# Trends in Aroma Research and Gas Chromatography-Olfactometry

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This paper looks at two aspects of aroma research. First, we'll review the last 20 years of research concerning volatile constituents in foods and beverages, particularly certain volatile constituents formed from sugars and amino acids during food processing. Then we'll give a brief overview of recent developments in gas chromatography-olfactometry.

## Volatile Constituents

During the first Weurman symposium in 1975, Rijkens and Boelens[1] discussed the future of aroma research regarding the number of identified volatile constituents in foods and beverages. More than 5,000 such constituents were expected to be present. Twenty years later, our analysis of the identified volatile constituents in foods and beverages[2] reveals that up to 6,600 have been reported so far. These compounds can be grouped into hydrocarbons, oxygen derivatives, nitrogen derivatives and sulfur derivatives.

It is general knowledge that many flavor components are formed from sugars and amino acids during food processing.[3] During this processing, the Maillard reaction occurs, followed by the Heinz rearrangement and/or the Amadori rearrangement, leading finally to the formation of a series of volatile flavor compounds.[5] Some important groups of these flavor compounds are the esters, the disulfides and methylthio derivatives, and the oxazoles and thiazoles. Trends in each of these three groups will be discussed. We'll also discuss trends in natural stereoisomers and in the formation of new volatile nitrogen compounds and sulfur compounds, such as the 1,3,5-dithiazines.

***Esters:*** Volatile esters are important flavor components, especially in fruit flavors. In 1974 Rijkens and Boelens[1] noticed that the number of published volatile esters, 450, was low compared to the 230 acids then known. By 1993 about 1,180 volatile esters had been published, while the number of published acids had increased to 330. The ratio of number of esters to number of acids increased from 2 to 3.3. The number of published volatile alcohols is about 580.[2] So, theoretically, 191,400 (or 330 x 580) esters can exist, based on the number of identified acids and alcohols. With respect to the number of acids and the number of alcohols in a single given fruit, the total number of existing esters could be a factor of 2 higher (about 2,500).

In Table I, the numbers of published esters from acids with even carbon numbers are shown. It is clear from these figures that many more esters, not yet published, exist. In

**Table I. Published esters of acids with even numbers of carbons[2]**

| Series of esters | Published number |
|---|---|
| acetates | 214 |
| butanoates | 58 |
| hexanoates | 48 |
| octanoates | 27 |
| decanoates | 15 |
| dodecanoates | 11 |
| tetradecanoates | 11 |
| hexadecanoates | 10 |

**Table II. Occurrence of alcohols, acids and esters in some fruits**

| Fruit | Alcohols | Acids | Esters published[2] | Esters (calc.) |
|---|---|---|---|---|
| apple | 60 | 43 | 125 | (2,580) |
| banana | 56 | 39 | 84 | (2,184) |
| guava | 66 | 55 | 73 | (3,630) |
| grape | 66 | 42 | 58 | (2,772) |
| papaya | 49 | 60 | 47 | (2,940) |
| raspberry | 42 | 33 | 26 | (1,386) |
| strawberry | 42 | 34 | 117 | (1,428) |

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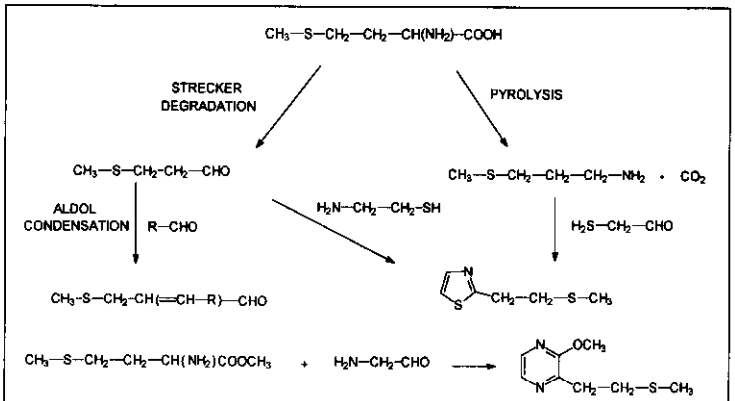


