

Molecular Methods of Plant Analysis

Editors:

J.F. Jackson (Managing Editor)

H.F. Linskens

R.B. Inman

Volume 21

Springer-Verlag Berlin Heidelberg GmbH

*Volumes Already Published in this Series
(formerly "Modern Methods of Plant Analysis"):*

- Volume 1:* Cell Components
1985, ISBN 3-540-15822-7
- Volume 2:* Nuclear Magnetic Resonance
1986, ISBN 3-540-15910-X
- Volume 3:* Gas Chromatography/Mass Spectrometry
1986, ISBN 3-540-15911-8
- Volume 4:* Immunology in Plant Sciences
1986, ISBN 3-540-16842-7
- Volume 5:* High Performance Liquid Chromatography in Plant Sciences
1986, ISBN 3-540-17243-2
- Volume 6:* Wine Analysis
1988, ISBN 3-540-18819-3
- Volume 7:* Beer Analysis
1988, ISBN 3-540-18308-6
- Volume 8:* Analysis of Nonalcoholic Beverages
1988, ISBN 3-540-18820-7
- Volume 9:* Gases in Plant and Microbial Cells
1989, ISBN 3-540-18821-5
- Volume 10:* Plant Fibers
1989, ISBN 3-540-18822-3
- Volume 11:* Physical Methods in Plant Sciences
1990, ISBN 3-540-50332-3
- Volume 12:* Essential Oils and Waxes
1991, ISBN 3-540-51915-7
- Volume 13:* Plant Toxin Analysis
1992, ISBN 3-540-52328-6
- Volume 14:* Seed Analysis
1992, ISBN 3-540-52737-0
- Volume 15:* Alkaloids
1994, ISBN 3-540-52738-9
- Volume 16:* Vegetables and Vegetable Products
1994, ISBN 3-540-55843-8
- Volume 17:* Plant Cell Wall Analysis
1996, ISBN 3-540-59406-X
- Volume 18:* Fruit Analysis
1995, ISBN 3-540-59118-4
- Volume 19:* Plant Volatile Analysis
1997, ISBN 3-540-61589-X
- Volume 20:* Analysis of Plant Waste Materials
1999, ISBN 3-540-64669-8
- Volume 21:* Analysis of Taste and Aroma
2002, ISBN 3-540-41753-2

Analysis of Taste and Aroma

Edited by
J.F. Jackson and H.F. Linskens

With 72 Figures



Springer

Prof. JOHN F. JACKSON
Dept. of Horticulture,
Viticulture and Oenology
Waite Campus
SA 5064 Glen Osmond
Australia

Prof. HANS F. LINSKENS
Goldberglein 7
91056 Erlangen
Germany

Prof. Ross B. INMAN
Dept. of Molecular Virology and Biochemistry
University of Wisconsin
Madison, Wisconsin
USA

ISBN 978-3-642-07513-1 ISBN 978-3-662-04857-3 (eBook)
DOI 10.1007/978-3-662-04857-3

Library of Congress Cataloging-in-Publication Data

Analysis of taste and aroma / edited by J.F. Jackson and H.F. Linskens.

p. cm. - (Molecular methods of plant analysis ; v. 21)

Includes bibliographical references.

1. Taste - Molecular aspects. 2. Smell - Molecular aspects. 3. Plants - Analysis. 4. Botanical chemistry. I. Jackson, J.F. (John F.), 1935- II. Linskens, H.F. (Hans F.), 1921- III. Series.
QK865 .M57 vol. 21
[QP456]
571.2'028 s - dc21
[572'.362] 2001042002

2001042002

This work is subject to copyright. All rights are reserved, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilm or in any other way, and storage in data banks. Duplication of this publication or parts thereof is permitted only under the provisions of the German Copyright Law of September 9, 1965, in its current version, and permission for use must always be obtained from Springer-Verlag. Violations are liable for prosecution under the German Copyright Law.

<http://www.springer.de>

© Springer-Verlag Berlin Heidelberg 2002

Originally published by Springer-Verlag Berlin Heidelberg New York in 2002.

