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VOLATILE COMPOUNDS IN CEREALS: SEPARATION AND CONDENSATION FOR CHROMATOGRAPHIC ANALYSIS AND DESCRIPTION OF THEIR AROMATIC CHARACTERISTICS

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Key words: distillation-extraction, gas chromatography, aromatic characterization

The distillation-extraction method was used to separate and condense volatile compounds from standard solutions and from cereal products. Condensed extracts of cereal volatiles were separated in gas chromatograph columns. Subsequently, the eluate was subjected to aromatic characterization.

INTRODUCTION

So far volatile compounds in cereals and their products were analyzed by means of the "head space" technique as well as by distillation and extraction methods.

Hougen et al. [4] used the "head space" technique to study odours of cereals. In order to increase volatile compounds vapour pressure over the product they heated a grain sample in sealed bottles for 2 hrs up to 120°C. Mulders et al. [13, 14] also applied the "head space" technique to investigate volatiles of bread. A sample of bread was heated up for 45 min at 36°C.

Direct analyses of vapours with that technique is not perfect since some chemical compounds have very low concentrations in vapours and are not always detected by gas chromatography. On the other hand, these concentrations may be sufficient for detection during sensory analyses. Consequently, the "head space" technique is mostly used to analyze one or more chemical compounds occurring in the highest concentrations and only in some food products [10]. Gas chromatography analysis is possible only with condensed vapours of examined products.

Presently more and more often condensation of vapours is carried out by adsorption of volatile compounds on the surface of Chromosorb, Teenax, Porapak or glass beads [13, 16, 21]. Most frequently, however, separation and condensation of foodstuff volatiles is performed with distillation methods combined with winterizing of vapours at 0° to minus 196°C [3, 7, 10, 12, 22, 25, 26, 27, 28].

Vapours winterized at -40, -80, -196°C are extracted with organic solvents: ethyl ether, pentane, hexane, methylene chloride. It is done in special apparatus for extraction. Resulting extracts are condensed by evaporation of excess solvent.

Sydow and Anjou [22] distilled rye-crisps volatiles with steam. The distillate was extracted with ethyl ether. After drying the extract was evaporated to the final volume of 0.1 cc and then analyzed in gas chromatography and mass spectrography. Wick et al. [25] distilled volatiles from white bread at 14-28 Hg pressure, cooling distillation receivers to -80°C and -196°C. The volatiles in distillates were extracted with ethyl ether, which was subsequently evaporated by fractionated distillation, and the resultant concentrate was analyzed with gas chromatography. Folkes and Gramshaw [3] extracted volatiles of bread crust with ethyl ether. Acids were removed from extracts, solids filtered, ether evaporated, and volatile compounds distilled from the rest in a closed system at 0.01 Hg pressure.

Mulders et at. [13, 15] extracted bread volatiles with water. Water extract was condensed 10 times by distillation in 32°C at 30 mm Hg pressure. Excess water was winterized and the resultant concentrate extracted with a mixture of pentane and ether (2:1). The extract was dried, condensed and analyzed with gas chromatography and mass spectrography.

The fundamental positive feature of separating volatile compounds by distillation and extraction is the rendering of a concentrate of volatiles in condensation sufficient for gas chromatography and mass spectrography analyses.

It follows from the above review of the literature that there is a great variety of methods of separation and condensation of volatile compounds in foodstuffs, including cereal products.

The aim of the present study was to check the effectiveness of distillation and extraction in separation and condensation of volatile compounds from cereal grain, malt, bread, and for subsequent gas chromatography analysis.

MATERIALS AND METHODS

MATERIALS

Raw materials:

— Dankowskie złote (golden) rye variety; crop 1976,
— roasted rye malt (initial roasting temp. 135°C, final — 210°C; time — 60 min.)
— whole-meal rye bread (Experimental Bakery, Poznań AU).

Reagents:

The following standards of volatile compounds were used in model studies:
5 ketones (Koch-Light (boiling p. 63.5°-246°C))
5 esters (Merck (b.p. 110°-240°C))
6 alcohols (Poly Science (b.p. 97°-215°C))

In each category of the investigated standards equal volumes of mixture were prepared. They were diluted with ethyl ether-pentane (1:1) to 0.04% v/v concentration of each component.

SOLUTION OF INTERNAL STANDARDS

The model study chromatographic analysis was made with internal standards: hexanone-3 and isoamyl caprylate in pentane-ethyl ether (1:1) solution — 40% of each component.

METHODS

SEPARATION AND CONDENSATION OF VOLATILE COMPOUNDS

Volatile compounds of the investigated materials were separated and condensed in a glass apparatus constructed after Linkens and Nickerson [9].

Flask 5 was filled with 500 g of crushed product or with 5 cc mixture of standards and 2 litres of freshly distilled bi-distilled water. Flask 1 was filled with 40 cc mixture of 1:1 mixture of pentane and ethyl ether; U-tube — with water up to the height of the right-hand section. The flask with solvents was kept at 45°C. The coolant was the "Borygo" fluid (-8°C). Distillation and simultaneous extraction time — 2 hrs.

When distillation and extraction were over the extract was dried with anhydrous sodium sulphate. It was then condensed in a nitrogen stream to final volume of 250 µl.

