A C T A A L I M E N T A R I A P O L O N I C A

Vol. V (XXIX), No. 3 1979

EDWARD KAMIŃSKI
STANISŁAW STAWICKI *)
ERWIN WĄSOWICZ
HELENA GIEBEL *)
ROMAN PRZYBYLSKI
RENATA ZAWIRSKA
ROMUALD ZALEWSKI

VOLATILE FLAVOUR COMPOUNDS PRODUCED BY BACTERIA CULTIVATED ON GRAIN MEDIA

Department of Food Technology of Plant Origin, Agricultural University, Poznań

*) Chair of Technology and Hygiene of Human Nutrition, Agricultural University, Poznań

Key words: volatile odour compounds, gas chromatography, mass spectrometry, sensoric characteristic, bacteria, wheat and corn grain, identification of odour compounds.

The bacteria growing on wheat and corn grain produce a number of volatile chemical compounds responsible for the unpleasant sour, sour-milk and putrid smells. With the aid of GLC/MS analysis, 46 chemical compounds produced by bacteria on cereal grain have been identified. The following compounds were produced by bacteria in greatest quantities: acetoin, acetone, acetoin acetate, methylallylacetate, vinyl acetate, volatile fatty acids and amines.

It happens quite often in the flour-milling industry that grain is unfit for processing due to its strange, undesirable odour, although its other qualitative properties meet the specifications of appropriate standards.

In the USA, the Grain Standard envisages the evaluation of grain odour which is determined as good, fusty or sour [7]. In the USSR a broader grain odour evaluation scale including corn, malt, honey, fruit, fusty and putrid smells is in use [1]. The main cause of development of a strange odour in cereal grain is the microflora which rapidly develops on wet grain. With the modern grain harvesting techniques presently in use, the content of moisture in grain may often be as high as 30%.

According to data presented by various authors, the content of moisture in grain freshly collected by combine harvesters can vary within the following range: 18-24% in Britain, 25% often quoted for the USA and 17-26% in the USSR depending on where the grain is harvested [1]. Not always grain is dried immediately after harvest and often it lies wet for a few days or more.

Among the rich microflora that can be found on grain in the first days of storage, the bacteria develop most rapidly. According to Semeniuk [15], field bacteria grow quickly in grain containing 22% water. A number of bacteria, mainly those of secondary infection may, however, grow on grain with a lower moisture content. Janicki and Stawicki [8] observed the growth of certain bacteria at a water content of 14%, and Mishustin a. Trisviátski [11] found that the growth of bacteria might take place in grain with a low content of water if external conditions favour water vapour condensation on grain surface. Among the bacteria occurring in grain after harvest some 90% are strains of the *Pseudomonas*, *Xantomonas*, *Flavobacterium* and *Brevibacterium* variety [11, 2, 12, 13].

Microorganisms from the *Enterobacteriaceae*, *Micrococcaceae* and *Bacillaceae* families constitute the dominating bacterial flora on grain stored [3, 20, 4, 16, 17, 18]. Most of the grain bacterial microflora produce a number of volatile metabolites of specific odour.

The purpose of this study was::

— an analysis of flavour compounds by pure bacterial cultures on wheat and corn grain using gas chromatography and mass spectrometry.

MATERIALS AND METHODS

Volatile produce of the following microorganisms: *Pseudomonas trifoli*, *Pseudomonas fluorescens*, *Lactobacillus plantarum*, *Propionibacterium sp.*, *Escherichia coli*, *Sarcina sp.*, *Bacillus subtilis*, *Bacillus megaterium* and *Clostridium sp.*, were studied. These bacteria strains dominated on the grain of wheat of Polish origin. The *Bacillus subtilis*, *Bacillus megaterium* and *Clostridium strains* were isolated for investigations from the grain of wheat stored for 14 months under laboratory conditions (10-12°C, relative humidity 70-80%); the other strains were taken from grain surface immediately after harvest.

