Flavour and Authenticity Studies at the TNO-CIVO Food Analysis Institute

By Henk Maarse, TNO-CIVO Food Analysis Institute, Zeist, The Netherlands

Flavour research at TNO has been in progress for more than thirty years. It actually started with mainly qualitative studies. Nowadays increasing emphasis is being laid on quantitative aspects although sensory analysis still plays a very important role in our studies. A new field of research has been entered upon in the form of authenticity studies of fruit juice and wines. Knowledge of the composition of the products we are studying is essential in our work and updated reference material is compiled in our publication *Volatile Compounds in Food*. In the following further information about this publication will be given as well as examples of our studies on authenticity of fruit juices and wines, and off-flavours.

Volatile Compounds in Food

In 1962 Dr. C. Weurman initiated what is now called the "TNO list" or more officially "Volatile Compounds in Food—Qualitative Data." Twenty years later we started a second series, "Volatile Compounds in Food—Qualitative Data."[2]

The presence of many volatile organic compounds in food and beverage products has been established over more than twenty years. The application of combined gas chromatography and mass spectrometry in flavour research in the early sixties was the beginning of a rapid increase in the number of identified compounds, in fact hundreds of compounds per product. Some examples are presented in Table I.

Table I. Number of Volatile Compounds in Food Products

Product	Number of Compounds	Product	Number of Compounds
Bread (wheaten)	297	Coffee	711
Beef (heated)	533	Beer	533
Orange	245	Wine (white)	556
Strawberry	312	Peanut (roasted)	364

Data taken from Reference 1

Quantitative data of 70 products have been published in 5 volumes. An increased interest in these data has been shown after the introduction by Stofberg of the so-called consumption ratio.[3] This is the ratio between the quantity of a flavouring material consumed as an ingredient of basic and traditional foods and the quantity of that same flavouring material consumed as a component of added flavourings by the same population over the same period. If the consumption ratio of a flavouring substance is higher than one, this substance is consumed predominantly as an ingredient of traditional foods, called "food predominant" by Stofberg.

The consumption ratio has been generally accepted as a useful tool for setting priorities for the safety evaluation of flavouring materials. Useful quantitative data are difficult to find in the literature. Frequently only peak area percentages or concentrations in the head space are given. Furthermore many data are not published by the flavour industry for obvious reasons. However, part of these data are undoubtedly not secret at all, and I would like to encourage everybody to publish as many quantitative data as possible. They are not only valuable because of the consumption ratio but also for other reasons. In the recent wine scandal, for example, it was important to know the natural levels of methanol in several wines, as mentioned in Volume 3 of our quantitative series.

Authenticity Studies

In the last few years we have been working on the authenticity of food products. I will give you two examples. The first one concerns one of the fruit juices, namely, black currant juice and related black currant products.

Black Currants

The authenticity of fruit juices and juice concentrates is controlled by measuring a large number of parameters, including inorganic and non-volatile organic compounds (e.g., amino acids, sugars, organic acids and trace elements). The average quality of a number of juice types (e.g., apple and orange) has undoubtedly improved in the last decade, because the producers were aware of the risk of being traced when they continued adulterating the juices. Another explanation of this quality improvement may be that producers, knowing the criteria, adulterated their products in a more sophisticated way. This professional adulteration can only be prevented if constituents are selected which are too expensive to be added. If these compounds are not

found, constituents are preferred which when added in too low or too high quantities, interfere with the typical and specific flavour of a juice.[4]

So far only limited attention has been paid to the use of volatile compounds of juices and juice concentrates, to control their authenticity. This is very surprising since the aroma composition of all fruit juices is rather complex. When volatile odorous compounds are added to a juice to upgrade its sensorial quality, the so-called gas chromatographic fingerprint will be characteristically different from that of a natural juice. Also the volatile compounds of a product added to the juice for economical reasons can be detected in this way. However, extensive knowledge of the composition of the relevant fruit is necessary. The composition of the volatile compounds depends on variety, year and site of production and ripeness of the fruit. For concentrates an additional factor is the loss of volatiles during the concentration process and possible changes due to enzymatic action or heat treatment.