**Figure 1. Formation of methylthio derivatives**

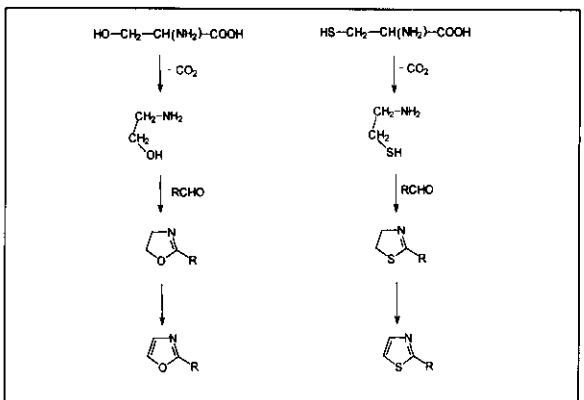


**Figure 2. Formation of 2-alkyloxazoles and 2-alkylthiazoles**

**Table III. Oxazoles and thiazoles found in foods and beverages[2]**

| Oxazoles | | Thiazoles | |
|---|---|---|---|
| **Type** | **Number** | **Type** | **Number** |
| alkyl-substituted | 57 | alkyl-substituted | 69 |
| 2-alkyl-substituted | 2 | 2-alkyl-substituted | 9 |
| benzoxazoles | 3 | benzothiazoles | 11 |
| functionalized | 3 | functionalized | 3 |

**Table IV. Published optically active volatile compounds derived from isoleucine[1]**

| Compound | Number |
|---|---|
| 2-methylbutanol, 2-methylbutanal, 2-methylbutanoic acid | 3 |
| 2-methylbutyl esters | 26 |
| 2-methylbutanoates | 23 |
| 2-methylbutyl ether | 1 |
| (2-methylbutoxy)-acetals | 6 |
| 2-methylbutyl, sec. butyl-pyrroles, pyridines, pyrazines | 13 |

Table II, the numbers of alcohols, acids and esters in some important fruits are shown.

***Disulfides and methylthio derivatives:*** About 10% of all published volatile compounds are sulfur compounds. One important group of these compounds contains the disulfides, of which only 55 have been found.[2] On the basis of the occurrence of 45 thiols, one could theoretically expect 1,035 disulfides based on the expression n(n+1)/2, where n is 45. Although not all 45 thiols occur in any one given food or beverage, many more disulfides will exist.

Another aspect of published volatile sulfur compounds is the frequency of occurrence of the methylthio group in heterocyclic molecules such as pyrazines and thiazoles. For instance, of the 185 pyrazines detected in food, only one contains a methylthio substituent; several more will exist and will be found in the future (Figure 1).

***Oxazoles and thiazoles:*** So far, 63 oxazoles and benzoxazoles, and 83 thiazoles and benzothiazoles have been identified in foods and beverages (Table III).[2] Oxazoles are chemically less stable than thiazoles.

It is surprising that up to now only 2-alkyl-substituted benzothiazoles have been found. 2-Alkylthiazoles (Figure 2) can easily be formed during food processing, especially during frying and roasting, via the corresponding thiazolidines from cysteamine and aldehydes.

***Optical isomers:*** The sensory properties of volatile compounds are largely affected by their stereo structure; that is, by their geometric or optical isomerism. During the last decade, increasing interest in natural optical isomers (enantiomers and chiral compounds) has resulted in a large number of publications. This interest will continue in the forthcoming decade.

Consider, for example, the series of optically active volatile compounds derived from isoleucine by Strecker degradation and secondary reactions. These are compounds such as 2-methylbutanol, 2-methylbutanal, 2-methylbutanoic acid, acetals, esters and bases (Table IV). Up to 1993, the published literature had described about 23 2-methylbutanoates, all with (R)- and (S)-forms having different sensory qualities.

A lot of work has to be done on separating and isolating these natural stereoisomers and on determining their sensory properties.