GROWTH OF BACTERIA ON COARSE WHEAT AND CORN MEAL

High-quality wheat and corn cultivated in Poland and free of sub--surface microflora was used to prepare the coarse meal. The surface infection degree per 1 g of grain was: 120 000 non-sporing bacteria, 600 aerobic bacteria spores of *Bacillus* variety and 2000 mould spores for wheat, and 260 000 non-sporing bacteria, 900 aerobic bacteria spores and 2400 mould spores for corn. The coarse meal was wetted up to 65-70% of water content, and sterilised at 121°C for 45 minutes. After cooling down to 30°C, the meal was inocculated with a bacteria suspension containinig 10^6 cells/cm^3. 100 cm^3 of suspension were used per 500 g of coarse wheat meal. Thus, initial infection amounted to some 200 000 cells per 1 g of meal.

The bacteria were grown in glass fermentors of 100 mm diameter and 400 mm high, at a temperature of 30°C for 72 hours. The culture was aerated by introducing to fermentors sterile, humidified air in an amound of 150 cm^3/min per 500 g of meal. The culture was checked after 24, 48 and 72 h by the plate quantitative inoculation method [19].

Volatile compounds from bacteria cultures were isolated by vacuum distillation in a glass aparatus [9]. Distillation was carried out under nitrogen of a pressure of 666 N/m^2 for a period of 4 hours. Bath temperature was 35°C, traps temperature -10°C and -30°C, and -40°C and -80°C. The distillate was extracted with CH_2Cl_2 and then concentrated to a volume of 100 nm^3.

CHROMATOGRAPHIC AND SENSORIC ANALYSIS

The separation of volatile compounds in concentrated distillates was carried out with the aid of a Varian Aerograph Moduline 2740 gas chromatograph equipped with a flame-ionization detector. Glass column i.d. 2 mm, length 3 m) packed with 15% Carbowax 20M TPA on Chromosorb W DMCS was used. The column operated for 6 minutes at 80°C and then the temperature was raised in a programmed way up to 170°C, at a rate of 4°/min. The temperature of 170°C was maintained for 15 minutes, detector temperature being 250°C. The percentage content of individual volatiles was calculated from the peak area on the chromatogram, the total area of all the peaks having been assumed as 100%. Kovats's indexes were calculated according to Schomburg and Dielmann [4].

A Finnigan quadrupole mass spectrometer combined with gas chromatograph, model 3300, was used for the identification of volatiles. Concentrated extracts were separated in a glass capillary column (id. 0.35 mm, length 50 m) with a stationary phase UCON HB 5100. Column temperature rise was programmed within the range from 40°C to 175°C at a rate of 2°C/min. The flow rate of helium used as the carrier gas was 2 cm^3/min. Electron energy was 70 eV. Mass spectrum sweeping rate varied from 10 to 250 amu.

The sensoric evaluation of the medium after the bacteria culture was carried out by a group of 5-6 persons who smelled the samples placed in special containers. The kind of odour and its intensity were determined using a O to 5 scores. The odours of volatiles in concentrated distillates separated chromatographically were evaluated by a group of 3-4 people at the detector flame extinguished, immediately after the compounds have left the column.

RESULTS

ODOUR OF MEDIUM AFTER GROWTH PURE CULTURES BACTERIA

The odour of the coarse wheat and corn meal was determined after 72 hours of growth of bacteria on it. Table 1 gives the results of these tests, i.e. the name of the percepptible odour and the number of points which characterises its intensity. The bacteria growing on wheat produced

Table 1. Characteristic of odors produced by the strains of aerobic and anaerobic bacteria grown for 72 hours in thermally treated coarse wheat meal under aeration (0.1-0.5 dcm^3 of air min/1 dcm^3 culture medium) or without aeration.