The volatile compounds are analysed by gas chromatography. Using black currant as an example, we describe the possibilities of this approach.

The influence of heat treatment is shown in figure 1. 4-Terpineol is a major component of black currants. Ethylcyclopentanone (peak 15) and the furan compounds are formed during processing and are not natural constituents of black currants. To improve or to upgrade the quality of black currant concentrates and flavours, Buchu oil is often added by the producer of the concentrates without any declaration or without informing the clients. An example is given in figure 2. In these chromatograms peak 34 is again 4-terpineol and peak 15, ethylcyclopentanone. Peak 29 and 30 are menthone and isomenthone; peaks 40 and 41, pseudo-diosphenol and diosphenol.

These four main components of Buchu oil are also the main components in the adulterated concentrate. Buchu oil, however, is not used because of these four main constituents but on account of the presence of a sulphur compound, 8-mercapto-p-menthan-3-one. The odour of this compound is similar to that of the catty odour of black currants. Furthermore Buchu oil was found in two carbonated lemonades (cassis drinks) and in a black currant juice. I like to emphasize that, according to the information on the labels, these products should only contain natural black currant compounds.

It was interesting to observe in an experiment the influence of these adulterations on the opinion of the consumer. We asked a panel of ten
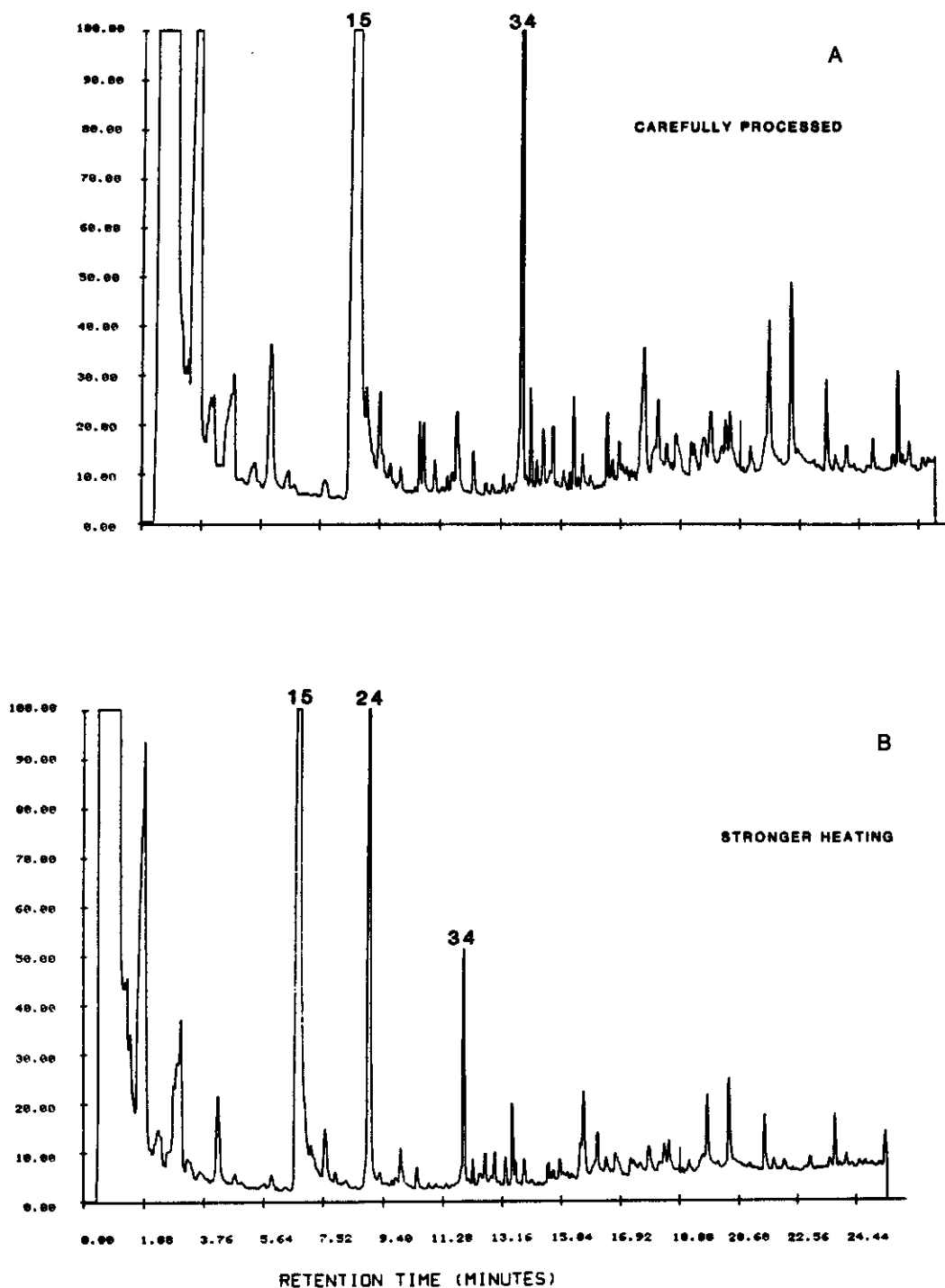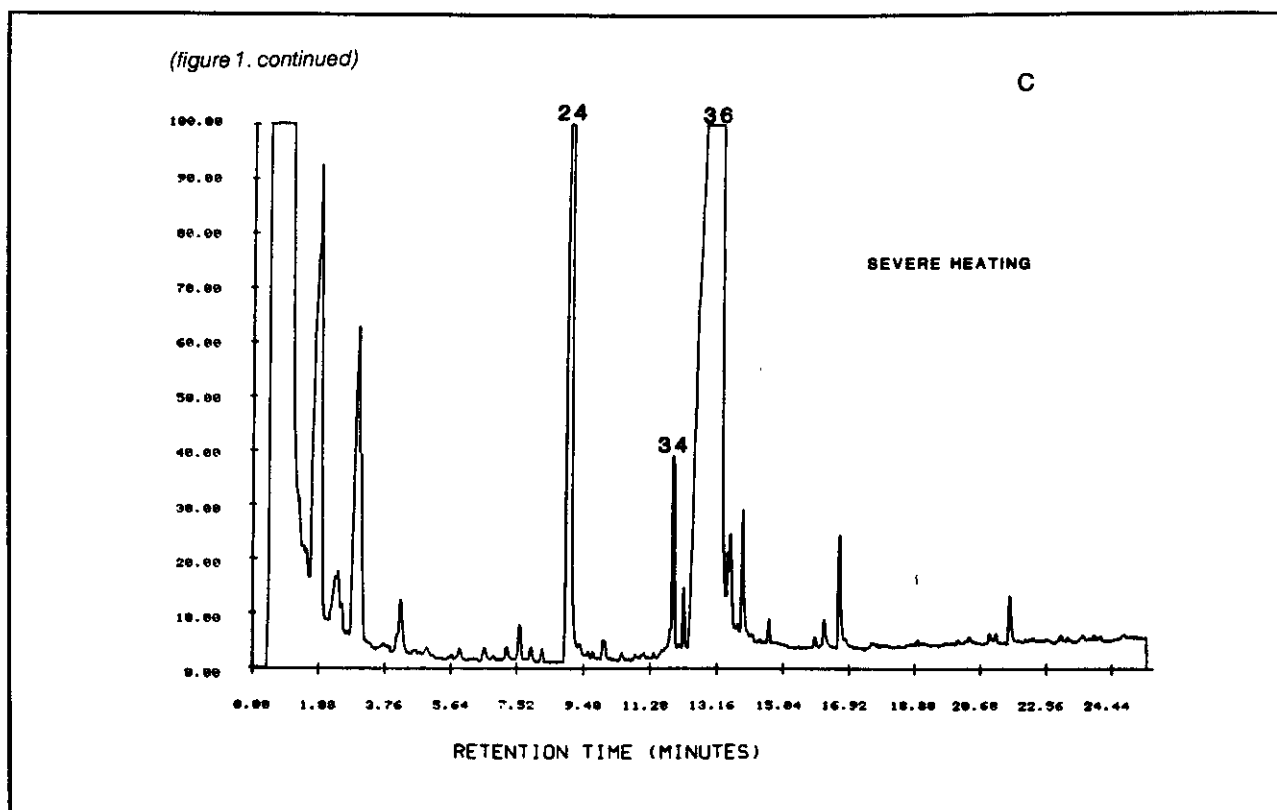
Figure 1.

