H. Maarse and R. Belz

Isolation, Separation and Identification of Volatile Compounds in Aroma Research

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By Dr. H. MAARSE and Drs. R. BELZ

Division for Nutrition and Food Research TNO
Institute CIVO-Analysis, Zeist, The Netherlands

With cooperation by

J. M. H. BEMELMANS
J. H. DHONT
L. J. VAN GEMERT
P. J. GROENEN
C. VAN INGEN
M. C. TEN NOEVER DE BRAUW
J. SCHAEFER
S. VAN STRATEN

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Isolation, Separation and Identification of Volatile Compounds in Aroma Research

Handbuch der Aromaforschung

Unter Mitarbeit namhafter Wissenschaftler des In- und Auslandes entstandene Sammlung von Monographien auf dem Gebiet der Aromaforschung

herausgegeben von
MANFRED ROTHE
Potsdam-Rehbrücke

Foreword

In 1973 Dr. M. ROTHE approached Dr. C. WEURMAN — at the time head of the Flavour Section, Central Institute for Nutrition and Food Research TNO (CIVO TNO) — on whether he would be willing to compose a series of monographs on techniques applied in aroma research. Dr. WEURMAN and his co-workers accepted the proposal, but the actual writing was considerably delayed because of Dr. WEURMAN's death in January 1975.
After some hesitation his colleagues decided to nevertheless carry the work out. This, however, proved to be far more time consuming than expected.

At CIVO TNO work on aroma problems already began in 1956. The original objectives were the development of analytical methods and instruments for the chemical investigation of the aroma of food products and the application of such techniques in unravelling complex aromas at the request of interested industries. Although the emphasis of aroma research has shifted somewhat to what are called negative quality aspects, such as off-flavours, toxicants and odour pollution of air, the relevant techniques do not essentially differ from those used in food aroma research in the strict sense.
After the initial years, during which the purely instrumental facilities were built up, sensory analysis has become an integrated part of aroma research and of off-flavour and odour pollution studies.

Prof. Ir. B. KROL,
Director, Division for
Nutrition and Food Research TNO

Preface

This monograph has been written by research workers of the Flavour Section and the Section of Instrumental Analysis of Institute CIVO-Analysis TNO.
Being editors as well as co-authors, it was our task to try and integrate the relevant contributions and seeing to it that the whole subject was covered and no overlapping occurred. Co-authors and other colleagues rendered valuable assistance in critically perusing the manuscripts. In this connection we would specially mention Dr. Ir. H. T. BADINGS of the Netherlands Institute of Dairy Research at Ede.
We are also grateful to many collaborators of the Flavour Section and the Section for Instrumental Analysis who assisted in selecting the illustrations, arranging lists of references, etc. The co-operation of the TNO translation units that took an active part in checking the manuscripts on the correct use of the English language is also much appreciated.
Finally, we would like to mention the enthusiasm with which Mrs. J. C. DE BEAUVESER organized and supervised the typing of the manuscripts.

September 1978

The Editors
H. MAARSE
R. BELZ

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1. Introduction

H. MAARSE

One of the aims of aroma research is to identify the compounds that play a part in the aroma of a food product. Because of the low concentrations in which the majority of these compounds occur, identification was difficult to achieve before the beginning of the era of modern instrumental aroma research some twenty years ago. From the mid-fifties to the mid-sixties emphasis was laid on identifying as many compounds as possible. The development of gas chromatography in those years and its ensuing use in combination with mass spectrometry (GC-MS) tremendously accelerated the output rate in aroma research (fig. 1—1). It was rather a disappointment that many newly identified compounds proved to be unimportant for the odour of a product.

From about the late sixties, identification work became more systematic and was increasingly directed to the really important compounds by proper use of sensory evaluation. Those who tried to set up a complete survey of all volatile compounds ever encountered in foods, as we did (1), will agree that impressive results have been obtained. Figure 1-1 shows considerable increase of the number of identified compounds over the years.

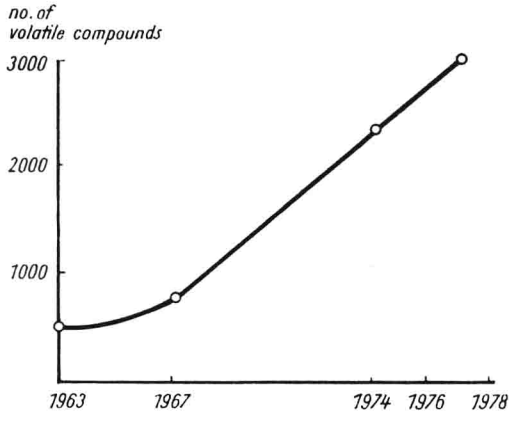

Fig. 1—1
Number of volatile compounds reported to occur in food products (1).

