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## The odour of white bread



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## Abstract

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Volatile constituents of white bread were investigated. Different methods were used for isolating and concentrating components to avoid artefacts as far as possible. Especially good was enlarged vapour analysis. Ninety-four components were identified, including hydrocarbons, alcohols, aldehydes, ketones, acids, esters as well as nitrogen, sulphur and miscellaneous compounds. The concentration of the main components in the vapour above white bread was determined by direct vapour analysis. The odour threshold values of these components in aqueous solution were determined, and the odour values calculated as the ratio of concentration to odour threshold value to estimate their contribution to the total odour. The Maillard reaction of the cysteine/cystine-ribose system was investigated in a search for components which can be expected in heat-processed food products, and to find out whether during this reaction compounds possessing bread-like odours were formed. Forty-five components were identified, including thiophenes, thiazoles, thiols, pyrazines, pyrroles, amines, furans, aldehydes, ketones and miscellaneous compounds. Possible pathways for the formation of 2-acyl thiazoles and of 3-methyl, and 5-methyl substituted 2-formylthiophenes are proposed.

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- E. J. Mulders, H. Maarse & C. Weurman, 1972. The odour of white bread. I. Analysis of volatile constituents in the vapour and aqueous extracts.  
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- E. J. Mulders, M. C. ten Noever de Brauw & S. van Straten, 1973. The odour of white bread. II. Identification of components in pentane-ether extracts.  
Z. Lebensmittelunters. u. -Forsch. 150: 306 - 310.
- E. J. Mulders & J. H. Dhont, 1972. The odour of white bread. III. Identification of volatile carbonyl compounds and fatty acids.  
Z. Lebensmittelunters. u. -Forsch. 150: 228 - 232.
- E. J. Mulders, 1973. The odour of white bread. IV. Quantitative determination of constituents in the vapour and their odour values.  
Z. Lebensmittelunters. u. -Forsch. 151: 310 - 317.
- E. J. Mulders, 1973. Volatile components from the non-enzymic browning reaction of the cysteine/cystine-ribose system.  
Z. Lebensmittelunters. u. -Forsch., in press.

# 1 Introduction

*Research on bread aroma* The odour of white bread depends on its ingredients and the way it is made. The importance of both fermentation and baking process in the formation of odorous constituents has been shown by Baker & Mize (1939, 1941). They established that bread baked from a dough without proper fermentation, as well as bread prepared from a normally fermented dough but baked by a process which prevented crust formation, had an unacceptable odour. During fermentation many compounds such as acids, alcohols, esters and carbonyl compounds are formed, which volatilize in part during baking. The precursors of the ultimate odour of bread, which are involved in the non-enzymic browning or Maillard reaction, are also formed. The characteristics of the Maillard reaction have been described by several investigators, e.g. Hodge (1953, 1967), Hodge et al. (1972), and Reynolds (1963, 1965, 1970).

During the last 20 years the odour of white bread has been the subject of a number of studies (Baker et al., 1953; Croes, 1958; Linko et al., 1962; Ng et al., 1960; Rothe & Thomas, 1963; Salem et al., 1967; Wick et al., 1964; Wiseblatt, 1960; Wiseblatt & Kohn, 1960). Papers on bread aroma have been reviewed by Buré (1965), Johnson et al. (1966) and Coffman (1967). As a result of these studies some 70 compounds have been identified in pre-ferments, dough, oven vapours and bread. Surprisingly, however, a study of these compounds showed that only 36 of them were present in white bread and some of these could be artefacts. A number of the papers on the aroma of white bread include quantitative data (Linko et al., 1962; Ng et al., 1960; Rothe & Thomas, 1963; Wiseblatt, 1960; Wiseblatt & Kohn, 1960). Nevertheless, no correct and complete picture of the odour can as yet be presented.

During the last couple of years studies have been reported in which compounds were mentioned, arising from Maillard reactions on model systems, and possessing a bread-like or cracker-like odour: 1-acetonylpyrroline by Kobayasi & Fujimaki (1965), and possibly the same compound by Morimoto & Johnson (1966); 2-acetyl-1-methylpyrrolidine and 1-azabicyclo[3.3.0]octan-4-one by Hunter et al. (1966); and 2-acetyl-1,4,5,6-tetrahydropyridine by Hunter et al. (1969). However, none of these compounds was positively identified in white bread.