RETENTION TIME (MINUTES)

Figure 1. Influence of Heat Treatment on the Composition of Black Currant Concentrates. A = Carefully Processed; B = Stronger Heating; C = Severe Heating. Peak 15 = Ethylcyclopentanone, 24 = Methyl furoate, 34 = 4-Terpineol, 36 = 5-Hydroxymethylfurfural

figure 1. continued

(figure 1. continued)

C

SEVERE HEATING

RETENTION TIME (MINUTES)

members to score the characteristic black currant flavour of five concentrates, one of which had been adulterated with Buchu oil. Half of the panel indicated the adulterated one as the sample with a true black currant flavour. I call this the "cassis effect."

Wine

A second example of our authenticity studies concerned the work on wine. Although experienced wine tasters have a remarkable ability to identify coded wine samples, incorrect conclusions are sometimes drawn because of changes in vinology and differentiation between crops. This implies that more objective methods are needed based on the difference in composition.

To study the possibilities of the use of pattern recognition techniques in the classification of wines, we chose two German white wines from the Rhinegauer district, namely, Müller-Thurgau and Riesling. The determined parameters were volatile compounds, non-volatile acids and amino acids. The result of the data analysis is shown in figure 3.

The wines belonging to category 3 occupy a separate position on the left hand side of the plot. The other two categories show a slight overlap. This means that on the basis of these parameters, the two wines can be partly separated, and the

Table II. Threshold Values and Detection Limits of Chloroanisoles

	Pentachloro-anisole	2,3,4,6-Tetra-chloroanisole	2,4,6-Tri-chloroanisole
Odour threshold value ($\mu g.kg^{-1}$water)	4	4×10^{-3}	3×10^{-5}
Detection limit ($\mu g.kg^{-1}$)	$10^{-3}-10^{-1}$	$10^{-3}-10^{-1}$	$10^{-3}-10^{-1}$

*Dependent on the product/medium

Riesling-Auslese can be correctly classified as a subgroup.

We also used pattern recognition techniques to classify fifty-one Riesling wines produced in two different regions in Germany, namely, in the Mosel and Rhine-Pfalz district. Assuming that the soil composition influences the concentration of trace elements in the wines, we analysed fourteen different trace elements. Results of data analysis showed that a 100% correct classification of the wines could be obtained. A distinction could even be made between wines from the northern and southern Rhine-Pfalz district.

Off-Flavours

About twenty years ago TNO investigators identified chloroanisoles as the compounds responsible for a musty taint in eggs. Since then these compounds have often been found to cause such taints in numerous food products. Chloroanisoles are extremely potent odour compounds which have a negative influence on the odour of food products, even in very low concentrations. They are formed from chlorophenols by fungal methylation. The most frequently occurring chloroanisoles formed from pentachlorophenol are pentachloroanisole, 2,3,4,6-tetrachloroanisole and 2,4,6-trichloroanisole (see figure 4).