Softcover reprint of the hardcover 1st edition 2002

The use of general descriptive names, registered names, trademarks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.

Production: PRO EDIT GmbH, Heidelberg, Germany

Cover design: design & production GmbH, Heidelberg, Germany

Typesetting: Best-set Typesetter Ltd., Hong Kong

Printed on acid-free paper

SPIN 10795738

11/3130/Di

5 4 3 2 1 0

Preface

Molecular Methods of Plant Analysis

Concept of the Series

The powerful recombinant DNA technology and related developments have had an enormous impact on molecular biology. Any treatment of plant analysis must make use of these new methods. Developments have been so fast and the methods so powerful that the editors of *Modern Methods of Plant Analysis* have now decided to rename the series *Molecular Methods of Plant Analysis*. This will not change the general aims of the series, but best describes the thrust and content of the series as we go forward into the new millennium. This does not mean that all chapters a priori deal only with the methods of molecular biology, but rather that these methods are to be found in many chapters together with the more traditional methods of analysis which have seen recent advances. The numbering of the volumes of the series therefore continues on from 20, which is the most recently published volume under the title *Modern Methods of Plant Analysis*.

As indicated for previous volumes, the methods to be found in *Molecular Methods of Plant Analysis* are described critically, with hints as to their limitations, references to original papers and authors being given, and the chapters written so that there is little need to consult other texts to carry out the methods of analysis described. All authors have been chosen because of their special experience in handling plant material and/or their expertise with the methods described. The volumes of the series published up to now fall into three groups: Volumes 1–5 and Volume 11 dealing with some basic principles of methods, Volumes 6, 7, 8, 10, 14, 16, 18 and 20 being a group determined by the raw plant material being analysed, and a third group comprising Volumes 9, 12, 13, 15, 17 and 19 which are separated from the other volumes in that the class of substances being analysed for is indicated in the volume title. Volume 21 and future volumes of *Molecular Methods of Plant Analysis* will continue in a similar vein but will include more chapters involved with the methods of molecular biology.

Development of the Series

The handbook, *Modern Methods of Plant Analysis*, was first introduced in 1954, and was immediately successful, seven volumes appearing between 1956 and 1964.

This first series was initiated by Michael Tracey of Rothamsted and Karl Paech of Tübingen. The so-called *New Series of Modern Methods of Plant Analysis*, Volumes 1–20, began in 1985 and has been edited by Paech's successor, H.F. Linskens of Nijmegen, The Netherlands, and John F. Jackson of Adelaide, South Australia. These same editors have now teamed up with a third, Ross B. Inman of Madison, Wisconsin, USA, to produce the renamed series *Molecular Methods of Plant Analysis*. As before, the editors are convinced that there is a real need for a collection of reliable, up-to-date methods of plant analysis covering large areas of applied biology ranging from agricultural and horticultural enterprises to pharmaceutical and technical organizations concerned with material of plant origin.

Future volumes will include such topics as Testing for Genetic Manipulation in Plants, Genetic Transformation of Plants and Various Aspects of Plant Genomics.

Volume 21: Taste and Aroma

Chapters dealing in many cases with analytical procedures involving molecular biology are presented in Volume 21, beginning with an introductory chapter on the molecular biology of human taste and aroma receptors with implications for taste and aroma of plant products.

A subsequent chapter reports the use of DNA microarrays in identifying genes involved in strawberry flavour formation; further chapters deal with taste and flavour of beer, soybean and other plant products, hop aroma extraction and analysis, wine olfactometry evaluation, and analysis of citrus flavours.

The use of antisense genes in depressing certain aromas in fruits is also described, and articles on the use of electroantennography in analysing flower volatiles, analysis of rose flower volatiles, analysis of flavour by GC olfactometry (finger span method and solid phase microextraction method) and methods describing RNA gel blot analysis in determining floral scent gene expression round off this volume.

