ANALYSIS OF FOODS AND BEVERAGES

Headspace Techniques

EDITED BY

GEORGE CHARALAMBOUS

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Anheuser-Busch, Inc.
Technical Center
St. Louis, Missouri

ACADEMIC PRESS **New York San Francisco London** **1978**
A Subsidiary of Harcourt Brace Jovanovich, Publishers

ACADEMIC PRESS, INC.
111 Fifth Avenue, New York, New York 10003

United Kingdom Edition published by
ACADEMIC PRESS, INC. (LONDON) LTD.
24/28 Oval Road, London NW1 7DX

Library of Congress Cataloging in Publication Data

Main entry under title:

Analysis of foods and beverages.

Proceedings of a symposium organized by the Flavor Subdivision of the Agricultural and Food Chemistry Division of American Chemical Society at the 174th national meeting, Chicago, Aug. 29-Sept. 2, 1977.

1. Food—Analysis—Congresses. 2. Beverages—Analysis—Congresses. 3. Flavor—Congresses. 4. Gas chromatography—Congresses. I. Charalambous, George, Date II. American Chemical Society. Division of Agricultural and Food Chemistry. Flavor Subdivision. III. Title: Headspace techniques.
TX545.A5 664'.07 77-28841
ISBN 0-12-169050-4

PRINTED IN THE UNITED STATES OF AMERICA

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FOREWORD

Headspace sampling for gas chromatographic analysis, which in its truest sense implies the direct injection of the mixture of vapors in equilibrium with a sample held within a confined space, possesses a desirable and appealing simplicity. It also offers a distinct advantage in that sample work-up procedures have been avoided. These latter usually involve distillation, extraction, and/or adsorption processes, and almost invariably engender quantitative and, frequently lead to, qualitative changes in the composition of the sample, which are certainly good reasons to favor simple headspace injections.

Unfortunately, these simple headspace injections also suffer a disadvantage: To obtain optimum chromatographic results, it is necessary that the injected sample occupy a minimum length of column as the chromatographic process begins. If this concept is violated, one pays the price in broad, poorly resolved peaks; hence the size of gas sample that can be injected is seriously limited.

This in turn poses another problem: This limitation in the size of sample that can be injected also limits the components that can be detected. Only those components that, by virtue of their concentration and relative volatility, are present in quantities sufficient to activate the detector will be detected. Relatively low molecular weight and highly volatile compounds (e.g., C_2–C_8 esters, aldehydes, ketones) can be readily detected by the direct injection of these restricted quantities of headspace gas; larger or less volatile constituents cannot. The amounts present in the small volume of gas injected are simply too low for detection in most cases. While precolumn concentrations or splitless injection (in which the solvent effect is utilized to achieve a narrowband concentration of trace components on the head of the column) can sometimes be used to overcome these limitations, these techniques, however, are not universally adaptable.

The swing toward high-resolution gas chromatography, encouraged by the availability of columns possessing 2500–3500 effective theoretical plates per meter, draws renewed attention to this old problem, because the sample capacity of these high resolution systems is still smaller, reducing still further the size of sample that can be injected.

But headspace sampling is an area that most of us are loath to abandon.

In many cases, headspace compositions are much more meaningful than the total volatile analysis resulting from distillation or extraction procedures.

Fortunately, the situation is not hopeless; many investigators have been working on these problems, and many interesting results are beginning to emerge. This symposium is an attempt to bring some of those investigators together, and to explore methods of headspace concentration and headspace sampling that are producing results on a variety of products and model systems. The content of the following papers will, I believe, convince most readers that the prognosis for a bright future in headspace sampling is highly favorable.

WALTER JENNINGS
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PREFACE

The merits and demerits of direct vapor analysis, or as it is more popularly known, the headspace method, have long been debated. Proponents of this technique cite the ease with which vapor analyses may be performed: The determination of volatile flavor components by direct chromatographic analysis is preferred by many to the classic methods involving distillation, adsorption, extraction, etc. On the other hand, the gain resulting from direct vapor analysis in eliminating the variability associated with multistep methods may be offset by nonreproducibility arising from inefficient sample preparation, even in a single-step procedure.