Pentachlorophenols have many applications. Particularly their use as fungicides in wood preservatives, adhesives and glues often cause musty off-flavours in food products.

The possibilities of detecting chloroanisoles have greatly improved, enabling researchers to perform a more or less routine examination of off-flavoured products. Isolation is carried out by combined steam distillation-extraction in a Likens-Nickerson apparatus. The extract is fractionated by high resolution capillary gas chromatography. Detection is performed by mass spectrometry or by an electron-capture detector.

In the last few years, the sensitivity of the detection increased as a result of selective ion monitoring techniques being used. Recently, further improvement was achieved by using chemical ionization. In Table II the threshold values and the detection limits are presented.

At our institute many off-flavours caused by chloroanisoles have been investigated in the last ten years. Three examples will illustrate some aspects, and finally some ways to prevent or at least to reduce the occurrence of musty off-flavours will be discussed.

Rice in Jute Sacks

Several complaints about mustiness of rice have been investigated by us. In all cases the rice was contaminated with chloroanisoles. This contamination may occur at any time between planting of the rice and consumption of the finished product. The soil in which the rice is planted may already be contaminated with chlorophenols and/or -anisoles. Other contaminated sources can be jute sacks used for transportation from the exporting country, containers of ships, warehouses, wooden pallets, kraft paper bags and cardboard boxes.

Table III. Chloroanisoles ($\mu g\ kg^{-1}$) in Jute Sacks (1978-1984)