This condensate of extracts was subjected to gas chromatography analysis. When extracts of standard mixtures were analyzed 10 µl solu-

tion of internal standard was added prior to chromatography, and recovery of volatile compounds was determined. Condensation of 2 litres of water solution with each standard concentration of 0.002% was carried out. After completion of distillation and extraction as well as condensation, 250 µl of extract were obtained. Concentration of particular standards was about 1% v/v.

Purity of the solvents and bi-distilled water was blind-tested. Chromatographic analysis of the test extract showed no peaks deriving from impurities.

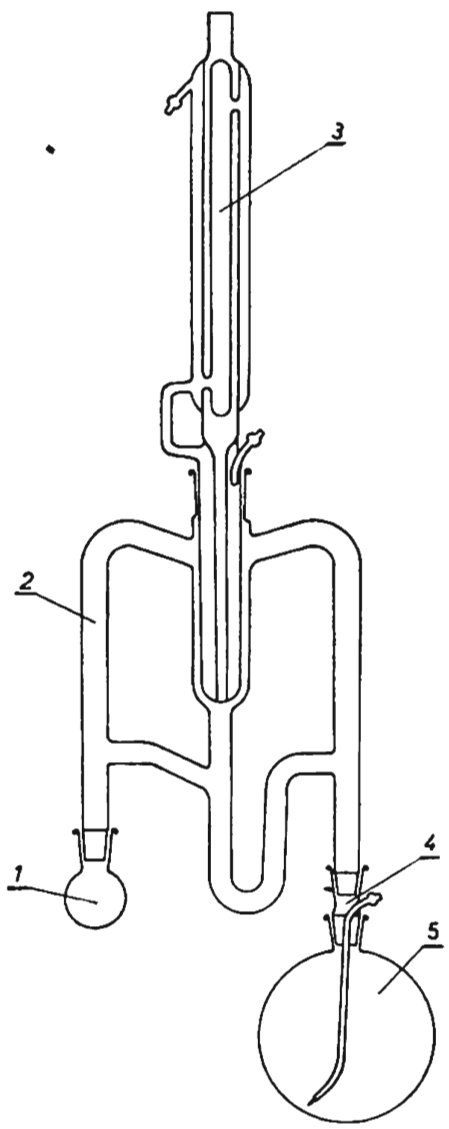

Fig. 1. Block diagram of the apparatus for isolating volatile compounds by distillation and extraction; 1 — solvent flask; 2 — distillation-extraction apparatus; 3 — double-jacketed cooler with cold finger; 4 — adapter with steam tube; 5 — product flask

FRACTIONATION OF THE CONDENSED VOLATILE COMPOUNDS

The whole-meal rye bread volatiles extract obtained by distillation and extraction was separated into basic, acidic, phenolic and neutral fractions, according to the diagram in Fig. 2. The received fractions were condensed to final volume of 100 µl in an apparatus after Kamiński et al. [5].