J.F. JACKSON, Managing Editor, H.F. LINSKENS, R.B. INMAN

Contents

1 Molecular Biology of Taste and Aroma Receptors: Implications for Taste and Aroma of Plant Products

| | |
|--|---|
| J.F. JACKSON | 1 |
| 1.1 Introduction | 1 |
| 1.2 Taste Buds and Receptor Cells | 1 |
| 1.3 Taste Receptors | 2 |
| 1.4 Taste Receptor Expression Patterns | 2 |
| 1.5 Conclusions for Taste Modality | 3 |
| 1.6 Aroma Detection in Mammals | 3 |
| 1.7 Model for the Olfactory System | 4 |
| 1.8 Conclusions for Aroma Perception in Humans | 4 |
| References | 5 |

2 Use of DNA Microarrays in the Identification of Genes Involved in Strawberry Flavor Formation

| | |
|--|----|
| A. AHARONI and A.P. O'CONNELL | 7 |
| 2.1 Introduction | 7 |
| 2.2 The Microarray Method | 9 |
| 2.2.1 Principle | 9 |
| 2.2.2 Microarray Procedure | 9 |
| 2.2.2.1 Array Fabrication | 9 |
| 2.2.2.2 Preparation of Targets and Hybridization | 10 |
| 2.2.2.3 Image Analysis and Data Extraction and Mining | 12 |
| 2.2.3 Key Microarray Applications | 12 |
| 2.2.3.1 Monitoring Gene Expression (mRNA Abundance) | 12 |
| 2.2.3.2 DNA Variation | 13 |
| 2.2.3.3 Arrays Containing Other Types of Bio-molecules | 14 |
| 2.3 Strawberry and Flavor Formation | 14 |
| 2.3.1 Strawberry Fruit | 14 |
| 2.3.2 Main Flavor and Aroma Components in Strawberry and Their Biosynthesis | 15 |
| 2.3.3 Alcohol Acyltransferases and Ester Formation | 18 |

| | |
|--|----|
| 2.4 From Expression to Function: | |
| Identification of Strawberry AAT (<i>SAAT</i>) | 18 |
| 2.4.1 Gene Expression During Development and Ripening | 18 |
| 2.4.2 Identification of the <i>SAAT</i> Gene | 19 |
| 2.4.3 <i>SAAT</i> Encodes the Ester-Forming Enzyme from Strawberry Fruit | 20 |
| 2.4.4 Other Candidate Genes Associated with Flavor Formation in Strawberry | 23 |
| 2.5 Conclusion and Future Prospects | 23 |
| References | 25 |
| 3 Testing for Taste and Flavour of Beer | |
| T. YONEZAWA and T. FUSHIKI | 29 |
| 3.1 Introduction | 29 |
| 3.2 Characteristics of Taste and Flavour Compounds in Beer | 30 |
| 3.2.1 Taste and Flavour Substances in Beer | 30 |
| 3.2.2 Threshold..... | 31 |
| 3.2.3 Flavour Units..... | 31 |
| 3.2.4 Bitterness from Hops..... | 32 |
| 3.2.5 Hop Aroma..... | 33 |
| 3.2.6 Alcohols..... | 33 |
| 3.2.7 Acids | 34 |
| 3.2.8 Esters..... | 34 |
| 3.2.9 Ketones and Aldehydes..... | 34 |
| 3.2.10 Sulfur Compounds | 36 |
| 3.2.11 Some Notes on Thresholds | 37 |
| 3.2.12 Effects of Carbonation | 38 |
| 3.3 Sensory Testing for Taste and Flavour of Beer | 38 |
| 3.3.1 Descriptive Terminology | 38 |
| 3.3.2 Standard Terminology for Beer Flavour..... | 39 |
| 3.3.3 Descriptive Test..... | 39 |
| 3.3.4 Difference Tests..... | 41 |
| 3.3.5 Bias in Sensory Verdicts | 41 |
| 3.3.6 Application of Taste Sensor | 41 |
| 3.3.7 Preference Test..... | 42 |
| 3.3.8 Drinkability Test..... | 42 |
| 3.4 Conclusions | 43 |
| References | 44 |
| 4 Taste Evaluation for Peptides in Protein Hydrolysates from Soybean and Other Plants | |
| K. MAEHASHI and S. ARAI | 47 |
| 4.1 Introduction | 47 |
| 4.2 Bitterness of Peptides from Soybean Protein | 48 |