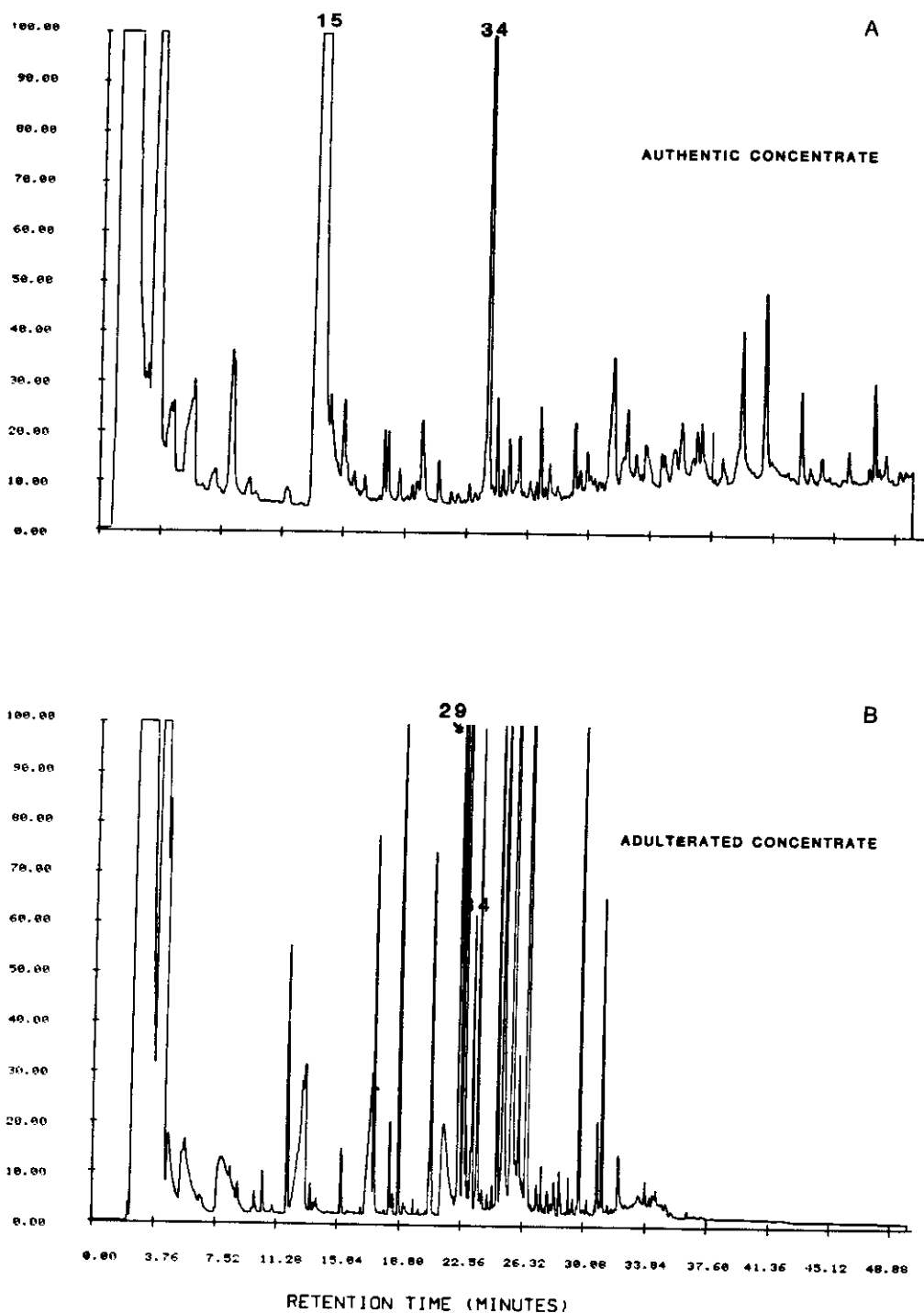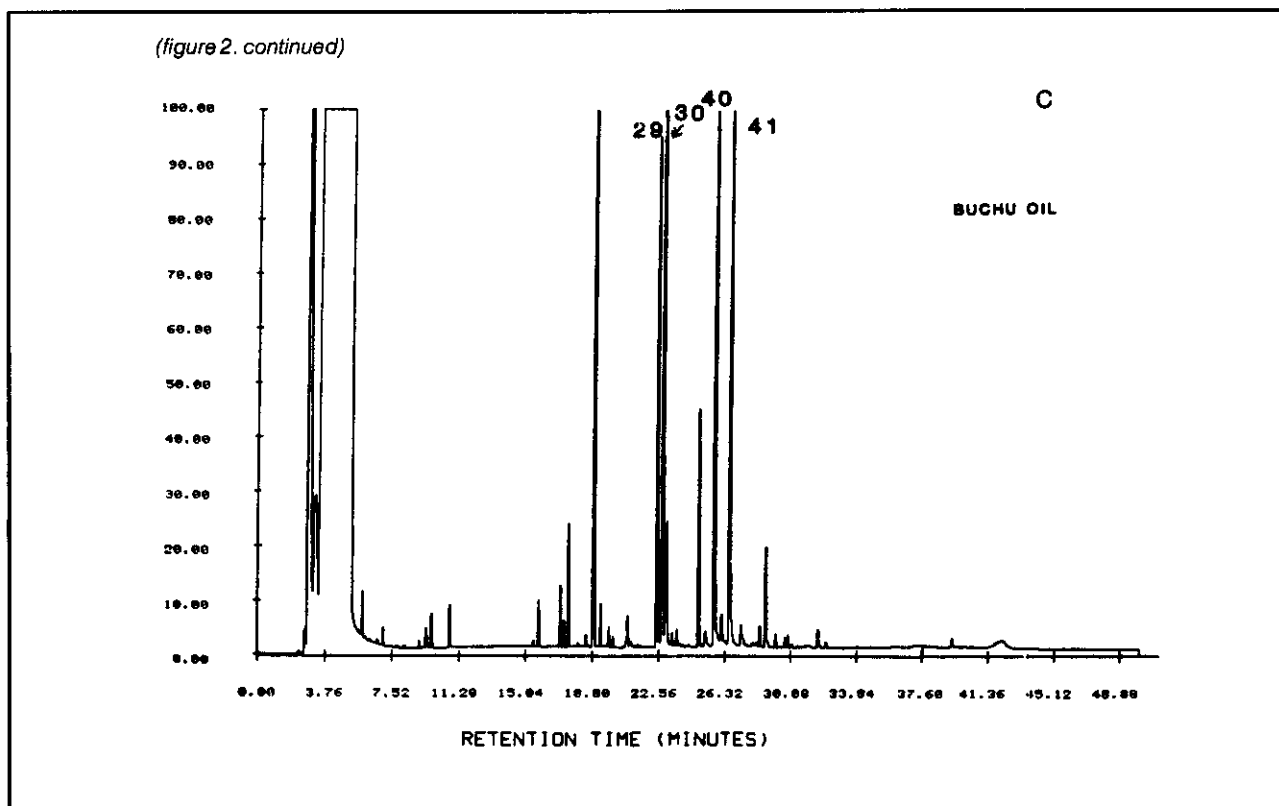|		Chloroanisole Isomer	
Sample No.	2,4,6-Tri-	2,3,4,6- and/or 2,3,5,6-Tetra-	Penta
1	1.9	1.4	1.7
3	0.3	0.3	0.3
5	0.5	0.4	3.3
9	0.1	0.4	0.3
13	0.5	-	-
15	18.6	10.2	5.1
16	6.6	1.5	3.8