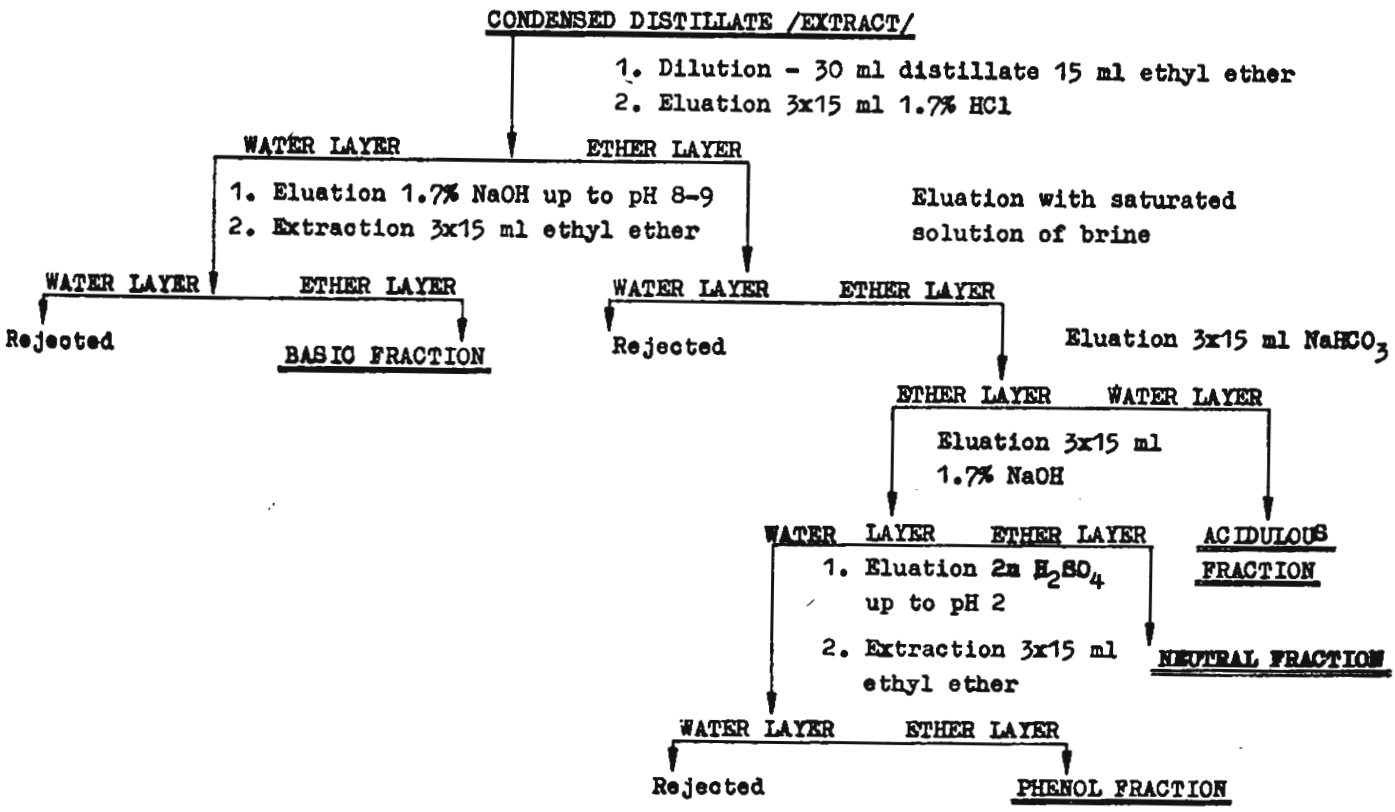

Fig. 2. Diagram of condensed distillate fractionation

CHROMATOGRAPHIC ANALYSIS AND AROMATIC CHARACTERISTICS

The chromatographic separation of standard mixtures and condensed volatile compounds of the investigated products was done with a gas chromatographs Finnigan 9610 and W. Giede 18.3 with flame ionization detectors.

Specifications of the chromatographic analysis are given in Table 1. At the column outlet, with detector flame extinguished, odours of successive eluate fractions were assessed.

Table 1. Operating parameters for the gas liquid chromatographs

Col. No	Column	Column temperature	Carrier gas flow rate
1.	2 m × 2 mm i.d. glass with Carbowax 20 M on Chromosorb P (1)	programmed from 60°C to 180°C at 6°C/ min	N_2, 20 cm^3/min
2.	3 m × 3 mm i.d. glass with 15% Carbowax 20 M TPA on Chromosorb w AW	programmed from 100°C to 210°C at 3°C/min	N_2, 20 cm^3/min
3.	50 m × 0.3 mm i.d. coated with SE-52	programmed from 70°C to 220°C at 2°C/min	He, 2 cm^3/min

COMPUTATION OF RESULTS

Concentration of volatile compound standards in the condensed extract was computed on the basis of peak heights with the use of the equation given by Shelley et al. [20].

RESULTS AND DISCUSSION

SEPARATION AND CONDENSATION OF VOLATILE COMPOUND STANDARDS FROM WATER SOLUTIONS

Recovery of volatile compounds by distillation and extraction for three groups of standards were investigated: six alcohols (boilling points from 97°C to 215°C); five esters (b.p. from 110°C to 240°C; five ketones (b.p. from 63.5°C to 246°C).

Table 2 presents exact data on recovery of the investigated standards after their condensation by distillation and extraction. For alcohols the range was 77.8 to 86.4%; for esters — 59.3 to 86.9%, and for ketones — 70.9 to 92.6%. Good repeatability (recurrence) of recovery results were obtained with all of the investigated standards.

Table 2. Recovery of volatile standards by distillation extraction method (recevery in ratio of the initial volume of standard)

No.	Compound	Recovery; %	Multiplication of condensation
	Alcohols:		
1	Propyl (b.p. 97°C)	77.8±4.96*)	6200
2	Butyl (b.p. 118°C)	84.5±4.27	6700
3	Amyl (b.p. 137°C)	86.4±3.59	6900
4	Hexyl (b.p. 157°C)	77.9±2.96	6200
5	Octyl (b.p. 194°C)	84.2±3.79	6700
6	Nonyl (b.p. 215°C)	81.5±3.39	6500
	Esters:		
7	Ethyl isobutyrate (b.p. 110°C)	86.9±4.67	6900
8	Ethyl metacrylate (b.p. 118°C)	82.9±3.39	6600
9	Ethyl capreate (b.p. 166°C)	61.4±1.52	4900
10	Ethyl caprilate (b.p. 207°C)	89.5±1.13	7100
11	Diethyl adipate (b.p. 245°C)	59.3±1.42	4700
	Ketones		
12	Ethyl-propyl (b.p. 63.5°C)	70.9±2.14	5600
13	Ethyl-amyl (b.p. 107°C)	82.5±3.21	6600
14	Methyl-tertbutyl (b.p. 169°C)	90.3±2.35	7260
15	Butyl-heptyl (b.p. 195.5°C)	92.6±2.80	7400
16	Methyl-decyl (b.p. 246°C)	86.8±3.04	0900

*) arithmetic mean ± standard deviation calculated for 12 results

The positive aspect of the method described above is a possibility to separate volatile compounds of high boiling point and attain good level of recovery (e.g., for methyldecyl ketone (b.p. 246°C) the recovery was 86.8%).