| | |
|--|----|
| 4.2.1 Theory for the Bitterness of Protein Hydrolysate | 48 |
| 4.2.2 Tastes of Soybean Protein Hydrolysate | 48 |
| 4.2.3 Debittering of Peptides | 50 |
| 4.3 Protein Hydrolysates from Soybean and Other Plant Foods | 51 |
| 4.3.1 Fermented Foods | 51 |
| 4.3.2 Other Plant Protein Hydrolysates | 52 |
| 4.4 Acidic Oligopeptides | 53 |
| 4.4.1 Taste of α -L-Glutamyl Oligopeptides | 53 |
| 4.4.2 Taste Properties of Food Protein Hydrolysates | 54 |
| 4.5 Isolation of Peptides from Protein Hydrolysate | 57 |
| 4.5.1 Enzymatic Digestion | 57 |
| 4.5.2 Gel Filtration | 57 |
| 4.5.3 Ion-Exchange Chromatography | 59 |
| 4.5.3.1 Group Fractionation | 59 |
| 4.5.3.2 Ion-Exchange Chromatography by a Gradient Elution | 60 |
| 4.5.4 Thin Layer Chromatography | 60 |
| 4.5.5 Reverse-Phase HPLC | 61 |
| 4.6 Sensory Evaluation | 61 |
| 4.6.1 Detection of Tasty Peptides in Purification Steps | 61 |
| 4.6.2 Determination of Recognition Threshold | 63 |
| 4.6.3 Synergism Among Savory Peptides | 63 |
| 4.6.4 Effect on Five Basic Tastes | 64 |
| 4.6.5 Taste Duration-Intensity Curve | 64 |
| 4.6.6 Buffer Capacity of Peptide | 64 |
| 4.7 Conclusions and Vista | 65 |
| References | 65 |
| | |
| 5 Hop Aroma Extraction and Analysis | |
| G. LERMUSIEAU and S. COLLIN | 69 |
| 5.1 Introduction | 69 |
| 5.2 Hop Aroma | 72 |
| 5.2.1 Terpenic Compounds | 72 |
| 5.2.2 Oxidation and Hydrolysis Products from Sesquiterpenes | 77 |
| 5.2.3 Alcohols, Carbonyles, Acids and Esters | 77 |
| 5.2.4 Hop Aroma Glycosides | 82 |
| 5.3 Varietal Discrimination of Hop Cultivars According to Their Oil Content | 83 |
| References | 86 |
| | |
| 6 Olfactometry and Aroma Extract Dilution Analysis of Wines | |
| V. FERREIRA, R. LÓPEZ, and M. AZNAR | 89 |
| 6.1 Introduction | 89 |
| 6.2 A Review of Wine Olfactometry | 89 |

| | |
|---|-----|
| 6.3 Wine Olfactometry: An Overview | 95 |
| 6.4 Methodological Aspects | 109 |
| 6.4.1 Headspace or Total Extraction? | 109 |
| 6.4.2 Obtaining an Extract | 110 |
| 6.4.3 Evaluation of the Representativity of the Extract | 111 |
| 6.4.4 Concentration of the Extracts | 112 |
| 6.4.5 The Chromatographic System for Olfactometry | 113 |
| 6.5 Techniques for Processing the Olfactometric Signal | 114 |
| 6.6 Final Remarks | 116 |
| References | 117 |

7 Analysis of Volatile Components of Citrus Fruit Essential Oils

| | |
|---|-----|
| G. RUBERTO | 123 |
| 7.1 Introduction | 123 |
| 7.2 Chemical Composition of Citrus Peel Essential Oils | 125 |
| 7.3 Analysis of <i>Citrus</i> Peel Essential Oils | 134 |
| 7.3.1 High Resolution Gas Chromatography (HRGC) | 134 |
| 7.3.2 High Resolution Gas Chromatography-Mass Spectrometry (HRGC-MS) | 135 |
| 7.3.3 High Resolution Gas Chromatography-Fourier Transform IR Spectroscopy (HRGC-FTIR) | 138 |
| 7.3.4 Liquid Chromatography-High Resolution Gas Chromatography-Mass Spectrometry (LC-HRGC-MS) | 141 |
| 7.3.5 Multidimensional Gas Chromatography (MDGC) | 143 |
| 7.4 Deterpenation of <i>Citrus</i> Essential Oils | 147 |
| 7.5 Novel <i>Citrus</i> Fruits | 150 |
| References | 153 |