The strains of bacteria	Counts of bacterial per 1 g medium	The odor description
Pseudomonas trifoli	$7 \cdot 10^{10}$	sour (3) milky (1) granary (1)
Pseudomonas fluorescens	$8 \cdot 10^{9}$	malty (3) sour (1)
*Lactobacillus plantarum**	$7 \cdot 10^{7}$	milky (3) sour (2)
*Propionibacterium sp.**	$5 \cdot 10^{7}$	sour (5)
Escherichia coli	$6 \cdot 10^{15}$	putrid (4) musty (1)
Sarcina sp.	$8 \cdot 10^{7}$	granary (2) fruity (2) sour (1)
Bacillus subtilis	10^{11}	putrid (3) sour (2)
Bacillus megaterium	$2 \cdot 10^{9}$	sour (4) putrid (1)
*Clostridium sp.**	$8 \cdot 10^{8}$	sour (2) putrid (1)

* — anaerobic bacterium, grown without aeration (score intensity of odors 0—5)

the following odours: sour, sour-milky, sour-putrid, malty-sour and putrid-fusty. Bacteria of the *Sarcina sp.* produced a specific fruity-sour smell. The odour of the coarse wheat and corn meal sterilised before cultivation was determined as one characteristic of the steamed grain.

CHARACTERISTIC OF VOLATILE COMPOUNDS PRODUCED BY BACTERIA ON COARSE WHEAT AND CORN MEAL

The volatiles produced by bacteria under study on wheat and corn were characterised, after separation in the gas chromatograph column, with respect of their odour.

Table 2 gives the Kovats indices and the relative percentage share of the peak area, and the corresponding odour in a fresh, thermally sterilised wheat sample, as well as the odour of the same wheat after

Table 2. Odor determination of gas chromatographic peaks after separation of volatile components occuring in concentrated distillates from thermally treated wheat and Bacillus subtilis cultivated in wheat

Wheat			*Bacillus subtilis* in wheat		
Peak area %	Kovats index	The odor description	Peak area %	Kovats index	The odor description
1.03	1138	herbal	0.75	1166	buttery
13.46	1170	oily undesirable	1.26	1190	herbal
10.08	1241	buttery	0.25	1223	burnt
12.42	1285	resinous	77.29	1296	like acetoin
1.81	1340	raw potato	0.08	1337	buttery
0.64	1356	raw potato	0.03	1349	buttery
4.53	1372	musty	3.54	1372	acid-burnt
11.39	1398	roasted potato	1.71	1393	roast potato
2.76	1435	not detectable	0.93	1424	musty potato
12.34	1483	not detectable	0.20	1444	musty potato
0.51	1536	floral, undesirable	0.05	1482	roasted potato
7.24	1572	potato	0.03	1510	ethereal
6.21	1579	granary	0.04	1529	herbal
0.17	1612	nutty	0.07	1544	herbal
1.55	1633	strawy	0.08	1554	burnt
0.51	1661	sweet	0.15	1572	burnt
1.29	1703	not detectable	6.92	1624	burnt, etheral, like mouse
0.64	1790	not detectable	0.48	1640	herbal
0.75	1875	not detectable	0.97	1670	granary
0.64	1902	strawy	0.25	1706	herbal-sweet
0.43	1918	strawy	0.11	1726	strawy-sweet
1.94	1971	not detectable	0.15	1748	anise
0.34	2066	not detectable	1.43	1790	herbal-sweet
1.55	2131	not detectable	0.45	1841	medicinal
0.51	2164	spicy	0.07	1893	herbal-sweet
1.06	2255	not detectable	0.08	1942	etheral
2.33	2303	not detectable	0.32	1964	etheral
1.81	2351	not detectable	0.89	1985	medicinal
			0.52	2041	herbal
			0.34	2078	not detectable
			0.14	2106	not detectable
			0.02	2190	not detectable
			0.02	2216	cinnamon
			0.04	2227	not detectable
			0.28	2262	like vanillin
			0.23	2277	not detectable
			0.14	2337	not detectable

the pure *Bacillus subtilis* culture had been cultivated on it. As results from the data, 28 fractions have been obtained from fresh wheat and 37 fractions from wheat after the cultivation of *Bacillus subtilis* on it. The samples also differed by the character of odour of individual fractions. In case of sound wheat, some volatile fractions mainly featured the following odours: buttery, resinous, potato and granary. A number of fractions had no odour at all. Samples from a *Bacillus subtilis* culture on wheat, in addition to odour fractions as in sound wheat, displayed other fractions such as acetoin, acid, herbal, burnt, etheral, cinnamon, anise, vanilin, etc. The fraction of the acetoin-like odour was the most characteristic and one of the biggest (77.3% of the sum of all fractions in this study).