CHROMATOGRAPHIC SEPARATION AND AROMATIC CHARACTERIZATION OF VOLATILE COMPOUNDS FROM CEREAL PRODUCTS

Distillation and extraction were used to separate and condense volatile compounds of rye, roasted rye malt and wholemeal rye bread.

All resultant extracts revealed concentrated aromas of the initial raw materials. Fig. 3 presents a chromatogram of separated rye volatiles. An olfactory assessment of the column eluate proved that the most frequently recurring odour was that of green plants corresponding to peaks Nos. 3, 4, 5, 24, 25; fruity — Nos. 10, 11, 16, 35, 36; flowery — 20, 23; hay-like — 31 and 42 (Table 3). In this concentrate there were some detected compounds of non-typical odours such as stale — peak No. 17, oily — peaks No. 18 and 29, and mushroom-like — peaks No. 14 and 33.

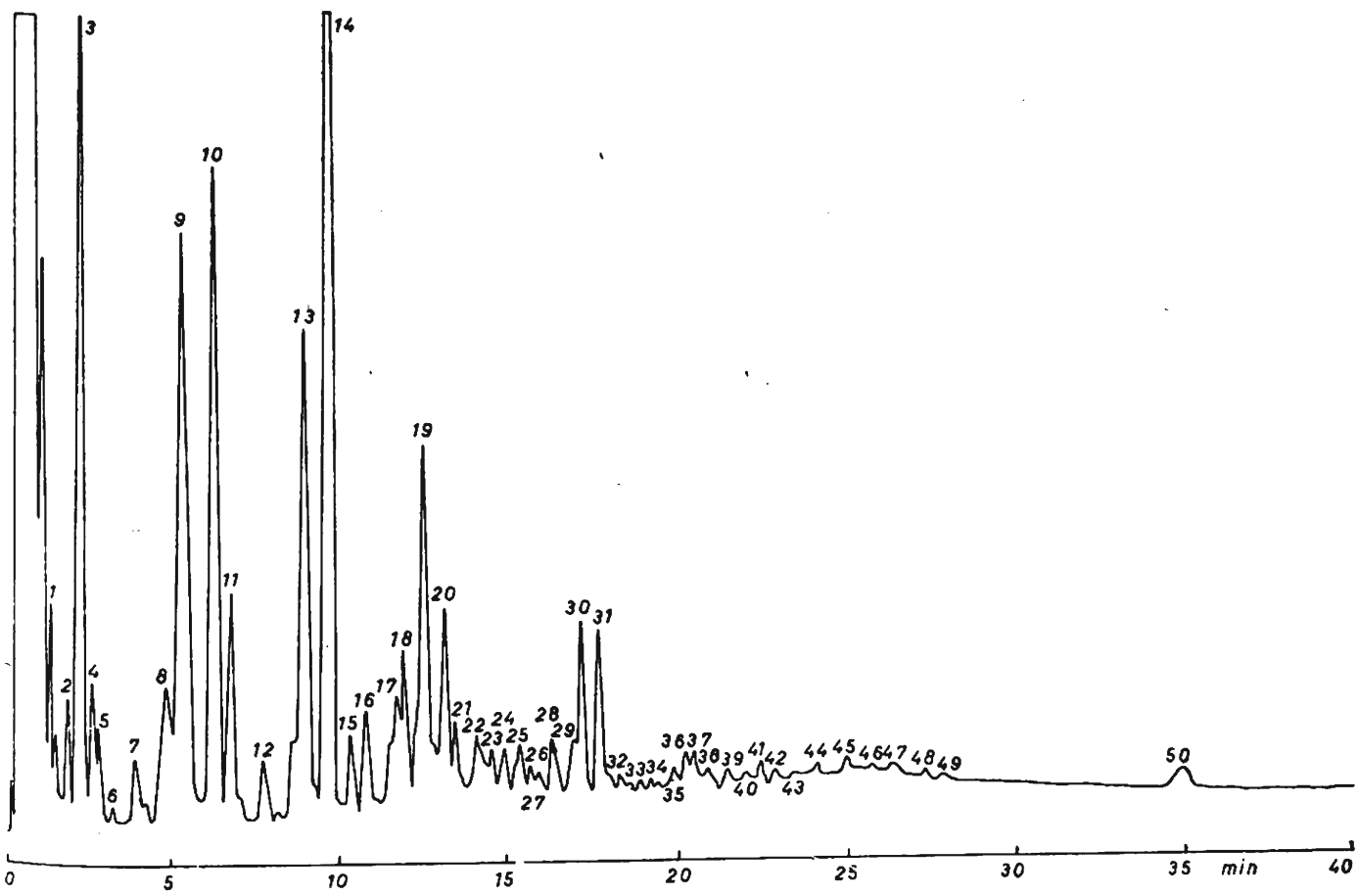

Fig. 3. Chromatogram of volatile compounds separated from rye grain. Column (2 m) filled with Carbowax 20 M on Chromosorb P

The roasted rye-malt chromatogram of its volatiles is given in Fig. 4. There were 51 peaks corresponding to a green plant aroma (peaks: 10, 11, 12, 13, 19, 24, 26) and the smell of roasted cereals (peaks: 2, 3, 30, 33, 34, 40). Non-typical odours were also detected: peak No. 8 — rancid oil, peak No. 23 — stale.