- = not detected

Figure 2. Composition of Black Currant Concentrate Adulterated with Buchu Oil.
A = Authentic Concentrate; B = Adulterated Concentrate; C = Buchu Oil. Peak
15 = Ethylcyclopentanone, 29 = Menthone, 30 = Isomenthone, 34 = 4-Terpineol,
40 = Pseudo-Diosphenol, 41 = Diosphenol

(figure 2. continued)

(figure 2. continued)

We know by experience that especially the jute sacks are responsible for the occurrence of the musty taint in rice. It was proved that jute sacks in which rice had been transported contained chloroanisoles in rather high concentrations. The first complaint dealt with by our institute in 1974 induced us to control jute sacks regularly on the presence of chloroanisoles.

Since 1978 chloroanisoles in jute sacks may be quantified by a gas chromatograph/mass spectrometer/computer combination. Before that time extracts of jute sacks made by combined steam distillation/extraction according to Likens and Nickerson were directly brought into the evaporation chamber of a mass spectrometer. A survey of the results obtained is presented in Table III.

Although chloroanisoles were found in all sacks, some did not give rise to complaints. In order to determine at which concentration of chloroanisoles complaints are to be expected, some model experiments were performed.

Small sacks were made from jute sacks imported from India and treated with solutions of 2,3,6-trichloroanisole. This compound was chosen because of its low odour threshold value. The concentrations in the treated sacks were determined analytically and varied from 1-1000 ppb.

After four months the rice stored in these sacks at 20°C was sensorially evaluated by a trained panel of five persons. A musty taint was readily observed in the rice at a concentration of 5 ppb 2,3,6-trichloroanisole in the jute sacks. This corresponds with the fact that only the use of sacks 15 and 16 for the transport of rice resulted in complaints about mustiness (see Table III).

Chlorophenols form a potential source of chloroanisoles. It has therefore to be concluded that to prove that new jute sacks are fit for transport of food products, not only the concentration of chloroanisoles but also the concentration of chlorophenols has to be determined.

Cork Taint in Wine and Brandy

Chloroanisoles are also responsible for the so-called cork taint in wines. This was proved by Tanner in 1981.[5] The cork taint is observed very soon after bottling of the wine. On the question of the origin of chloroanisoles opinions are divided. Tanner states that this taint is caused by chemical reactions between compounds originating from the cork and chlorine used for bleaching and disinfecting the corks before use.