*Purpose of the study* As has already been mentioned, our knowledge of the aroma of white bread, one of our major foodstuffs, is rather small. Moreover, it has been suggested that the pleasant and specific odour of fresh baked bread is gradually losing some of its character, possibly by changes in the procedure of breadmaking.

These considerations formed the reasons for an investigation<sup>1</sup> on the composition of the volatile components in regular, fresh white bread and of its odorous vapours.

The Maillard reaction of the cysteine/cystine-ribose system was studied in a search for components possessing bread-like odours, and, generally, to investigate which volatile components can be expected in heat-processed food products containing these substances. A sulphur-containing amino acid was chosen, because in the literature a sulphur compound has been described as possessing a strong bread-like odour (Tonsbeek et al., 1971), and because sulphur compounds in general are very potent flavouring constituents. Ribose was chosen because of its high reactivity in browning reactions.

1. This study was carried out in the Aroma Department of the Central Institute for Nutrition and Food Research TNO, Zeist, The Netherlands. The investigation was supported in part by the Netherlands Association of Flour Millers, The Hague, The Netherlands.

The author thanks all who have contributed to the realization of this thesis.

## 2 Materials and methods

*Breadmaking* The white bread was prepared at the Institute for Cereals, Flour and Bread TNO, Wageningen, Netherlands. A rigorously standardized manual procedure was used which guaranteed optimum aroma development on the basis of a recipe, in which only those ingredients essential to proper baking were included (Mulders et al., 1972).

*Qualitative analysis of the volatile components of white bread* Several methods of isolation and identification of the volatile constituents of white bread were applied under conditions of minimum artefact formation.

1. A vapour enrichment technique was developed, enabling direct identification of the main components in vapour samples of up to 1 l by combined gas chromatography - mass spectrometry and measurement of their Kovats indices (Mulders et al., 1972).
2. Volatile components were stripped under vacuum from an aqueous slurry of white bread, then the distillate was concentrated by freezing and the concentrate was extracted with a mixture of pentane and ether; components were identified by mass spectrometry and measurement of their Kovats indices (Mulders et al., 1972).
3. Volatiles were isolated by pentane-ether extraction from powdered bread and identified by mass and infrared spectrometry and measurement of their Kovats indices (Mulders et al., 1973).
4. Carbonyl compounds and carboxylic acids were extracted from powdered bread by low boiling solvents and isolated by formation of derivatives. Carbonyl compounds were identified by thin-layer chromatography of their 2,4-dinitrophenylhydrazones, and acids by gas chromatography after regeneration (Mulders & Dhont, 1972).

*Quantitative analysis of constituents in the vapour of white bread and their odour values* The quantitative composition of the main components in the vapour above white bread was determined by gas chromatography. On the basis of the data obtained, a synthetic mixture in water was prepared in such a manner that the chromatogram of its vapour was identical to the average chromatogram of the vapour of white bread. The odour threshold values of the bread components were determined, and the odour values calculated as the ratio of the concentration of the component to its odour threshold value (Mulders, 1973a).

*Qualitative analysis of the browning system* Volatile constituents of the cysteine/cystine-ribose browning system were analysed by gas and thin-layer chromatography. The reaction conditions were as follows: 0.01 mol L-cysteine, 0.01 mol L-cystine, 0.025 mol D-ribose and 35 ml phosphate buffer solution, pH 5.6, in 200 ml diethylene glycol. This mixture was refluxed for 24 hours at 125 °C. Components were identified by mass, infrared, and nuclear magnetic resonance spectrometry (n.m.r.), and measurement of their Kovats indices. Vapours above the reaction mixture were partitioned by gas chromatography and detected by flame photometry for the study of volatile sulphur compounds (Mulders, 1973b).



### 3 Results

The results of the qualitative analysis of the odour of white bread are given in Table 1. The components mentioned in this table were identified in the following ways: 15 components in the vapour above white bread and 12 in the aqueous extract (Mulders et al., 1972); 52 components in the pentane-ether extract (Mulders et al., 1973); 24 carbonyl compounds and 13 carboxylic acids isolated from extracts by formation of derivatives (Mulders & Dhont, 1972). Some of these compounds were identified by several methods.

Table 2 shows the results of the gas chromatographic determination of the composition of the compounds in the vapour above white bread (Mulders, 1973a).