Table 3. Aromatic characteristics of rye grain and rye bread volatiles in eluate from chromatograph column

Rye grain		Rye bread	
peak No. (Fig. 3)	aroma	peak No. (Fig. 5)	aroma
1	cream	1	fatty
3	vegetable (weak)	2	fruity
4	vegetable	5	hay-like
5	vegetable (weak)	7	fruity
6	sweet (weak)	8	fruity
7	sweet	9	cocoa-like
8	burnt-like	10	fatty
9	burnt rubber	11	fatty
10	fruity	13	fruity
11	fruity	14	acetic acid-like
12	sweet	15	potato-like
13	dried potatoes	16	fried potatoes
14	mushroom-like	17	mushroom-like
15	burnt	19	fried potatoes
16	fruity	21	flowery-herbal
17	musty	22	potato-like
18	oily	23	oily
19	nutty	25	bread-like
20	flowery	26	flowery
21	etheric	27	flowery
23	flowery	28	fruity
24	vegetable	29	sour
25	vegetable	30	fruity
26	burnt-like	31	fruity-herbal
27	etheric	34	fatty
28	rancid	35	rancid butter
29	oily	36	etheric
31	hay-like	37	hay-like
32	spinach-like	38	hay-like
33	mushroom-like (weak)	41	fruitty
34	vegetable	44	vegetable
35	fruity	45	herbal
36	fruity	46	herbal
37	sweet	47	fruity
38	burnt-like	48	fatty
39	glue-like	49	vegetable
41	cereal-like (weak)	50	broth-like
42	hay-like	51	flowery
43	burnt-like (weak)	52	vanilla-like
44	nutty	53	fruity
45	vegetable	54	bread-like
46		56	fruity
47		57	fruity

Separation of rye bread volatiles rendered 57 peaks (Fig. 5) of which only Nos. 25 and 54 corresponded to a typical bread aroma (Table 3). The most recurrent aromas: fruity, flowery, potato-like and sour (peaks No. 14 and 29).

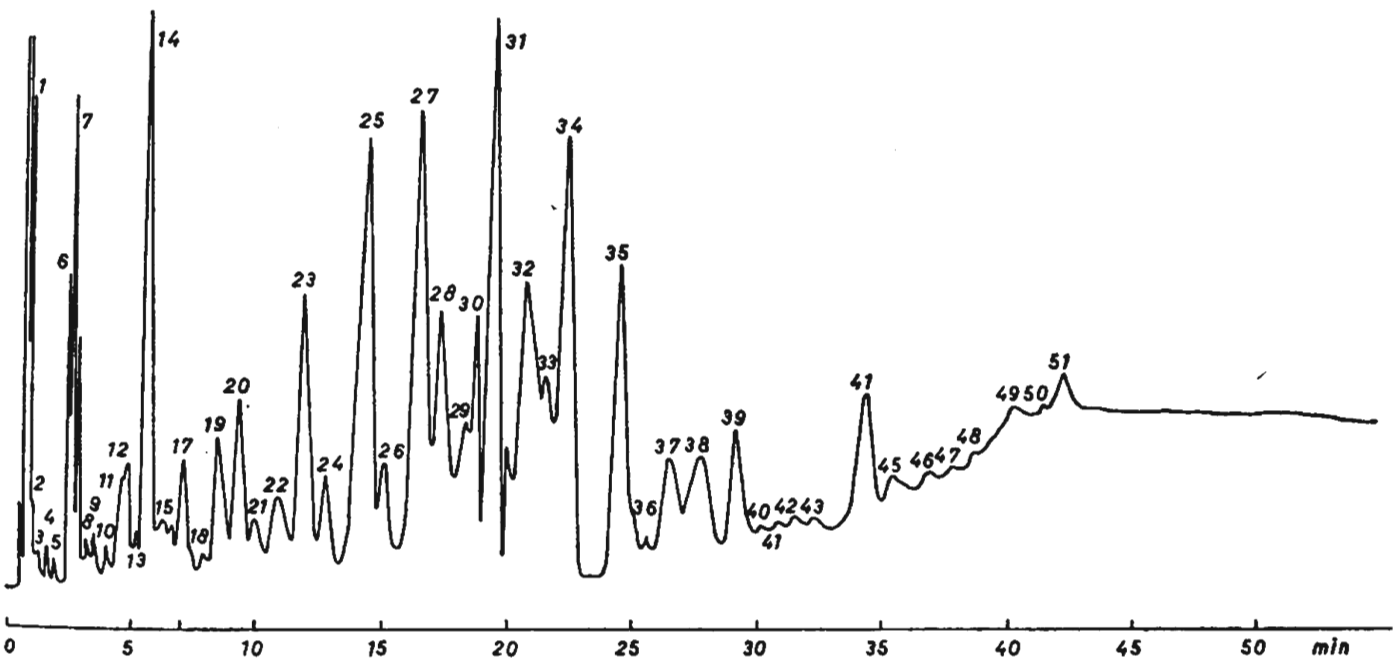

Fig. 4. Chromatogram of volatile compounds separated from roasted rye malt. Column (3 m) filled with Carbowax 20 MTPA on Chromosorb W AW