8 Aroma Volatiles in Fruits in Which Ethylene Production Is Depressed by Antisense Technology

| | |
|---|-----|
| A.D. BAUCHOT, D.S. MOTTRAM, and P. JOHN | 159 |
| 8.1 Why Use Antisense Technology to Study Fruit Aroma? | 159 |
| 8.1.1 Successful Inhibition of Ethylene Biosynthesis in Fruit | 160 |
| 8.1.2 Studying Fruit Aroma in Ethylene-Depleted Fruit | 160 |
| 8.1.3 Fruit Volatile Compound Analyses | 161 |
| 8.2 Methods | 162 |
| 8.2.1 Inhibition of Ethylene Biosynthesis: Fruit Transformation | 162 |
| 8.2.1.1 Tissue Regeneration | 163 |
| 8.2.1.2 <i>Agrobacterium</i> Transformation | 163 |
| 8.2.1.3 Generation of Transformed Plants | 164 |
| 8.2.1.4 Production of Hybrids | 164 |
| 8.2.2 Volatile Analyses | 165 |
| 8.2.2.1 Solvent Extraction | 165 |

| | |
|--|-----|
| 8.2.2.2 Headspace Sampling | 165 |
| 8.2.2.3 Gas Chromatography-Mass Spectrometry | 167 |
| 8.3 Illustration: Our Results | 168 |
| 8.4 Conclusions | 170 |
| References | 171 |
| 9 Detection of Physiologically Active Flower Volatiles Using Gas Chromatography Coupled with Electroantennography | |
| F.P. SCHIESTL and F. MARION-POLL | 173 |
| 9.1 Introduction | 173 |
| 9.2 Collection of Floral Scent | 174 |
| 9.2.1 Location of Floral Scent Emission | 175 |
| 9.2.2 Variation of Scent Emission | 175 |
| 9.2.3 Choice of Type and Amount of Adsorbent Material | 176 |
| 9.3 Gas Chromatography | 177 |
| 9.3.1 Fractionation of Samples | 177 |
| 9.3.2 Injector Types | 178 |
| 9.3.3 Columns | 178 |
| 9.3.4 Coupling the GC with the Electroantennographic Detector (EAD) | 179 |
| 9.3.4.1 Split | 179 |
| 9.3.4.2 Heating of the Transfer Line | 180 |
| 9.3.4.3 Air Flow Over the Antenna | 180 |
| 9.4 Electrophysiology | 181 |
| 9.4.1 Olfactory System | 181 |
| 9.4.2 EAG | 182 |
| 9.4.2.1 EAG Preparations | 182 |
| 9.4.2.2 Recording an EAG | 183 |
| 9.4.3 GC-SSR (GC-SCR) | 184 |
| 9.4.3.1 Technique | 184 |
| 9.4.3.2 Signal Measurement | 184 |
| 9.4.4 Overcoming Problems of Low Sensitivity | 185 |
| 9.4.5 Comparison of EAG, GC-EAD, and GC-SSR | 186 |
| 9.5 Behavioural Tests | 187 |
| 9.5.1 Attraction Tests | 187 |
| 9.5.2 Proboscis Extension | 188 |
| 9.6 Compilation of Results | 188 |
| 9.7 Concluding Remarks | 188 |
| References | 194 |
| 10 Analysis of Rhythmic Emission of Volatile Compounds of Rose Flowers | |
| J.P.F.G. HELSPER, J.A. DAVIES, and F.W.A. VERSTAPPEN | 199 |
| 10.1 Introduction: Rhythmicity in Emission of Volatile Compounds, How and Why | 199 |