The composition of the synthetic mixture, the concentration in the vapour, the odour threshold values and odour values for a number of the bread components are listed in Table 3 (Mulders, 1973a).

The components identified in the reaction mixture of the cysteine/cystine-ribose system are summarized in Table 4 (Mulders, 1973b).



Table 1, continued.

<i>Sulphur compounds</i>	<i>Miscellaneous compounds</i>
Dimethyl sulphide (4)	Furan (4)
Dimethyl disulphide (4)	2-Methylfuran (4)
3-Acetylthiophene (5)	a Pentylfuran <sup>a</sup> (5)
	Ethyl furfuryl ether (4, 5)
	1,1-Diethoxyethane (4)
a. Tentative.	
1. Baker et al., 1953.	7. Ng et al., 1960.
2. Croes, 1958.	8. Rothe & Thomas, 1963.
3. Linko et al., 1962.	9. Salem et al., 1967.
4. Mulders et al., 1972.	10. Wick et al., 1964.
5. Mulders et al., 1973.	11. Wiseblatt, 1960.
6. Mulders & Dhont, 1972.	12. Wiseblatt & Kohn, 1960.

Table 2. Composition of the vapour above white bread by g.l.c.-analysis on the LAC-1-R-296 column of samples of 6½ ml (Mulders, 1973a).

Peak no.	Component	Average of retention time × peak height	Standard deviation in %	Relative amount in %
4	Acetaldehyde	327	45	2.4
	Dimethyl sulphide			
5	Furan	68	37	0.5
6	Ethyl formate	288	32	2.1
	2-Methylpropanal			
7	2-Propanone	52	94	0.4
8	2-Methylfuran	65	40	0.5
9	Ethanol	137100	12	988
	Ethyl acetate			
	1,1-Diethoxyethane			
10	1-Propanol	175	23	1.3
	2,3-Butanedione			
12	2-Methyl-1-propanol	339	28	2.4
	Dimethyl disulphide			
13	Unknown	28	21	0.2
14	3-Methyl-1-butanol	377	25	2.7

Table 3. Composition, odour threshold values and odour values of components of the synthetic mixture (Mulders, 1973a).

Component	Concentration in synthetic mixture in ppm (v/v)	Odour threshold value in water in ppm (v/v)	Odour value	Concentra- tion in the vapour in µg/l	Relative amount in the vapour in %
Ethanol	10 <sup>4</sup>	900	11.1	1970	981
1-Propanol	9	40	0.2	2.2	1.1
2-Methyl-1-propanol	7	3.2	2.2	2.5	1.25
3-Methyl-1-butanol	7.5	0.77	9.7	2.5	1.25
Acetaldehyde	6.8	0.12	57	18	9
2-Methylpropanal	0.1	0.01	10	1.5	0.75
3-Methylbutanal	0.02	0.007	2.9	0.1	0.05
2-Propanone	1.4	300	≪1	2.8	1.4
2,3-Butanedione	0.005	0.0065	0.8	0.2	0.1
Ethyl formate	0.6	17	≪1	4	2
Ethyl acetate	0.25	6.2	≪1	1.7	0.85
1,1-Diethoxyethane	0.15	0.042	3.6	0.7	0.35
Furan	0.016	4.5	≪1	0.8	0.4
2-Methylfuran	0.013	3.5	≪1	0.7	0.35
Dimethyl sulphide	0.005	0.001	5	0.2	0.1
Dimethyl disulphide	0.0025	0.00016	15.6	0.2	0.1
Total mixture	10032.8615	80	125.4 <sup>a</sup> 118.1 <sup>b</sup>		

a. Determined.

b. Summarized.

Table 4. Volatile components identified in the cysteine/cystine-ribose browning system (Mulders, 1973b).