Table 3. Percentage share of fraction of the „like acetoine" odour in individual bacteria cultures

Name of bacteria strain	Percentage share	
	on wheat	on corn
Pseudomonas trifoli	63.5	64.0
Bacillus subtilis	77.3	38.4
Bacillus megaterium	58.9	—
Sarcina	63.4	—
Propionibacterium sp.	37.3	—
Lactobacillus plantarum	19.6	—

Tests with volatiles from other bacteria cultures after chromatographic separation indicate that the number of fractions varied depending on culture. The lowest number of fractions, 26, was obtained for *Escherichia coli* on wheat; the highest, i.e. 26, for the *Lactobacillus plantarum* culture. The kind of odour was a more characteristic feature than the number of fractions. In all the pure bacteria culture examined, the fraction with the acetoin-like odour had the highest percentage share. In addition to the odour mentioned in the case of *Bacillus subtilis*, the following characteristic odours were found in other cultures: fruity, dairy, acid--dairy, oily, fungal, rencid cheese, mouse-like, hay and flowery. Two fractions of the rancid cheese odour separated in the *Lactobacillus plantarum* culture constituted 20% of the sum of all fractions.

Investigations of the volatile compounds obtained from corn meal and after the cultivation of individual bacteria strains on that meal show that a number of fractions with different odours were found, like in the case of wheat. Also the character of odour was, in most cases, similar to that described for wheat; and the biggest fractions had the acetoin--like smell, as well. Both in fresh, sound wheat and in corn, no fractions having that odour were found.

Because of the characteristic fraction of the acetoin-like odour separated in all the bacteria cultures, the percentage share of this fraction in partical cultures in given in Table 3.

IDENTIFIATION OF VOLATILE COMPOUNDS PRODUCED BY BACTERIA ON GRAIN

The identification of volatiles produced by bacteria on wheat and corn was carried out with the help of GLC-MS basing on the catalogue spectra [14]. Data obtained from identification are specified in Table 4. All in all, 46 chemical compounds were identified in all the bacteria cultures examined. The mass-to-charge ratio for these compounds is given in Table 4 (intensities are specified only for the eight greatest peaks of the mass spectrum).

Fig. presents an exemplary chromatogram from the separation, in a capillary column, of the volatile compounds from the culture of *Bacillus subtilis* on wheat. On the basis of this chromatogram, and the remaining ones, acetoin has been found to be the biggest fraction. In addition to compounds specified in Table 4, volatile fatty acids and amines were found in the cultures investigated using other analytical techniques [10]. Besides the nitric acid detected by means of GLC/MS, the following volatile acids have been found to be present: propionic, isobutyric, butyric, isovaleric and caproic [10]. The amines such as methylamine, propylamine, n-butylamine, iso-amylamine, dimethylamine, diethylamine, diisobutylamine and trimethylamine were found in the bacteria cultures by a special technique of separating these amines and converting them into hydrochlorides, followed by isolating them from these salts prior to the introduction into the chromatographic column [10].

DISCUSSION OF RESULTS

The studies have shown that the bacteria growing on grain produce a relatively great quantity of volatiles of highly differentiated odour. These compounds may occur in various amounts depending on the degree and kind of grain infection with bacteria. Acetoin is one of the most characteristic compounds produced by pure bacteria cultures on grain. All the examined bacteria which grown on grain give acetoin in the greatest quantity. No smell typical of acetoin was detected in grain left after bacteria cultures which evolved odours termed as sour milky, malty-sour and putrid. The remaining volatile compounds produced by bacteria specified in Table 4 have also their influence on this character of odours. The organoleptic evaluation of the odour of these volatiles depends both on their concentration in the medium and on their perceptibility thresholds. Some volatile compounds, occurring in large quanti-

Table 4. Identified volatile compounds by GLC/MS produced by bacteria in the thermally treated wheat and corn

