Wiley Series on Mass Spectrometry
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Mass Spectrometry in Grape and Wine Chemistry

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WILEY

MASS SPECTROMETRY IN GRAPE AND WINE CHEMISTRY

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WILEY
A JOHN WILEY & SONS, INC., PUBLICATION

Published by John Wiley & Sons, Inc., Hoboken, New Jersey
Published simultaneously in Canada

Library of Congress Cataloging-in-Publication Data:
Flamini, Riccardo, 1968–
Mass spectrometry in grape and wine chemistry / Riccardo Flamini, Pietro Traldi.
p. cm.
Includes bibliographical references and index.
ISBN 978-0-470-39247-8 (cloth)
1. Wine and wine making–Analysis. 2. Wine and wine making–Chemistry. 3. Mass spectrometry. I. Traldi, Pietro. II. Title.
TP548.5.A5T73 2010
663'.200284–dc22

2009019923

Printed in the United States of America

10 9 8 7 6 5 4 3 2 1

PREFACE

Science is based on the transfer of knowledge on specific subjects. Only by comparison of results and experiences can some fixed points be defined. These points represent the foundation of further investigations. This finding is particularly true when the knowledge is found in different research areas: here the researcher interests operate in a collaborative effort, which leads to a feedback process between the two groups. Often it seems that the verb *to collaborate* has a different meaning from that given by Webster's dictionary; that is, *to work jointly with others especially in an intellectual endeavour*. This definition implies a transfer of knowledge between the collaborating groups in two directions, but what generally happens is that one direction is highly privileged. The right balance between the two arrows is due to the coscience of each partner of the efforts, difficulties, and views of the other: once this has been reached the collaboration becomes more complete and completes the professional relationship in a friendly manner.

This book was written by two friends, who are different in age, experiences, and knowledge, who started to collaborate many years ago on the application of mass spectrometry to the field of grape and wine chemistry. The availability of new mass spectrometric approaches and the desire to test their capabilities in the analysis of complex natural matrices, such as grape and wine, led the authors to undertake a series of research, projects, which give a more detailed view of the chemistry involved in these natural substrates.

In the last decades, the increased consumption of table grapes and wines has been encouraged by the amply demonstrated beneficial effects of these substances on human illness, such as cardiovascular diseases, brain degeneration, and certain carcinogenic diseases. Improving the quality of grapes is achieved by selecting the best clones and varieties, the use of more appropriate growing techniques, and taking into account the environmental effects on the vineyard. The quality of wines is increased by optimizing the wine-making processes, such as extraction of grape compounds, alcoholic fermentation, malolactic fermentation, and barrel– and bottle aging.

The legislation of the European Community (EC) and of single countries is devoted to protecting consumer health and internal markets from the sometimes harmful effects that may be caused by low-quality products. Legal limits are defined and quality certificates are often required (for pesticides, toxins, etc.).

In this framework, knowledge of the chemical composition of grapes and wines is essential. Mass spectrometry (MS) is proving to be the most powerful tool with which to achieve this result: This book presents the match between the high structural identification power of MS techniques and the variegated chemistry of grape and wine.

The volume is divided into two parts: Part I (Chapters 1–3) gives a general view of the mass spectrometric methods usually employed in the field of interest; Part II (Chapters 4–10) is divided into seven chapters by subject and describes the grape and wine chemistry, as well as both the traditional and more recent applications of MS.

This book was perceived as both an up-to-date source for students beginning work in the field of oenological (and in general of foods) analytical chemistry, and as support to Research and Quality Control Laboratories.

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ACKNOWLEDGMENTS

We want to thank all the contributors whose work made this volume possible: Laura Molin and Luca Raveane for their kind support in preparing the first part of the book, Mirko De Rosso, Annarita Panighel, and Antonio Dalla Vedova for their support in organizing the second section, and Professor Rocco Di Stefano of Palermo University and Professor Paolo Cabras of Cagliari University for the kindly provided data.

Lastly, we want to thank John Wiley & Sons, Inc. for giving us the opportunity to prepare this book.

CONTENTS

INTRODUCTION

Nowadays, mass spectrometry (MS) strongly interacts with most chemical research areas, from studies of gas-phase reactivity of ions of interest to biomedical investigations. This finding is the result of the many efforts from different research groups around the world, working to develop instrumental arrangements suitable for specific analytical and fundamental studies.

Half a century ago mass spectrometers were considered (and really they were!) very complex and expensive instruments, requiring well-experienced personnel for their management. Then, "mass spectrometry labs" were present at the departmental level and were used mainly by research groups operating in the field of organic chemistry. Now the situation is completely different: The (relatively) low prices and ease of instrumental management has moved mass spectrometers from dedicated labs to the utilizer environment, resulting in a capillary diffusion of medium high-performances instruments.

Surely, this is the result on one hand to the development of ionization methods alternative to electron ionization, able to generate ions from highly polar, high-mass molecules and are easily coupled with chromatographic systems. This aspect has been well recognized by the entire scientific community with the assignment in 2002 of the Nobel

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Prize for Chemistry to John B. Fenn and Koichi Tanaka for "*their development of soft ionization methods for mass spectrometric analyses of biological macromolecules*".

On the other hand, the development of compact mass analyzers, which are easy to use and fully controlled by data systems, led to mass spectrometers no longer covering an area of some square meters (as the early magnetic sector-based ones), but bench top machines, whose dimensions are sometimes smaller than those of the chromatographic devices with which they are coupled. Most of these instruments are based on the interaction of ions with quadrupolar electrical fields and were developed by the W. Paul (Nobel Prize for Physics, 1989) group at Bonn University.