A symposium on the analysis of foods and beverages by headspace techniques was organized by the Flavor Subdivision of the Agricultural and Food Chemistry Division of American Chemical Society at its 174th National Meeting, August 29–September 2, 1977, in Chicago, Illinois, with the purpose of reviewing the latest developments in this field. This volume presents the proceedings of that symposium.

The current state-of-the-art points to a productive combination of techniques leading to the enrichment of headspace vapor components with gas chromatographic resolution followed by mass spectrometric identification. Such concentration techniques obviate the need for an increased sample size with its attendant drawback of a decrease in the minimum detectable amounts of flavor compounds of low vapor pressure. It may be concluded that headspace analysis is alive and well.

Flavor chemists in industry and academia from Europe, the United States, and Japan have contributed recent findings that cover the analysis by headspace techniques of mouth odors, vegetable flavors, lipoxygenase catalyzed reactions, the vanilla bean, coffee, tea, cocoa, beer, wine, and sake. Other contributors have dealt with general considerations such as the use and abuse of headspace sampling, statistical treatments of GLC headspace data, as well as quantitative aspects, new instrumentation, and techniques.

On behalf of the Flavor Subdivision, the editor wishes to thank the speakers, all experts in their fields, whose outstanding presentations made this symposium a considerable success; the papers presented in this volume will be of great value to the advancement of flavor research. The editor is

also grateful to the contributors for their valiant and courteous responses to the numerous demands made on them for the preparation of this volume. He is particularly grateful to Professor Walter Jennings for contributing the foreword and to the publishers for their guidance and assistance.

CONTENTS

HEADSPACE SAMPLING: USE AND ABUSE

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ABSTRACT

As applied to gas chromatographic sampling methodology, many of us frequently misuse the term "headspace". Most precisely, it should denote that mixture of vapors existing in equilibrium with a sample held in a closed system. Because only the more abundant and more volatile compounds will exist at detectable levels in the small samples that can be used for direct injection, a variety of methods for achieving headspace concentration have been proposed. When the vapor is removed at a rate faster than the equilibrium can be maintained, changes in the relative concentrations of individual components can be expected to occur. Compositional changes can also be engendered by discriminatory trapping; some trapping substrates exhibit lower affinities for specific compounds, and similarly, discrimination can be experienced in the recovery step.

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[1]To whom all inquiries should be directed.

I. INTRODUCTION

The analysis of the headspace vapors above foodstuffs by gas chromatography or gas chromatography-mass spectrometry (GC-MS) has been widely applied in flavor chemistry. A variety of methods have been used to sample and/or isolate the trace volatiles present in such headspace. However, the term "headspace" as applied to these sampling techniques has been used to convey a number of different meanings. In the context of this work we have defined headspace as the gaseous mixture surrounding a sample within a closed system at equilibrium.

Some workers have suggested the use of displacement procedures to obtain larger volumes of a "true" headspace sample. These have ranged from the displacement of a gaseous sample within a confined space via a moveable piston or a liquid to the inflation of a balloon within that space. When such procedures are used, attention should be directed to the possibility of preferential solubility or absorption of sample components in the rubber, lubricant or other parts of the system. Preferential adsorption on the walls of the container or sampling system can also yield variable results. Chromatograms of simple syringe injections from a standard headspace source frequently exhibit considerable scatter unless the syringe has been filled and emptied several times to satisfy its adsorptive demands.

Many "headspace" determinations involve the passage of a non-condensible gas over the sample to sweep the volatiles into a trapping device. Under these non-equilibrium conditions the composition of the sample as subsequently determined--i.e. the ratios of the individual volatiles--may bear little relation to the true headspace composition. Additionally, the manipulations required to transfer the sample from the trap to the chromatograph may also cause compositional changes.

Since in many cases the levels of volatiles in a headspace sample are very low and since these volatiles are almost always dominated by water, some method of preconcentration and cleanup before analysis is usually required. The use of porous polymers to affect these steps is now widespread (1, 2, 3, 4) and considerable work has been carried out on the relevant properties of the available materials (5, 6).

Less attention has been paid, however, to the influence of these adsorbants and of the sample manipulations required for their use, on the composition of the sample. Of particular concern is whether the adsorption and desorption processes are quantitative under the conditions employed and the influence of the subsequent sample collection procedure.