M. W.	Formula	Name of compounds	Mass to charge ratio/Intensities								
1	2	3	4								
44	CH_2O	Acetaldehyde	29	44	26	28	43	27	25	42	
			100	27	21	20	16	11	11	6	
58	C_3H_6O	Acetone	43	15	58	42	27	44	29	26	
			100	78	18	17	17	16	18	9	
60	$C_2H_4O_2$	Acetic acid	43	45	29	15	60	42	31	28	
			100	97	60	100	32	25	19	15	
71	C_4H_6O	Pyr-rolidine	43	27	55	41	70	28	39	42	
			100	23	21	21	16	16	16	13	
74	$C_4H_{10}O$	1-Butanol	31	43	41	27	33	42	29	39	
			100	96	86	71	56	56	43	15	
74	$C_4H_{10}O$	2-Butanol	29	27	45	31	43	57	26	59	
			100	94	86	46	37	34	24	24	
74	$C_4H_{10}O$	iso-Butanol	43	41	31	42	27	33	39	29	
			100	83	82	61	60	53	37	34	
79	C_5H_5N	Pyridine	79	52	26	50	51	27	78	39	
			100	100	87	70	63	28	22	20	
86	CH_4N_5	5-Aminotetrazole	28	27	29	26	42	41	39	40	86
			100	98	82	73	71	47	29	24	16
86	$C_4H_6O_2$	Vinyl acetate	43	15	42	86	41	26	29	28	
			100	95	15	6	5	5	5	3	
86	$C_5H_{10}O$	2-Pentanone	43	27	29	72	71	86	57	45	
			100	23	23	17	13	12	11	9	
86	$C_5H_{10}O$	2-Methylbutanal	29	27	57	41	39	43	31	55	86
			100	96	83	33	39	27	16	12	1
88	$C_4H_8O_2$	Acetoin	45	43	15	19	27	29	42	88	
			100	96	55	27	23	19	7	5	
88	$C_5H_{12}O$	2-Methylbutanol	29	41	27	31	39	15	57	56	
			100	83	82	79	46	45	43	38	
88	$C_5H_{12}O$	3-Methylbutanol	27	29	31	41	42	15	55	39	
			100	99	96	70	63	55	47	38	
88	$C_5H_{12}O$	1-Pentanol	31	42	27	41	29	55	43	70	
			100	95	90	86	83	58	39	24	
94	C_6H_6O	Phenol	94	66	39	65	51	63	38	40	
			100	65	62	47	32	32	29	18	
96	$C_5H_4O_2$	2-Furylaldehyde	39	95	96	29	38	37	40	42	
			100	72	69	54	44	28	13	13	
96	C_6H_8O	2,4-Hexedienal	96	67	27	39	81	53	95	41	
			100	90	87	80	79	65	52	50	
98	$C_5H_6O_2$	2-Furylalcohol	39	41	29	53	27	69	98	80	
100	$C_6H_{12}O$	1-Hexen-3-ol	57	29	27	43	41	55	42	39	
			100	67	61	39	30	24	18	18	
100	$C_6H_{12}O$	trans-2,4-Dimethyl-	85	41	43	39	56	55	57	42	100
		tetrahydrofurane	100	38	23	21	21	19	15	9	4
102	$C_5H_{10}O_2$	2-Methyl-3-keto-	31	43	27	29	59	41	45	39	100
		2-butanol	100	85	78	73	47	35	34	20	0,7