<i>Alcohols</i>	2-Acetyl-3-methylpyrazine <sup>b</sup>
Furfuryl alcohol	
<i>Aldehydes</i>	<i>Sulphur compounds</i>
Acetaldehyde	Hydrogen sulphide
2-Furaldehyde	Carbon disulphide
	Methanethiol
	Ethanethiol
<i>Ketones</i>	1-Propanethiol
2-Acetylfuran	2-Formylthiophene
1(2-Furyl)-2-propanone	2-Formyl-3-methylthiophene <sup>b</sup>
	2-Formyl-5-methylthiophene
<i>Nitrogen compounds</i>	3-Acetylthiophene
Methylamine	2-Acetyl-5-methylthiophene
Propylamine	3-Acetyl-2-methylthiophene <sup>b</sup>
n- or iso-Pentylamine	Thieno [3,2- <i>b</i> ] thiophene <sup>b</sup>
Pyrrole	Thieno [3,4- <i>b</i> ] thiophene <sup>a,b</sup>
2-Formyl-1-methylpyrrole	2-Methylthiazole <sup>b</sup>
2-Acetylpyrrole	5-Ethylthiazole <sup>b</sup>
1-Furfurylpyrrole	5-Ethyl-2-methylthiazole <sup>b</sup>
2-Methylpyridine	2,4-Dimethyl-5-ethylthiazole <sup>b</sup>
2-Methyl-5-ethylpyridine <sup>b</sup>	2,4,5-Trimethylthiazole <sup>b</sup>
2-Methylpyrazine	2-Acetylthiazole <sup>b</sup>
2,5-Dimethylpyrazine	5-Acetyl-2-methylthiazole <sup>b</sup>
2-Ethyl-6-methylpyrazine	1(2-Thiazolyl)-1-propanone <sup>b</sup>
2-Propylpyrazine	1(2-Thiazolyl)-1-butanone <sup>b</sup>
2-Allylpyrazine <sup>b</sup>	2-(2-Furyl)thiazole <sup>b</sup>
trans-2-Propenylpyrazine <sup>b</sup>	3-Methyl-1,2,4-trithiane <sup>a,b</sup>
2,6-Diethyl-3-methylpyrazine	
6,7-Dihydro-5 <i>H</i> -cyclopentapyrazine <sup>a</sup>	<i>Miscellaneous compounds</i>
2-Methyl-6,7-dihydro-5 <i>H</i> -cyclopentapyrazine <sup>a</sup>	trans-1,2-(2,2'-Difuryl)ethene <sup>a,b</sup>
2-Acetylpyrazine	

a. Tentative.

b. Compound has not yet been reported to occur in food products (Straten & Vrijer, 1973).

## 4 Discussion

*Qualitative analysis of the volatile components of white bread* In this investigation 94 different volatile components were identified, 5 of which tentatively (Table 1).

These include:

2 hydrocarbons	9 esters
14 alcohols	9 nitrogen compounds
23 aldehydes	3 sulphur compounds
16 ketones	5 miscellaneous compounds
13 acids	

Of these, 65 components have not been previously reported to occur in white bread; new types of components are sulphur compounds, pyrazines and pyrroles, while the number of furans is considerably increased.

It was shown that none of the components identified, originated from other sources than the product studied. The extracts as well as the total condensates of gas chromatographic effluents were regularly subjected to organoleptic evaluation. The extracts were used for further analysis only when the odour was very reminiscent of that of bread.

Different methods were used for isolating and concentrating components, to avoid artefacts as far as possible. Especially good was enlarged vapour analysis. In this method neither distillation nor extraction procedures were applied, and the only danger of artefact formation was that which is inevitably associated with gas chromatography.

Many of the components identified originate from the Maillard reaction between sugars and amino acids. For the formation of pyrazines a mechanism was proposed by Wang et al. (1969) and Rizzi (1972). The Strecker degradation of  $\alpha$ -amino acids leads to the formation of aldehydes of one carbon atom less (Schönberg & Moubacher, 1952). Furans arise, at least in part, from carbohydrate degradation reactions (Hodge, 1967; Fagerson, 1969). Although sulphur compounds are also formed biochemically during fermentation, most of them volatilize during baking. The main source of such compounds in bread, therefore, has to be sought in sulphur-containing amino acids, which degrade during the heating process (Schwimmer & Friedman, 1972).

*Quantitative analysis of the vapour of white bread* For several reasons direct vapour analysis, as opposed to distillation or extraction procedures, is very suitable in odour studies for the determination of quantities and ratios of the main components

in the odorous vapours. Non-volatile material does not interfere with the analyses, and the possibility of artefacts is minimized. Erroneous information due to changes in quantities of components as a consequence of extraction or concentration procedures is avoided.

