atmospheric pressure or by distillation under reduced pressure, including molecular distillation (Chapter 1). Gas chromatography (Chapter 7) is another separation technique based on the relative volatilities of substances. It takes place in packed or microcapillary-open tubular columns. The rate of migration along the column material is refined further by partitioning the substances to be separated between a stationary phase (liquid) and a mobile phase (inert gas). The separation of ionic compounds, of compounds that can be caused to

ionize by changing the pH or by selectively complexing with ionized compounds, can be undertaken with the help of ion-exchange chromatography (Chapter 2) - one of the oldest types of chromatography - or with the aid of newer techniques such as chromatofocusing chromatography or ligand-exchange chromatography (Chapter 2). Separation can further be achieved by partitioning volatile (Chapter 7) or nonvolatile compounds (Chapter 5) between two immiscible phases, in which one, the stationary phase, may be represented by some "inert" insoluble support material (silica or cellulose) and the other one, the mobile phase, is passed along the stationary phase using a planar (Chapter 5) or columnar (Chapters 2, 4, 6, and 7) mode. Separation by inversed-phase chromatography is readily achieved by first bonding a hydrophobic layer to the "inert" insoluble support material (Chapters 2, 5, and 6). Thin-layer chromatography (Chapter 5), a time-honored separation technique, can be used to separate most types of compounds rapidly, inexpensively, and at microgram, nanogram, or even picogram levels.

Membrane-separation processes are of increasing importance in food analysis as well as in food technology. Since there exist numerous membranes of various pore sizes and stabilities to pressure, heat, and chemical solvents, many food chemicals can be separated from each other with great ease. The principles and applications of membrane-separation processes are described in considerable detail in Chapter 3, particularly from an engineering point of view.

Gel filtration chromatography (Chapter 4) is a separation technique in which compounds are fractionated according to size, based on their relative distribution between the buffer phase outside the gel beads and the buffer phase in the interstices of the pores of the gel beads. Affinity chromatography (Chapter 2) utilizes the specific recognition that may exist between an immobilized ligand (attached to an insoluble, inert matrix) and one of the solutes in a food mixture. Recognition may be as strict as that of an enzyme-substrate or antigen-antibody interaction or as broad as the recognition of a group of compounds, e.g., dehydrogenases that all require NAD^+ as a cofactor. Affinity chromatography is one of the newest and most versatile chromatographic methods.

High-performance liquid chromatography (HPLC, Chapter 6) utilizes many of the separation principles pertaining to Chapters 2, 4, and 5. Its superior performance and attractiveness derive from the availability of noncompressible microbeads of uniform size that can be packed uniformly into stainless steel columns and used repeatedly at high pressures, and sometimes high temperatures, with a minimum of effort expended in the regeneration of the packing material. Sometimes separations by HPLC can be achieved within 5-30 min, minimizing thereby peak spreading due to diffusion, eddy effects, irregular packing, and so forth. While Moore and Stein in 1956 separated

the 20 amino acids normally found in protein hydrolysates by chromatography on sulfonated polystyrene polymers in the course of 22-24 h, HPLC separation succeeds in 20 min on the same column material. However, in spite of the undeniable advances in chromatography that have occurred since Tswett first described the technique in 1903 and Martin and Synge determined its principles of operation in 1941, no one doubts that many more major advances are bound to occur regarding this versatile separation technique.

We thank all the authors for their contributions to this volume.

Dieter W. Gruenwedel
John R. Whitaker

Contributors

Lave Fischer, Sangtec Medical AB, Stockholm, Sweden

Walter Jennings, Department of Food Science and Technology, University of California, Davis, California

J. P. Leppard*, Wolfson Bioanalytical Unit, Institute of Industrial and Environmental Health and Safety, University of Surrey, Guildford, England

Robert H. McCormick†, Department of Chemical Engineering, The Pennsylvania State University, University Park, Pennsylvania

Joseph Sherma, Department of Chemistry, Lafayette College, Easton, Pennsylvania

H. Strathmann, Fraunhofer-Institut für Grenzflächenund Bioverfahrenstechnik (IGB), Stuttgart, Federal Republic of Germany

John R. Whitaker, Department of Food Science and Technology, University of California, Davis, California

*Present address: Nyetimber, Oaklands Way, Southampton, England
†Present affiliation: Professor Emeritus