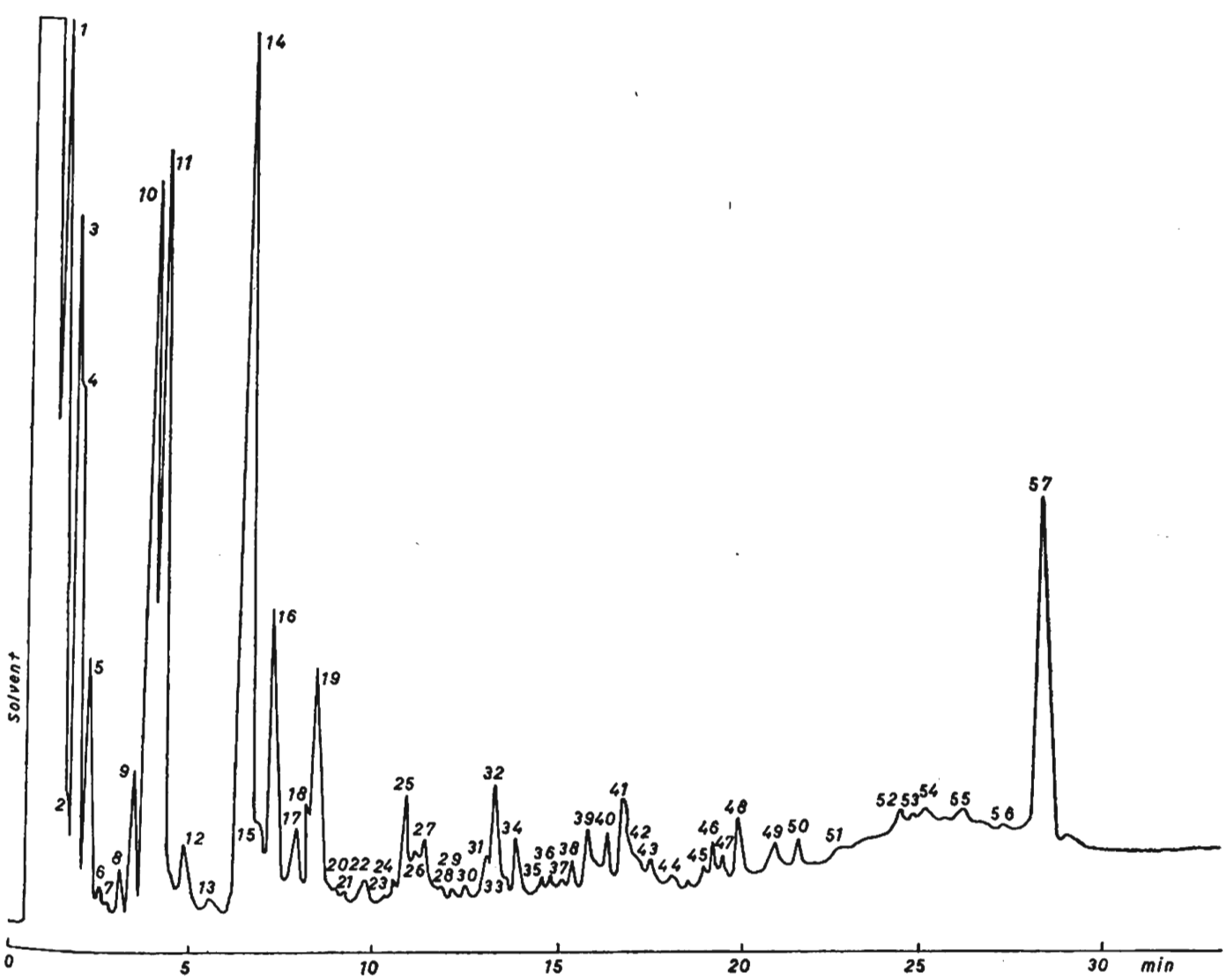

Fig. 5. Chromatogram of volatile compounds separated from rye bread. Column (2 m) filled with Carbowax 20 M on Chromosorb P

The extract of rye bread volatile compounds was separated into the following fractions: neutral, basic, acidic and phenolic. All of them except the acidic were of bread aroma of different intensities.

Fig. 6 presents a chromatogram of the rye-bread basic fraction volatiles (36 peaks). In this fraction the largest number of peaks corresponded to the aroma of bread (peaks 11, 13, 18, 20, 22).

The chromatographic separation of the rye-bread neutral fraction provided 51 peaks and 39 peaks in the phenolic fraction. In the sour fraction of the rye bread acetic acid and isobutyric acid were detected and identified. Total sum of peaks (128) from all fractions indicates that the initial concentrate was not completely separated in the analytical