Many of the techniques and methods described in this book were specially developed for the study of the odorous components of food products. The peculiar nature of this field of application becomes evident from the following facts:

- The number of volatile substances in foods is extremely high.
- There is great variety in these substances, both in physical and chemical properties and in the concentrations in which they occur, which range from picogrammes to milligrammes per kilogramme product.
- Components contributing most significantly to odour may well occur at the low end of the concentration range.
- Quantitative ratios among aroma components are often more important than the effects of individual ones.
- Many odorous components become unstable when they are isolated from their original environment. Some foods must be regarded as live systems in which the qualitative and quantitative composition of odorous compounds is regulated by biochemical processes; interference with these processes, as occurs in isolation procedures, e.g. maceration, generally induces changes in composition.
- In contrast to other fields of analysis, the concentrations in the vapour emanating from the product instead of those within the product are of primary importance.
- Volatile compounds can be identified and measured instrumentally, but only the human nose can estimate their actual contribution to the odour of a product.

The only handbook on methodology in aroma research appeared in 1971 (2). In its preface, the authours state inter al.: "Flavour research is an active and rapidly changing field. No doubt by the time this book reaches the reader many advances will be made". Seven years later it can indeed be stated that developments have continued ever since. In this period, some centres of attention have been: a) the mechanisms through which odorous compounds are formed; b) the relationship between concentrations of compounds within a food product and those in the vapour phase above it; c) the relation of sensory to instrumental analysis in the development of new methods for assessing the quality of a product. As regards chromatographic techniques, the increased use of glass instead of stainless steel capillary columns and the incipient use of high-pressure-liquid chromatography for aroma research are worth mentioning. The possibilities of GC-MS were further extended by the introduction of the computer as part of the combination.

In this book the following items will be treated successively:

- methods for the isolation and concentration of volatile compounds;
- separation methods;
- methods for the identification of volatile components;
- sensory analysis as a step-by-step control of the instrumental procedures.

Although this book is entirely devoted to the analysis of volatile compounds, it should be remembered that besides odour, taste is an equally important aspect of the quality of a food product.
Attention will be mainly focussed on recent developments and applications, while only a summary account of the theoretical part is given. For the fundamentals, the reader will be referred to the handbooks. As a rule, illustrative material has been

derived from the analysis of odorous compounds; only if no appropriate examples were available has material from other research areas been used.
The subject of thin-layer chromatography (TLC) is treated more extensively than the rest, rendering the possibility to incorporate unpublished work from the author's laboratory.

Since a separate monograph on sensory analysis will be published in this series, the chapter on sensory analysis is limited to the aspects relevant to the step-by-step control of the instrumental analysis, beginning with the sampling stage right through to the final identification of purified components. The importance of such guidance by sensory methods can be illustrated by the following example. When one relies solely on instrumental analysis, sensorially significant components, which after gas chromatographic separation are overlaid by other components in much higher concentration, often escape detection.

The final chapter deals with some applications of methods, developed by aroma investigators, in other fields of research that are more or less related to aroma research proper, viz. off-flavours, mal-odours of the environment, pheromones, aroma and toxic aspects of tobacco smoke, volatile toxic chemicals, and medical (clinical) chemistry.

In the past, misuse by flavour chemists of terms from the sensorial field, such as "odour", "flavour" and "aroma", often led to confusion. Being primarily interested in the physical and chemical properties of substances, they do not always carefully choose the terms to describe the organoleptic properties of isolated components or fractions. Speaking the same "language" actually will be conducive to a good cooperation between instrumental and sensorial analysts. In the last few years, attempts have been made to give sharp definitions of these terms and to discourage the use of others. In anticipation of the results of international consultations, which in the meantime resulted in two international standards and one draft international standard (3), the British Standards Institute (B.S.I.) has issued a comprehensive list of terms (4). A few of these are given below:

to smell	— to test for sensation by use of the olfactory organ
odour	— 1) the sensation perceived via the olfactory organ from certain volatile substances 2) quality of this particular sensation due to these substances
aroma	— an odour with a pleasant connotation
taste	— 1) the sensation perceived via the taste buds resulting from the presence of certain soluble substances 2) quality of this particular sensation due to these substances
flavour	— the combination of taste and odour. It may be influenced by sensations of pain, heat, cold (e.g. spices, horseradish and menthol) and by tactile sensations
off-flavour	— an atypical flavour, usually associated with deterioration
taint	— a taste or odour foreign to the product.

In the following chapters we will try to adhere to these definitions.

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2. Isolation and concentration of volatiles from foods

2.1. Isolation and concentration from the product phase

J. M. H. BEMELMANS

2.1.1. Introduction

Notwithstanding the spectacular development of analytical instruments in recent years, the human nose is as yet more sensitive to many odorous compounds than the detectors presently available for aroma analysis. This difference in sensitivity, the minute concentration of many odorous compounds in food and the amount of sample needed for their identification make it necessary to isolate the odorous compounds from the food and to concentrate them prior to the actual analysis.

An excellent review on methods for the isolation and concentration of food volatiles was given by WEURMAN (1). He pointed out that combinations of procedures used by some investigators to prepare odour concentrates were not always logical. Some isolation and concentration methods were also reviewed by FORSS (2) and by TERANISHI et al. (3).