Contents of Other Volumes

Volume 1. PHYSICAL CHARACTERIZATION

1. Statistical Evaluation of Experimental Data
   Edward B. Roessler

2. Sensory Techniques of Food Analysis
   Rose Marie V. Pangborn

3. Color Measurement
   Fergus M. Clydesdale

4. Rheological Techniques
   John R. Mitchell

5. Mechanical Properties of Foods
   John M. deMan and David W. Stanley

6. Automated Food Analysis Using Continuous-flow Analytical Systems
   Ram B. Roy

7. Laboratory Computers
   Charles F. Shoemaker

Index

Volume 2. PRINCIPLES AND TECHNIQUES

1. Temperature Measurements
James F. Schooley

2. Solubility
Kailas D. Thacker and Lee T. Grady

3. Viscosity
Rodney E. Harrington

4. The Light Microscope in Food Analysis
Clarence Sterling

5. Ultraviolet and Visible Spectrophotometry
Joachim H. von Elbe and Steven J. Schwartz

6. Optical Activity and Structure of Biological Molecules
W. Curtis Johnson, Jr.

7. Fluorescence and Phosphorescence
Ronald Otto Rahn

8. Electron Paramagnetic Resonance Spectroscopy
Mark G. McNamee

9. Atomic Absorption
W. John Price and Peter J. Whiteside

10. Infrared Spectrophotometry in Food Technology
Georg Zundel, Ulrich Böhner, Johannes Fritsch, Helmut Merz, and Bernd Vogt

11. The Application of Raman Spectroscopy to the Characterization of Food
Paul C. Painter

12. Size Exclusion Chromatography with Low-angle Laser Light-scattering Detection
Robert C. Jordan

Index

Volume 3. BIOLOGICAL TECHNIQUES

1. The Whole Animal as an Analytical Tool
Andrew J. Clifford

2. Use of Bacteria, Fungi, Protozoa, and Yeast as Analytical Tools in Food Analysis
Robert E. Landers and Dennis L. Gierhart

3. Analysis of Food Products for Microorganisms or Their Products by Nonculture Methods
James M. Jay

4. Cell and Tissue Culture Methodology in Food Research
Dieter W. Gruenwedel

5. Immunochemical Techniques
Marilynn E. Etzler

6. Analytical Uses of Enzymes
John W. Whitaker

Index

Contents

Introduction to the Treatise iii
Preface v
Contributors xiii
Contents of Other Volumes xv

1. **Distillation** 1

Robert H. McCormick

I. Introduction 1
II. Theory and Principles 5
III. Experimental Techniques, Apparatus Used to Determine Distillation Data, and Applications 20
IV. Summary 51
List of Symbols 53
References 53

2. Ion-Exchange and Affinity Chromatography 55

John R. Whitaker

I. General Introduction 55
II. Ion-Exchange Chromatography 62
III. Affinity Chromatography 90
References 124

3. Membrane Separation Processes 135

H. Strathmann

I. Introduction 135
II. Fundamentals of Membrane Separation Processes 137
III. Chemical Engineering Aspects of Membrane Separation Processes and System Designs 167
IV. Analytical Application of Membrane Separation Processes 187
V. Industrial Applications of Membrane Separation Processes 189
VI. Future Perspective of Membrane Processes 207
References 208

4. Exclusion Processes: Molecular-Sieve Adsorption and Gel Filtration Chromatography 219

Lave Fischer

I. Principles and Theory 219
II. Variables Characterizing Solutes and Chromatographic Systems 228
III. Gel Materials 244
IV. Experimental Techniques in Column Chromatography 255
V. Special Techniques 264
VI. Applications 268
References 276

5. Thin-Layer Chromatography 297

Joseph Sherma

I. Introduction and History 297
II. Techniques of TLC 299
III. Applications of TLC to Food Analysis 315
IV. The Future of TLC in Food Analysis 346
References 346

6. High-Pressure Liquid Chromatography 365

J. P. Leppard

I. Introduction 365
II. Principles 366
III. Instrumentation 380
IV. Applications 393
V. Needed Methodology and Research 401
VI. Conclusion 402
List of Symbols 402
References 403

7. Gas Chromatography 409

Walter Jennings

I. Introduction 409
II. Principles and Instrumentation 410
III. Applications 422
IV. Needed Methodology 433
V. Conclusions 434
References 434

Index 445