The usual procedures for sample recovery are heat desorption followed by collection in a cooled trap, or (less commonly) solvent extraction. It is likely that either of these manipulations will have an influence on the sample composition.

A number of investigators have described methods for the direct desorption of the collection traps in the inlet of a gas chromatograph (4, 8). While this method obviously minimizes sample handling losses, it does have some disadvantages, e.g., only one analytical run can be obtained from each collection. In our case we have been unable to obtain sufficiently rapid desorption to meet the injection requirements for good chromatography on small-bore capillary columns.

II. METHODS AND MATERIALS

A. Gas Chromatography

Analyses were performed on a Packard Model 427 gas chromatograph adapted to an improved version of a linear glass inlet splitter (9) and a wall-coated open-tubular (WCOT) glass capillary column, 0.24 mm i.d. X 40 m., coated with methyl

silicone SE-30 admixed with 7% Igepal CO 990. Inlet and detector temperatures were maintained at 200°C. Unless otherwise specified the column was programmed from 70 to 160°C at 6°C/min after an initial delay of 4 min. Because the peaks were narrow, sharp and symmetrical, and baseline separation was achieved, the sample compositions were calculated from the measurement of the peak heights.

B. Model Systems

Two model systems, whose components were selected to represent a range of functional groups and boiling point, were used. Their components, in order of elution on these columns, are shown in Table 1 and Table 2.

TABLE 1

Components of Model System 1

Ethanol
2-Pentanone
Heptane
Pentanol
Hexanol
Hexyl formate
2-Octanone
Limonene
Heptyl acetate
γ-Heptalactone

TABLE 2

Components of Model System 2

Ethyl butyrate
2-Pentanone
Hexanol
Hexyl acetate
Limonene

C. Porous Polymer Trapping Procedures

The traps were prepared by filling *ca.* 3 cm of a 15 cm length of 6 mm o.d. Pyrex tubing with either 80-100 mesh Porapak Q (Waters Associates) or 60-80 mesh Tenax GC (Enka, The Netherlands) between glass-wool plugs. Traps were conditioned at 180° at a flow rate of 60 ml/min of purified N_2, for 8 hr. A similar tube packed only with glass wool was utilized

for the sample collection efficiency experiments.

The desorption-collection system used is shown in Figure 1.

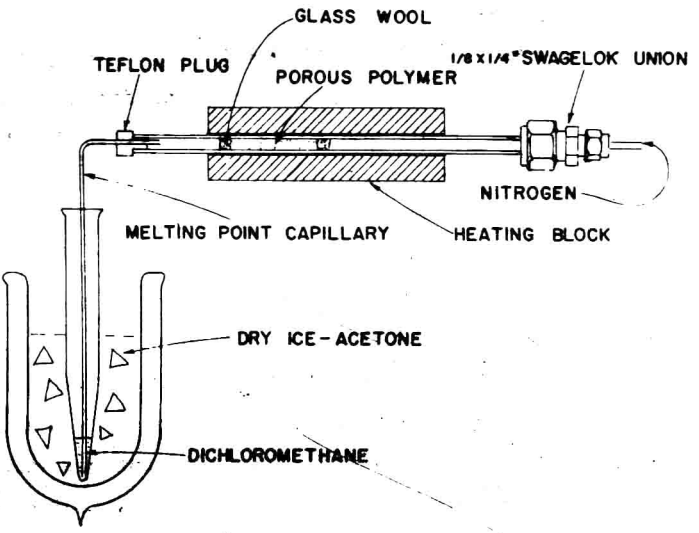

Fig. 1. Apparatus used for desorption-collection from the porous polymer traps. See text for details.

The heating block was maintained at 130°, the trap flow rate at 10 ml/min and the trapping time was 30 min unless otherwise specified.

For the through-flush experiments *ca.* 3 µl of the model system mixture was applied to one end of the trap with a microsyringe and the apparatus immediately assembled so that the gas flow swept the sample through the polymer trap, and the desorption-collection procedure commenced.

In the backflush experiments, the sample was applied as above but the sample was flushed into the polymer for 5 min then the trap reversed, placed in the desorption-collection apparatus and the sample collected as above. For some experiments the bent capillary methylene chloride trap was replaced with a straight length of capillary tube (uncut melting point