| | |
|---|-----|
| 10.2 Rhythmicity in Emitted Volatiles | 201 |
| 10.2.1 Methods..... | 201 |
| 10.2.1.1 Plant Containment | 201 |
| 10.2.1.2 Environmental Conditions | 201 |
| 10.2.1.3 Volatile Adsorption | 202 |
| 10.2.1.4 Volatile Desorption | 202 |
| 10.2.1.5 GC and GCMS Analysis | 203 |
| 10.2.1.6 Calibration Curves | 204 |
| 10.2.1.7 Quantification of Compounds for Which No Authentic Standard Is Available | 205 |
| 10.2.1.8 Recovery of Volatiles in the Experimental Setup from Plant to GCMS | 205 |
| 10.2.2 Circadian Rhythmicity in Emission of Volatile Compounds by Rose Flowers: Experimental Results and Discussion | 205 |
| 10.3 Rhythmicity in Precursors of Emitted Volatiles in Rose Petal Tissue | 211 |
| 10.3.1 Introduction | 211 |
| 10.3.2 Methods | 213 |
| 10.3.2.1 Plant Material | 213 |
| 10.3.2.2 Assay of Non-glucosylated Fragrance Compounds in Petal Tissue | 213 |
| 10.3.2.3 Assay of Glucosylated Fragrance Compounds in Petal Tissue | 214 |
| 10.3.3 Rhythmicity in Petal Concentrations of Precursors of Volatile Compounds: Experimental Results and Discussion | 215 |
| 10.4 General Conclusion | 218 |
| References | 220 |

11 Odour Intensity Evaluation in GC-Olfactometry by Finger Span Method

| | |
|---|-----|
| P.X. ETIÉVANT | 223 |
| 11.1 Introduction | 223 |
| 11.2 Description of the Finger Span Cross-Modality Matching Principle | 224 |
| 11.3 Selection and Training | 226 |
| 11.4 Performance of the Method | 229 |
| 11.5 Applications | 232 |
| 11.5.1 Sample Discrimination Based on Odour Intensity of Constituents | 232 |
| 11.5.2 Determination of Stevens' Coefficients | 234 |
| 11.6 Conclusion | 236 |
| References | 236 |

| | |
|--|-----|
| 12 Solid Phase Microextraction Application in GC/Olfactometry Dilution Analysis | |
| K.D. DEIBLER, E.H. LAVIN, and T.E. ACREE | 239 |
| 12.1 Introduction | 239 |
| 12.1.1 Aroma Chemistry | 239 |
| 12.1.2 Mouth Simulators | 240 |
| 12.1.3 Solid Phase Microextraction | 241 |
| 12.2 Description of Methods | 242 |
| 12.2.1 SPME Initialization | 242 |
| 12.2.2 SPME CharmAnalysis | 243 |
| 12.2.3 Quantification of SPME | 243 |
| 12.3 Example of SPME Dilution Analysis | 245 |
| 12.3.1 Methods | 245 |
| 12.3.1.1 SPME Extraction | 245 |
| 12.3.1.2 GC Parameters | 245 |
| 12.3.1.3 Optimization of Exposure Time | 245 |
| 12.3.1.4 Dilution Analysis | 246 |
| 12.3.1.5 CharmAnalysis of Coffee | 246 |
| 12.3.2 Results of Example | 246 |
| 12.4 Conclusions | 247 |
| References | 248 |
| 13 RNA Gel Blot Analysis to Determine Gene Expression of Floral Scents | |
| J. BOATRIGHT and N. DUDAREVA | 249 |
| 13.1 Introduction | 249 |
| 13.2 RNA Gel Blot Analysis | 251 |
| 13.2.1 RNA Isolation | 252 |
| 13.2.2 RNA Fractionation by Agarose-6 M Urea Gel Electrophoresis | 253 |
| 13.2.2.1 Preparation of Vertical Agarose-6 M Urea Gel | 255 |
| 13.2.2.2 Gel Electrophoresis | 255 |
| 13.2.3 Transfer RNA from Gel to Membrane | 256 |
| 13.2.4 Hybridization | 257 |
| References | 259 |
| Subject Index | 263 |