c.d. tab. 4

1	2	3	4								
102	$C_6H_{14}O$	3-Methylpentanol	29	27	41	56	31	55	39	43	
			100	88	80	62	59	45	43	43	
102	$C_6H_{14}O$	4-Methylpentanol	56	43	31	41	29	55	42	69	
			100	99	87	70	64	61	54	29	
106	C_7H_6O	Benzaldehyde	77	51	105	106	50	29	78	51	
			100	82	76	71	65	52	24	20	
108	C_7H_8O	Benzylalcohol	79	51	108	107	77	50	91	39	
			100	62	59	59	59	46	28	26	
108	C_7H_8O	p-Cresol	107	108	77	79	39	51	50	53	
			100	100	63	60	55	37	30	18	
108	$C_6H_8N_2$	2,5-Dimethylpyra-	42	43	39	27	108	28	26	40	
		zine	100	78	70	55	48	42	30	30	
110	$C_6H_6O_2$	2-Furyl methyl ke-	95	39	43	38	110	67	96	111	
		tone	100	82	63	37	35	12	6	5	
114	$C_7H_{14}O$	4-Heptanone or	43	71	27	29	58	41	114	57	
		2-Methyl-3-heksa-	100	40	39	32	18	14	14	10	
		none									
114	$C_7H_{14}O$	3-Heptanone	57	29	27	43	41	39	54	72	114
			100	69	50	41	32	21	16	8	0,7
114	$C_6H_{10}O_2$	Methylallylacetate	43	15	72	27	29	57	39	114	
			100	49	26	15	9	6	4	3	
115	$C_5H_9NO_2$	Propionic acid	43	15	44	29	27	115	57	60	
		N-acetyloamide	100	68	34	25	21	21	10	10	
120	C_8H_8O	Acetophenone	77	105	51	50	29	43	27	74	120
			100	90	70	47	42	30	18	17	7
122	$C_8H_{10}O$	Phenylethyl	91	92	65	122	39	43	51	31	
		alcohol	100	53	25	16	15	12	12	12	
122	$C_7H_6O_2$	Benzoic acid	105	77	27	122	29	41	51	39	
			100	98	61	50	49	43	39	27	
124	$C_8H_{12}O$	1-Cyclohexen-1-yl-	81	109	27	124	53	39	52	51	
		-methyl ketone	100	71	70	45	42	28	26	23	
124	$C_8H_{12}O$	3-Cyclohexen-1-yl-	43	81	109	27	124	72	53	71	
		-methyl ketone	100	29	22	19	16	13	10	9	
124	$C_7H_8O_2$	o-Hydroxybenzyl-	114	77	51	123	39	79	95	55	124
		alcohol	100	92	57	53	35	28	24	25	7
128	$C_8H_{16}O$	3-Methyl-2-hepta-	43	85	72	29	27	57	128	41	
		none	100	42	30	30	26	20	14	11	
130	$C_6H_{10}O_3$	Acetoine acetate	43	15	87	45	28	42	30	29	130
			100	37	7	5	4	4	3	2	1
130	$C_6H_{10}O_3$	Methyl-2-methyl-	43	88	42	29	27	45	59	55	130
		-3-ketobutyrate	100	19	12	12	11	10	10	9	0,4
142	$C_9H_{18}O$	2,6-Dimethyl-3-	43	15	27	55	99	29	45	56	142
		heptanone	100	43	17	15	14	10	10	8	3
156	$C_{10}H_{22}$	2-Methyldecane	43	58	41	27	29	15	19	42	156
			100	36	34	29	19	17	15	12	1
170	$C_{11}H_{22}O$	2-Undecanone	43	58	41	27	15	29	39	71	170
			100	35	33	23	20	20	14	13	1