This chapter will deal with distillation and extraction procedures used to isolate volatiles from foods directly. Their advantages and disadvantages in laboratory-scale experiments will be discussed. Attention will also be given to the various methods for preparing concentrates of the odorous compounds, followed by a discussion on some procedures to fractionate these concentrates.

Volatiles can also be isolated from the vapour phase in equilibrium with the food, or from gas flows led through the food. The relevant procedures are discussed in Chapter 2.2.

A major difficulty encountered in aroma investigations is that the amount of volatile compounds in foods is very small. Table 2—1 gives the concentration of the odorous compounds in some foods and shows that only minute quantities are present. Concentrations of individual compounds may be as low as 1 ppb or less, requiring large quantities of food to be processed in order to obtain sufficient material for analysis.

Table 2—1
Concentration of aroma compounds in various products according to WEURMAN (4)

product	concentration, ppm
banana	12—18
strawberry	2—8
grape	110
passion fruit	36
tomato	3—5
cocoa	100
beef	34
bread	6—10 (excl. ethanol)

Characteristic properties of food odours, summarized in Table 2-2, have always to be considered during isolation and concentration. Food odours usually contain between 50 and 250 different volatile compounds having a wide range of boiling points (7). Some products contain an even larger number: more than 700 have already been found in coffee.

Table 2—2
Characteristic properties of food odours are:*

1. the complexity of the mixture of odorous components present in most food products
2. the low concentration in which these components are generally present
3. the chemical reactivity and lability of the components
4. the polar character of many aroma compounds
5. the wide range of boiling points of the components
6. the large differences in the relative amount of the individual components
7. the presence of large quantities of volatile non-aroma compounds (a.o. water)
8. the strict relation between the quantitative composition of the mixture and the organoleptic properties
9. the differences in odour potencies of the different components

* According to WEURMAN and VAN LUNTEREN (5), but with additions from BADINGS (6)

Since some volatiles are heat sensitive or reactive, precautions have to be taken to ensure that they do not react during their isolation or concentration. Furthermore, it should be noted that foods themselves are complex matrices containing many chemical constituents, some of which might react and generate volatiles. The formation of new compounds (artifacts) should be prevented as much as possible, since it can lead to erroneous conclusions. This is also true for extraneous compounds (contaminants) introduced during analysis from e.g. solvents or glass-ware.
When isolating and concentrating food volatiles the above-mentioned factors must be taken into account; in this connection the following guide-lines are recommended:

1. The volatiles should be isolated from the food as completely as possible.
2. Artifacts and contaminants have to be kept down to a minimum: labile volatiles should be prevented from reacting or decomposing; no volatiles should be generated from the non-volatile food constituents.
3. Preferably, recovery data for the relevant methods should be ascertained.

In this chapter, the procedures are classified throughout as isolation and concentration methods, although with some of these the volatiles are simultaneously isolated and concentrated.

2.1.2. Selection and pretreatment of samples

Before analysis it should be clearly defined which odour characteristics of the product are to be studied. The sample of which the odour has to be analyzed must possess these odour notes as strongly as possible, while less desirable notes should be weak or absent. This implies that great care is required when selecting the sample which actually is to be submitted to analysis. Thus CHANG et al. (8) employed a panel for sample selection, using the profile method, and WOBBEN et al. (9) had their samples selected by experienced flavourists.

Usually the product under investigation has to be submitted to a pretreatment before the volatile constituents can be removed from the nonvolatile food matrix, unless it is a liquid of low viscosity. This pretreatment can comprise one or more of the following procedures: milling, homogenization, centrifugation, filtration or pressing. Solid products may have to be homogenized with water, so as to produce a slurry.

Formation of artifacts by chemical or biochemical reactions during pretreatment of a sample must be avoided. To minimize chemical reactions, it is usually necessary to ensure that the heat developed during milling or homogenization is dissipated (e.g. by cooling), so as not to expose the product to a high temperature. Also, exposure to light and/or oxygen must be excluded as much as possible.

Special procedures have to be applied for foods containing active enzymes. During maceration or homogenization, cell structures are disrupted, allowing enzymes and precursors to react. DRAWERT et al. (10) have shown that esters are hydrolyzed and that fast oxidative reactions occur in the presence of oxygen, as soon as the cell structure is disturbed. A large increase in the hexanal and hex-2-enal concentrations has been observed after homogenization of various fruit tissues (11). Similarly, FLEMING et al. (12) found a rapid formation of carbonyl compounds when cucumbers were homogenated in the presence of oxygen, while KAZENIAK and HALL (13) noted that the quantity of aldehydes in sliced tomatoes was markedly smaller than in homogenized ones. The latter phenomenon was confirmed by other investigators (14, 15). Cutting banana and potato tissues gave rise to an increased ethylene production (16).

When studying the volatiles of the undamaged tissue the enzymes have to be inactivated before or immediately after homogenization. DRAWERT et al. (17) have recommended that homogenization be carried out in the presence of methanol, thus inhibiting the enzyme activities; they followed this procedure for the isolation of apple volatiles. The same method was employed in studies of banana volatiles (18), and