Other authors, for instance Rigaud et al. claim that chloroanisoles are formed by microbiological conversion of chlorophenols.[6] They assume that cork oaks are treated with preservatives containing pentachlorophenol. To prove this they examined the bark of cork oaks. In all cases the presence of pentachlorophenol and chloro-
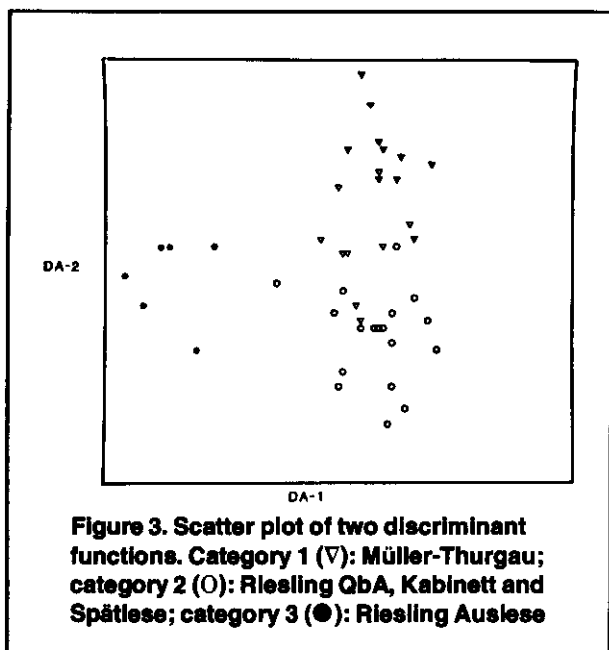
Figure 3. Scatter plot of two discriminant functions. Category 1 (▽): Müller-Thurgau; category 2 (○): Riesling QbA, Kabinett and Spätlese; category 3 (●): Riesling Auslese

anisoles in the bark could be established. The outside was found to contain the highest concentrations of chloroanisoles. One cork oak which had not been treated or barked for at least forty years contained neither chlorophenols nor chloroanisoles.

We support the ideas of Rigaud et al. because in case of chemical reactions one would expect the occurrence of mainly mono-, di- and trichlorophenols and the corresponding anisoles.[6]

According to Heiman et al. the cork taint affects about 2% of the wine in bottles closed with natural corks. If a chemical reaction would be the cause of formation of chloroanisoles, the percentage of off-flavours would have been much higher.[7]

A taint with a different character but also caused by 2,4,6-trichloroanisole originating from the cork was detected in a brandy at our institute. It is remarkable that the off-flavour was not described as musty but as walnut-like and heavy. It was followed by a dry mouthfeel. Even more re-

markable was that the off-flavour was not noticed immediately, but 5-10 minutes and sometimes 20-30 minutes after consumption of the brandy.

Analysis of the brandy showed the presence of only, 2,4,6-trichloroanisole (approximately 1 μg.kg^{-1}). Addition of the same amount of this compound to a brandy of good quality gave the same characteristic flavour and aftertaste.

In connection with this off-flavour problem, a number of cork samples of different quality and corks treated with different concentrations of chlorine used for bleaching were analysed for chloroanisoles. The results showed that almost all corks were contaminated with trichloroanisole and in some cases also with tetrachloroanisole and pentachloroanisole. The presence of these anisoles was in accordance with our expectations, based on the assumption that cork oaks are treated with pentachlorophenol.

Conclusions

To prevent accidental introduction of chlorophenols and anisoles in the future, more should be known about the production of food products and chlorophenols as such should not be used.

It is to be expected that chlorophenols and chloroanisoles will be present in food products in the near future. Therefore odour threshold values in various food products should be determined, and migration of phenols and anisoles from contaminated sources such as packaging materials into food products should be studied.

References

Address correspondence to Dr. Henk Maarse, TNO-CIVO Institutes, General Food Chemistry Dept., P.O. Box 360, 3700 AJ Ziest, The Netherlands

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pentachloroanisole   2,3,4,6-tetrachloroanisole   2,4,6-trichloroanisole

Figure 4. Most Frequently Occurring Chloroanisoles Produced by Fungal Methylation of Pentachlorophenol Fungicides