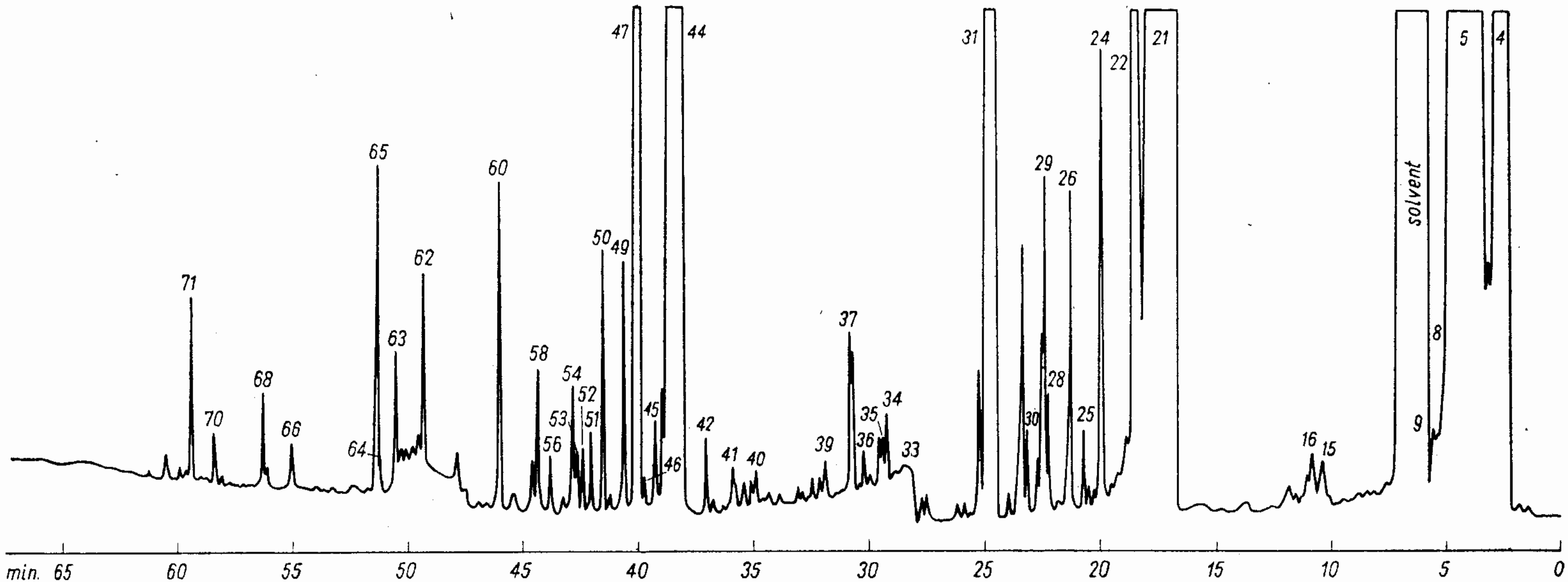

Fig. Gas chromatogram of volalites flavor compounds from concentrated distillates of *Bacillus subtilis* grown on thermally treated wheat. Identified compounds: 5 — acetone, 21 — acetoin, 22 — methylallylacetate, 31 — acetoine acetate, 37 — propionic acid N-acetyloamide, 47 — 2,4-hexedienal, 66 — phenol ties are, however, not detected sensorically. Thus, for example, the threshold for acetone is 450 ppm in a water solution, for acetic acid 54 ppm., and for butyric acid only 6.8 ppm [6].

Employing chromatographic methods in investigations into grain one can detect all the microorganism metabolites irrespective of treatment to which the grain has been subjected (e.g. to remove the microflora). This is why the microbiological evaluation not always confirms the usefulness of grain for technological purposes; because it happened that bread made of flour from grain showing a low infection level did not posess the desired organoleptic properties [10].

CONCLUSIONS

1. Bacteria growing on the wheat and corn grain produce a number of chemical compounds which are responsible for the strange, unpleasant odours such as sour, milk dairy and putrid.
2. The number of volatile compounds produced by bacteria on cereal grain detected using special concentration and separation techniques in a capillary column may amount to more than 100.
3. 46 volatile chemical compounds separated from single bacteria cultures on wheat and corn, have been identified with the aid of GLC/MS.
4. Chemical compounds such as acetoin, acetone, acetoin acetate, methylallyl acetate, vinyl acetate, volatile fatty acids and amines are the main metabolites of the bacteria growing on cereal grain.

ACKNOWLEDGEMENTS

This research was financed in part from a grant made by the U.S. Department of Agriculture, Agricultural Research Service authorised by Public Law 480, Grant No FG-Po-285. The authors are grateful to Miss Anna Bela for capable technical assistance.