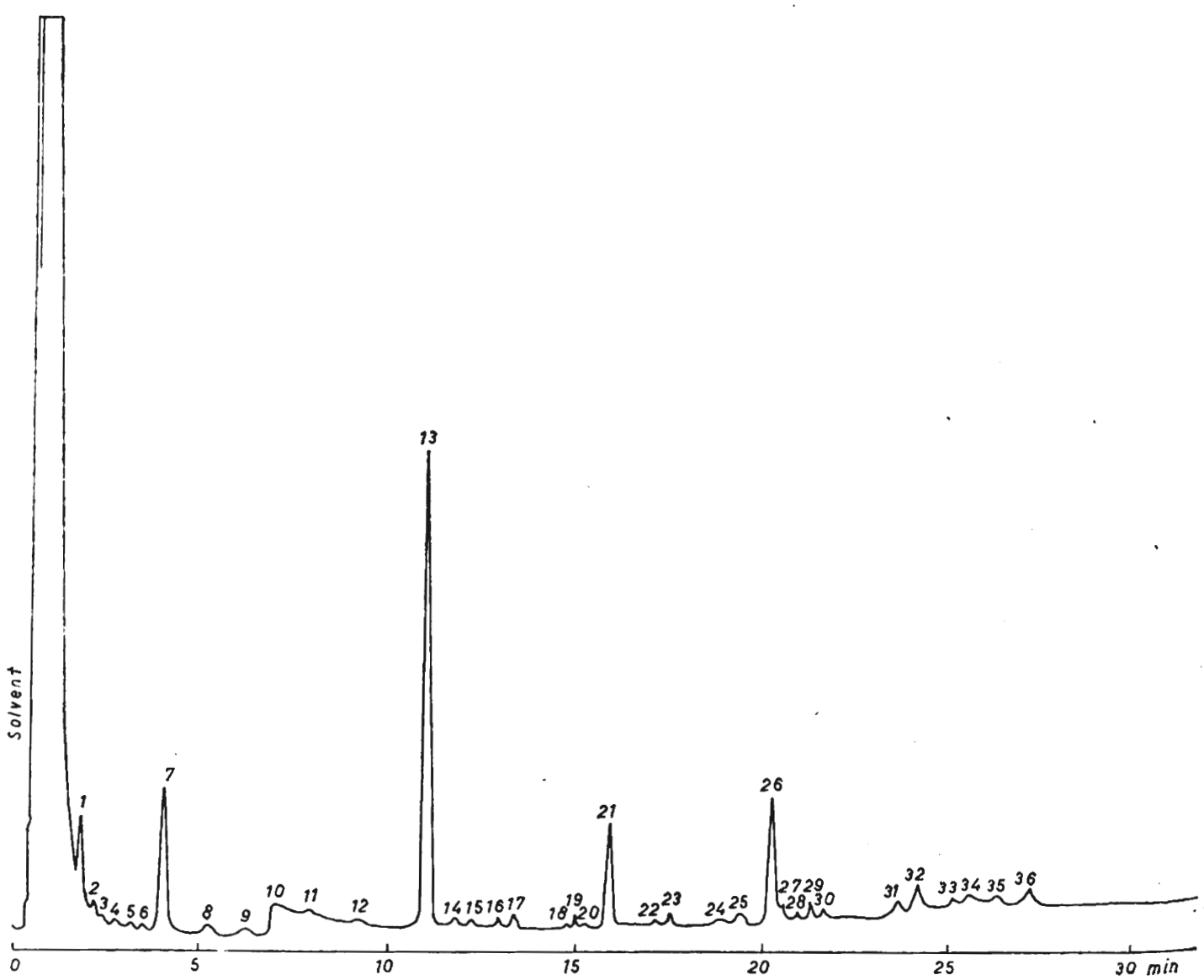

Fig. 6. Chromatogram of volatile compounds of the basic fraction separated from rye bread. Column (2 m) filled with carbowax 20 M on Chromosorb P

column (57 peaks). Fig. 7 presents a chromatogram of the rye-bread volatiles concentrate separated in a capillary column.

The separation in this column provided 155 peaks. As further work indicated, concentration of substances corresponding to individual peaks is sufficient to obtain a mass spectrum enabling their identification.