In spite of the rigorous standardization for the procedure of breadmaking, considerable quantitative differences in vapour composition were observed between individual loaves (Table 2). The synthetic aqueous solution, which was prepared to show the same gas chromatogram as the vapour of bread, had an odour which scarcely resembled that of white bread, but reminded one more of dough. Since all loaves had the same distinct odour, it seems appropriate to conclude that neither the components detected in direct vapour analysis, nor their ratios are characteristic for the odour of white bread. However, they must form the basis of the aroma of bread, although they have to be completed with other components from Table 1. No more information about the quantities of these components by direct vapour analysis could at present be obtained.

*Odour values* From the odour values of the bread components (Table 3), it is expected that acetaldehyde, dimethyl disulphide, ethanol, 2-methylpropanal, 3-methyl-1-butanol, dimethyl sulphide, 1,1-diethoxyethane, 3-methylbutanal and 2-methyl-1-propanol contribute considerably to the odour of the mixture, and must, therefore, also contribute to the odour of white bread. The sum of the odour values of the individual components is 118.1, accounting for about 94% of the determined odour value of the whole mixture. Consequently it is not likely that suppression or synergism effects between components of the mixture is significant.

*Qualitative analysis of the browning system* In the cysteine/cystine-ribose browning system and in its vapour 50 components were identified, 5 of which tentatively (Table 4).

These include:

- |             |                          |
|-------------|--------------------------|
| 1 alcohol   | 20 nitrogen compounds    |
| 2 aldehydes | 24 sulphur compounds     |
| 2 ketones   | 1 miscellaneous compound |

In chromatograms of blank samples subjected to the reflux and extraction procedure, no significant peaks were observed other than the solvent peaks and the diethylene glycol peak.

Among the sulphur compounds identified 8 were thiophenes, 10 thiazoles and 3 alkyl thiols. Among the nitrogen compounds 11 were pyrazines, 4 pyrroles, 2 pyridines and 3 alkyl amines. As far as known (Straten & Vrijer, 1973) 20 of the components identified in this study have not yet been reported to occur in the aroma of any food product. These compounds will probably be found in future investigations of heat-processed food products containing cysteine or cystine and ribose. Possibly the same or similar compounds will be found in products containing other sugars than ribose.

The odour of 2-acetylthiazole was described as cereal, biscuit and cracker-like, and that of 2-propionylthiazole (or 1(2-thiazolyl)-1-propanone) as burnt bread crust, cracker-like and wet biscuit. In our studies on the odour of white bread, however, these compounds have not yet been identified.

The pathways by which 2-acyl thiazoles might be formed have not yet been elucidated, but a possible reaction mechanism is proposed (Mulders, 1973b). Possible routes of formation for 2-formyl-5-methylthiophene and 2-formyl-3-methylthiophene, respectively, also identified in this study, are given (Mulders, 1973b).



## Summary

In this investigation the volatile constituents of white bread were studied. Different methods were used for isolating and concentrating components to avoid artefacts as far as possible. Especially good was enlarged vapour analysis. Components were identified by combined gas chromatography - mass spectrometry, infrared spectrometry and measurement of Kovats indices. Some carbonyl compounds were identified by thin-layer chromatography of their 2,4-dinitrophenylhydrazones.

In vapour and extracts of white bread, 94 components were identified including hydrocarbons, alcohols, aldehydes, ketones, acids, esters, as well as nitrogen, sulphur and miscellaneous compounds. Of these, 65 components have not been previously reported to occur in white bread; especially sulphur compounds, pyrazines, and pyrroles had not yet been found; many more furans were identified.

The concentration of the main components in the odour of white bread was determined by direct vapour analysis. Considerable quantitative differences in vapour composition were observed between individual loaves, despite rigorous standardization for breadmaking; their odours, however, were indistinguishable. An aqueous synthetic mixture, prepared so that the chromatogram of its vapour was identical to the average chromatogram of bread vapour, had an odour which scarcely resembled that of bread, but was rather dough-like. Therefore, the components detected in normal vapour samples cannot account for the characteristic odour of fresh white bread. Their odour threshold values were determined. From the odour values, calculated as the ratio of concentration to odour threshold value, the contribution of these compounds to the total odour could be estimated.