These developments [together with the availability of high-performance instruments, e.g., Fourier transform–mass spectrometry (FT–MS) and Orbitrap] make possible the application of MS in many different fields. The problem is to individualize the best instrumental choices and the related parameterization to obtain the analytically more valid results, which allows to propose new, highly specific analytical methods.

As complex as the analytical substrate of interest might be, specificity plays a fundamental role. This is the case for grape and wine, highly complex natural substrates, for which the use of different mass spectrometric techniques allowed to obtain a clear (but still not complete!) view of the chemical pathways present in them.

Viticulture and oenology play an important role in the economy of many countries, and considerable efforts are devoted to improve the quality of products and to match the broadest demands of the market. Many industrial processes are finalized to obtain products with peculiar characteristics: the inoculum of selected yeast permits a regular alcoholic fermentation with minimum secondary processes by other microorganisms, which favor formation of positive sensory compounds and limit the negative ones; extraction of grape components is enhanced by maceration of grape skins in controlled conditions during fermentation and addition of specific enzymes; malolactic fermentation to improve organoleptic characteristics and to add biological stability to the wine; barrel- and bottle-aging refines the final product (Flamini, 2003). European Community (EC) laws, as well as those of a single country, are devoted to protecting consumer health, rather than the market, from the introduction of low-quality products. This goal is achieved by accurate foods controls. Consequently, quality certificates are often required, for exporting wine and enological products. Of particular concern are the presence of pesticides, heavy metals, ethyl carbamate,

and toxins, for which legal limits are often defined. To prevent frauds and to confirm product identity, accordance between the real-product characteristics and the producer declarations (e.g., variety, geographic origin, quality, vintage), has to be verified. Researchers and control organism activities are devoted to developing new analytical methods. These methods are applied to verify the product origin (Ogrinc et al., 2001), to detect illegal additions and adulteration (sugar-beet, cane sugar or ethanol addition, watering) (Guillou et al., 2001), to protect the consumer health by determination of contaminants (Szpunar et al., 1998; MacDonald et al., 1999; Wong and Halverson, 1999).

On the other hand, to expand the worldwide market considerable efforts of the main wine producing countries are devoted to improve the image of products. Consequently, the product characteristics and origin have to be well defined. Research in viticulture and oenology tries to enhance the typical characteristics of grape varieties by selection of best clones, and to identify the more suitable parameters for product characterization (Di Stefano, 1996; Flamini et al., 2001). For the variety characterization, several parameters of plant and grape, such as deoxyribonucleic acid (DNA), amphelography, isoenzymes, and chemical compounds of grape, are studied (Costacurta et al., 2001). To define characteristics and identify products, secondary metabolites of grape and wine (compounds mainly linked to a specific variety, but not indispensable for the plant survivor, also if environmental and climatic variables can influence their contents in the fruit) are studied (Di Stefano, 1996). These compounds are included in the chemical classes of terpenes and terpenols, methoxypyrazines, volatile sulfur compounds, benzenoids, nor-isoprenoids, and polyphenols (e.g., flavanols, flavonols, anthocyanins, procyanidins, and tannins). Volatile compounds and polyphenols are transferred from the grape to the wine in winemaking conferring fragrance, taste, and color to the products.

The first structural studies by gas chromatography–mass spectrometry–electron impact (GC/MS–EI) of grape and wine compounds were performed in the early 1980s. A number of new volatile wine compounds formed by yeasts during alcoholic fermentation, and aroma compounds from grapes, were identified (Rapp and Knipser, 1979; Rapp et al., 1980; 1983; 1984; 1986; Williams et al., 1980; 1981; 1982; Shoseyov et al., 1990; Versini et al., 1991; Strauss et al., 1986; 1987a; 1987b; Winterhalter et al., 1990; Winterhalter, 1991; Humpf et al., 1991). It was confirmed that grape varieties with an evident floral aroma were classified as "aromatic varieties" (e.g., Muscats, Malvasie, Riesling, Müller-Thurgau, and Gewürztraminer) and are characterized by their high monoterpenol

contents. These characteristics increase during the final stages of ripening (Di Stefano, 1996), and during fermentation. Wine aging chemical transformations involving these compounds lead to formation of new monoterpenols (Williams et al., 1980; Di Stefano, 1989; Di Stefano et al., 1992). It was found also that several norisoprenoid compounds are important in the aroma formation of grapes and wines (Strauss et al., 1986; 1987a; 1987b; Winterhalter et al., 1990; Winterhalter, 1991; Humpf et al., 1991).

In the 1990s, studies of the Sauvignon grapes and wines revealed that several sulfur compounds and methoxypyrazines (grassy note) are typical aroma compounds of these varieties (Harris et al., 1987; Lacey et al., 1991; Allen et al., 1994; 1995; Tominaga et al., 1996; Bouchilloux et al., 1998).

Mass spectrometry is also applied in the control of pesticides and other contaminants (e.g., 2,4,6-trichloroanisole), detection of compounds formed by yeast and bacteria, determination of illegal additions to the wine. Liquid chromatography/mass spectroscopy (LC/MS) methods for determination of toxins in the wine (e.g., ochratoxin A) have been proposed (Zöllner et al., 2000; Flamini and Panighel, 2006; Flamini et al., 2007).

Currently, LC/MS and multiple mass spectrometry (MS/MS) have been used to study the grape polyphenols (anthocyanins, flavonols, tannins and proanthocyanidins, hydroxycinnamic, and hydroxycinnamoyltartaric acids), which allow to structurally characterize and understand the mechanisms involved in stabilizing the color in wines (Flamini, 2003).

To be able to estimate the potential of the grape and how it may be transferred to the wine, a good knowledge of enological chemistry is essential. In this framework, the MS played, and, by the new technologies introduced in the recent years, plays a fundamental role.

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