***Dithiazines:*** At high temperatures, approximating roasting conditions, amino acid pyrolysis is an important reaction and cysteine, for example, is transformed into mercaptoacetaldehyde, acetaldehyde, hydrogen sulfide, cysteamine and ethane-1,2-dithiol (Figure 3), which undergo aldol-type and other condensation reactions

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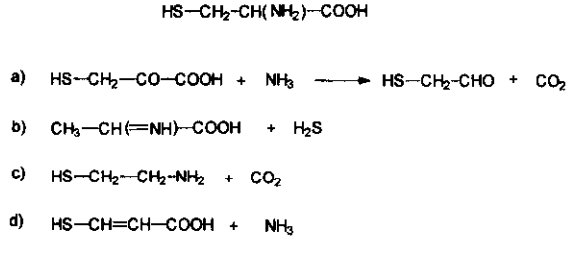


**Figure 3. Degradation of cysteine**

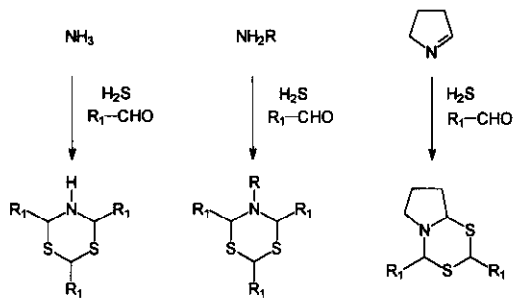


**Figure 4. Formation of 1,3,5-dithiazines**

with amino acids and sugar degradation products.

An important group of compounds formed by these reactions is the alkyl-1,3,5-dithiazines. An interesting example of this group is pyrrolidino[1,2-e]-4H-2,4-dimethyl-1,3,5-dithiazine, a character-impact compound of some shellfish (Figure 4). It can be formed from hydrogen sulfide, ethanal and 1-pyrroline. The threshold value in water of this compound was reported[4] to be at $1 \times 10^{-11}$ ppb. This extremely low figure begs for confirmation. The compound has three asymmetric carbons so eight stereoisomers exist, which are unknown up to now.

## Gas Chromatography-Olfactometry

***Sniffing at GC-columns:*** The name gas chromatography-olfactometry (GCO) is new, but sniffing at the exit ports of GC-columns is as old as GC itself. However, the first detectors used (thermal conductivity detectors) were rather insensitive and non-discriminating compared to the sensitivity of the human nose. Even with modern and more sensitive detectors like FID (flame ionization detectors) it still occurs that the chromatogram does not show a peak where an odor can be smelled at the exit of the column. For instance, recent publications mention odor-active regions of which 50% or more do not coincide with a peak.[6,7] These findings indicate that just paying attention to the "visible" volatile components might be misleading if one wants to find all important odorants.

***New GCO methods:*** Recently, much attention has been given to the development and application of GCO methods. At the latest Weurman symposium a workshop was devoted to GCO.[8] The best known methods are Aroma Extract Dilution Analysis (AEDA), CharmAnalysis and Osme. AEDA and CharmAnalysis, both based on the threshold, are relatively simple sensory procedures in which the assessor indicates only whether or not an odor can be perceived. Numerous injections are required for AEDA and CharmAnalysis in order to reach a dilution of the extract or concentrate in which odors are no longer perceived. The result of AEDA is expressed as the Flavor Dilution (FD) value;[9] the result of the CharmAnalysis is called Charm.[10]

Osme requires the measurement of the intensity of the emerging odor and can be done with one injection. This technique is a time-intensity measurement method. In most cases descriptions of the odor character are also requested. A computer for the registration of the responses is very practical, especially when a large number of GC runs have to be sniffed.

***Results of GCO:*** Application of GCO has resulted in knowledge about important odorants in a large number of products. For instance, a recent review by Grosch[9] lists a number of foods with their potent odorants identified on the basis of AEDA. It is expected that a consistent application of GCO will result in much more information about the important compounds responsible for the odor and flavor of foods and drinks.

***Problems in GCO:*** Little attention has been given to reproducibility within and between assessors.[7,10-12] It is unknown to what degree the sometime single sniffer is representative of a larger population. Application of selected and trained panels seems necessary in order to obtain results that can be analyzed statistically. One of the aims of the selection should be directed at partial anosmia in order to find assessors that have at least a normal sensitivity.[11]

Another point that deserves more interest is the representativeness of the concentrate regarding the odor and/or flavor of the product to be investigated. When performing odor and flavor studies, most researchers probably will have assessed their extracts and concentrates in an informal way, although descriptions of formal checks are rare in the literature.[13,14]

GCO methods are to be considered as screening procedures.[8] They are a first approach to determining the importance of individual compounds for the odor and flavor of a product. The question regarding which methods to apply in order to find the real importance of individual compounds remains unanswered.

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