The Maillard reaction of the cysteine/cystine-ribose system was studied in a search for components which might occur in heat-processed food products in general, when previous to heating, the ingredients contained these amino acids and this sugar. A second major objective was to investigate whether compounds with bread-like odours were formed. The reaction mixture was analysed by g.l.c. and t.l.c.; highly volatile sulphur compounds were partitioned by gas chromatography and detected by flame photometry.

By mass, infrared and n.m.r. spectrometry, and measurement of Kovats indices, 50 components were identified including thiophenes, thiazoles, thiols, pyrazines, pyrroles, amines, furans, aldehydes, ketones and miscellaneous compounds. Two acyl thiazoles found in the reaction mixture, had a bread-like odour. However, they have not yet been identified in white bread. Possible pathways for the formation of 2-acyl thiazoles, and of 3-methyl, and 5-methyl substituted 2-formyl-thiophenes are proposed.

## Samenvatting

Een onderzoek werd verricht naar de samenstelling van de vluchtige verbindingen in wittebrood. Voor de isolatie en concentratie van deze verbindingen werden verschillende methoden toegepast, waarvoor de mogelijkheid van artefactvorming verwaarloosbaar of althans sterk gereduceerd is; in het bijzonder kan de analyse van grote dampmonsters genoemd worden. De verbindingen werden geïdentificeerd door middel van gecombineerde gaschromatografie-massaspectrometrie, infrarood spectro-metrie en meting van de Kovats indices. Vele carbonylverbindingen werden geïdentificeerd met behulp van dunnelaagchromatografie van hun 2,4-dinitrofenylhydrazonen.

In de damp en in extracten van wittebrood werden 94 verbindingen geïdentificeerd. Hiertoe behoren koolwaterstoffen, alcoholen, aldehyden, ketonen, zuren, esters, stikstofverbindingen, zwavelverbindingen en verbindingen van andere aard. In de literatuur over wittebrood werden 65 van deze verbindingen nog niet eerder vermeld; met name zwavelverbindingen, pyrazinen en pyrrolen werden nog niet aangetoond, terwijl het aantal geïdentificeerde furanen belangrijk is toegenomen.

De hoeveelheid van de in kwantitatieve zin voornaamste verbindingen in de damp van wittebrood werd door middel van directe dampanalyse bepaald. Ondanks dat de methode van broodbereiding rigoureuus werd gestandaardiseerd, werden aanmerkelijke kwantitatieve verschillen in de damp-samenstelling tussen afzonderlijke broden waargenomen; de geur was evenwel gelijk. Een waterig synthetisch mengsel, op een zodanige wijze bereid dat het chromatogram van de damp gelijk was aan het gemiddelde chromatogram van de damp van brood, had een geur die nauwelijks aan die van brood deed denken, maar veeleer deegachtig was. De verbindingen, gedetecteerd in een normaal dampmonster, kunnen derhalve niet verantwoordelijk zijn voor de karakteristieke geur van vers wittebrood. De geurdrempelwaarden van deze brood-componenten werden bepaald. Met behulp van de geurwaarden, berekend als het quotiënt van de concentratie en de geurdrempelwaarde, kon de bijdrage van deze verbindingen aan de totale geur geschat worden.

De Maillardreactie van het cysteine/cystine-ribose systeem werd onderzocht teneinde na te gaan welke verbindingen in het algemeen verwacht kunnen worden in voedingsmiddelen die deze stoffen bevatten, en tijdens de bewerking verhit zijn. Een tweede, belangrijk, doel was na te gaan of er verbindingen met broodachtige geuren gevormd worden. De analyse van het reactiemengsel werd door middel van gas- en dunnelaagchromatografie uitgevoerd; de zeer vluchtige zwavelverbindingen werden gaschromatografisch met behulp van een vlamfotometerdetector onderzocht.

Er werden 45 verbindingen met behulp van massa-, infrarood- en n.m.r. spectro-

metrie en bepaling van de Kovats indices geïdentificeerd. Hiertoe behoren thiofenen, thiazolen, thiolen, pyrazinen, pyrrolen, aminen, furanen, aldehyden, ketonen en verbindingen van andere aard. Twee acylthiazolen hadden een broodachtige geur; in wittebrood werden zij echter nog niet aangetroffen. Mogelijke vormingswijzen van 2-acylthiazolen en van 3-methyl en 5-methyl gesubstitueerde 2-formylthiofenen zijn aangegeven.

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