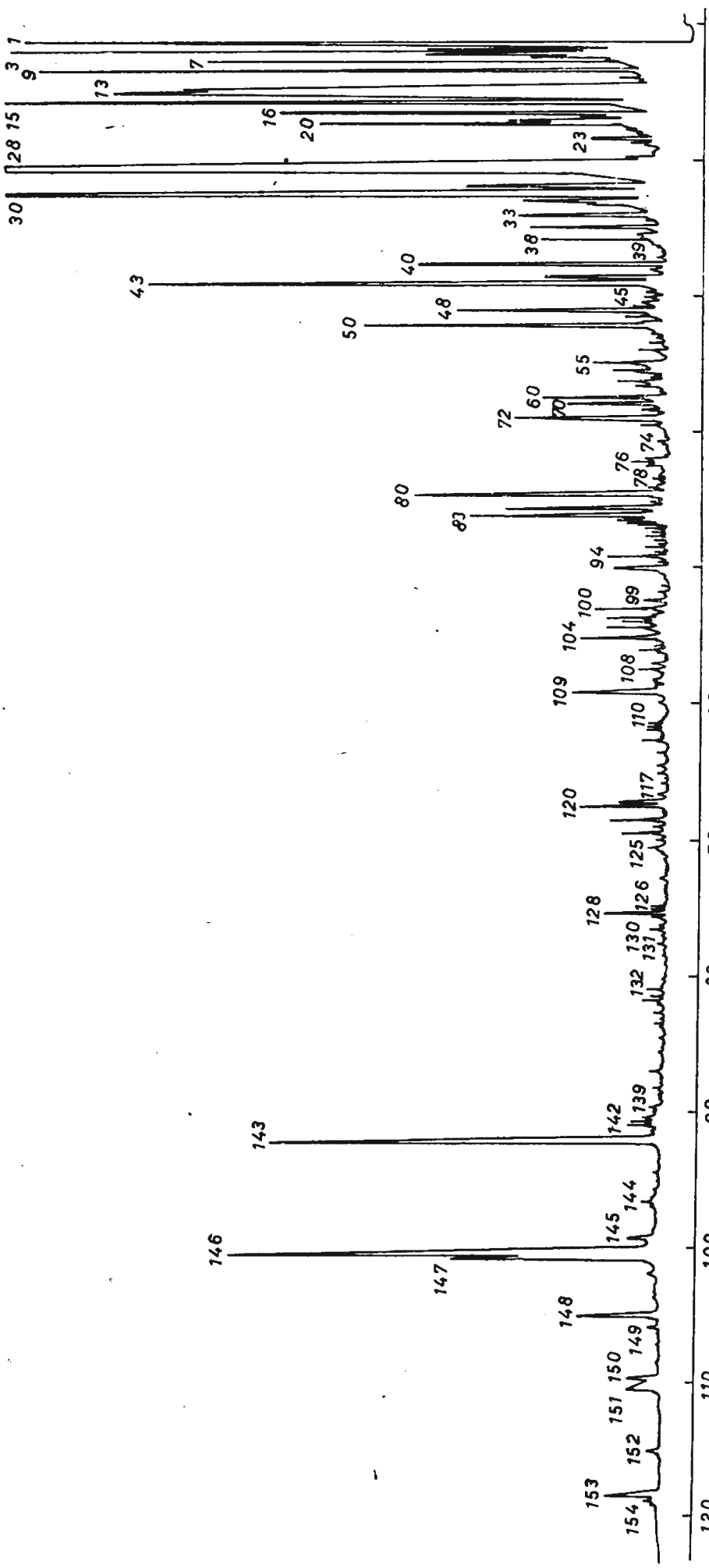

Fig. 7. Chromatogram of volatile compounds separated from rye bread. Capillary column (50) coated with SE-52 stationary phase

DISCUSSION

The use of the distillation-extraction method in this study rendered it is possible to exceed 70% recovery for most of the compounds. Somewhat lower (ca 60%) levels for certain esters may be due to formation of azeotropes with organic solvents and to unfavourable coefficients of division [19].

The obtained higher recoveries for ketones in comparison with ether groups of compounds can be accounted for by their high hydrophobicity and a favourable coefficient of division [19]. Similar results were arrived at by Schultz et al. [19] as well as Farmer et al. [2].

In this study recoveries were below 100%. It is likely that the greatest losses of the compounds take place during condensation. Schultz et al. [19] and Farmer et al. [2] had 100% recoveries without condensation. In the case of our investigations it was necessary to introduce condensation on account of the fact that in the received cereal product extracts certain volatile compounds were present in undetectable concentrations. It should be stressed that in spite of introduction of condensation the results show high repeatability with retention of an also high degree of recovery of the compounds.

In the model study the compounds used for analyses were those which most often appear in foodstuffs [17].

Application of volatile compounds fractionation made it possible to have a fuller analysis of the concentrate from cereal products. This mode of action is often used for analysis of aromatic compounds in diverse products [8, 21, 24].

The observed presence of large quantities of volatile components in roasted rye malt and in rye bread confirmed the reports provided by many analysts who continue their work in this field [10, 12, 13, 14, 15, 21]. Chromatographic analysis of volatiles in rye indicates identification of a greater number of compounds by means of the distillation-extraction method used for their separation than reported by Mc Williams and Mackey [12] and Hougen et al. [4].

CONCLUSIONS

1. The distillation-extraction method renders it is possible to obtain sufficiently condensed concentrate of aromatic compounds from cereal and cereal products to be used in chromatographic analysis and fractionation.

2. Fractionation of the cereal product volatile compounds extract enables their more exact analysis by means of gas chromatography and mass spectrography.

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WYODRĘBNIANIE I ZAGĘSZCZANIE LOTNYCH ZWIĄZKÓW Z PRODUKTÓW ZBOŻOWYCH DO ANALIZY CHROMATOGRAFICZNEJ

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Streszczenie

Związki lotne z wodnych roztworów standardowych alkoholi, estrów i ketonów wyodrębniano i zagęszczano metodą destylacyjno-ekstrakcyjną. Odzysk związków standardowych o różnych temperaturach wrzenia wahał się w granicach od 59,3% do 92,6%, a krotność zagęszczania od 4700 do 7400.

Metodą destylacyjno-ekstrakcyjną wyodrębniano związki lotne z normalnego ziarna żyta, prażonego żyta oraz chleba żytniego. Zagęszczone ekstrakty rozdzielano w kolumnach chromatografu gazowego. Ocena zapachowa eluatów z kolumn chromatograficznych wykazała dużą różnorodność zapachów rozdzielanych składników. Stosowanie rozdziału ekstraktu związków lotnych produktów zbożowych na frakcje, pozwoliło na ich dokładniejszy rozdział w kolumnie chromatograficznej.