LITERATURE

1. Akwis S. I.: Trudy WNIIZ 1960, **39**, 19.
2. Amos A., Kent-Jones D.: Modern cereal chemistry. Northern Publishing, Liverpool 1947.
3. Christensen C. M.: Cereal Chem., 1955, **32**, 507.
4. Düggeli M.: Zentralbl. f. Bakt., 1904, **12**, 602; **13**, 56.
5. Fight Peak Index of Mass Spectra vol. 1, 2, 3 second edition. Published by Mass Spectrometry Data Center AWRF, Aldermaston, Reading U. K., 1974.
6. Forss D. A.: Odor and flavor compounds from lipids. Progress in the chemistry of fats and other lipids. v. XIII Pergamon Press 1972.
7. Grain Inspection Manual Instruction No 918 (GR)-6. United States Department of Agriculture 1972.
8. Janicki J., Stawicki S.: Biul. Inform. CLTPPZ Warszawa, 7, 1963.

9. Kamiński E., Lis H., Wąsowicz E.: Chemia analityczna 1971, **16**, 1325.
10. Kamiński F., Stawicki S.: Characterization and identification of volatile odor substances produced by bacteria in cereal grain. Final Technical Report. Project number PL-ARS-21, FG-Po-285 Poznań 1977.
11. Miszustin F. N., Trisviatski L. A.: Mikrobiologia zjerna i muki. Chleboizdat, Moskwa 1960.
12. Oksention U. G., Polonskaja M. S.: Mikrobiologia 1964, **33**, 870.
13. Podjapolska P. O., Mirzojewa W. A.: Trudy WNIIZ, Wypusk XXX, 1955.
14. Schomburg J., Dielman G.: J. Chromatogr. Sci., 1973, **11**, 151.
15. Semeniuk G.: Microflora. Storage of grain and grain products. Moskwa 1956.
16. Spicher G.: Getreide u. Mehl 1963, **13**, 109.
17. Spicher G.: Getreide u. Mehl 1969, **19**, 65.
18. Spicher G.: Getreide u. Mehl 1970, **20**, 61.
19. Stawicki S.: Annals Acad. Agr. Poznań 1962, **13**, 185.
20. Trisviatski L. A.: Chranienije zierna, Zagotizd, Moskwa 1957.

Manuscript received: April, 1978.
Authors address: Wojska Polskiego 31, 60-624 Poznań.

*E. Kamiński, S. Stawicki *), E. Wąsowicz, H. Giebel *), R. Przybylski, R. Zawirska, R. Zalewski*

LOTNE ZWIĄZKI ZAPACHOWE PRODUKOWANE PRZEZ BAKTERIE ROSNĄCE NA ZBOŻU

Instytut Technologii Żywności Pochodzenia Roślinnego AR, Poznań
*) Instytut Żywienia Człowieka AR, Poznań

S t r e s z c z e n i e

Badano lotne związki wytwarzane przez czyste kultury bakterii rozwijające się na wysterylizowanej śrucie pszennej. Przebadano następujące drobnoustroje: *Pseudomonas trifoli, Pseudomonas fluorescens, Lactobacillus plantarum, Propionibacterium sp., Escherichia coli, Sarcina sp., Bacillus subtilis, Bacillus megaterium, Clostridium sp.*

Po hodowli bakterii na wymienionym podłożu określano organoleptycznie zapach podłoża (tab. 1). Dominowały zapachy „sour i malty". Również występowały takie zapachy, jak: „milky, putrid i fruity". Analizę wytwarzanych związków lotnych przeprowadzano w zagęszczonych destylatach z podłoża za pomocą chromatografii gazowej. Wykryto od 26-48 frakcji o różnych zapachach w zależności od szczepów bakterii (tab. 2).

Największa frakcja we wszystkich zagęszczonych destylatach miała zapach „like acetoine" (tab. 3). We frakcji tej za pomocą spektrometrii masowej wykryto acetoinę. Podobne frakcje wykryto w hodowlach tych samych szczepów bakterii na wysterylizowanej kukurydzy. Za pomocą analizy GLC-MS zidentyfikowano w tych samych destylatach 46 związków chemicznych (tab. 4, rys. 1). Poza związkami wymienionymi w tab. 4 w badanych kulturach wykryto jeszcze lotne kwasy tłuszczowe i aminy.