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Liquid products of meat and bone meal pyrolysis: comprehensive assessment by chromatographic methods

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Abstract:
Dorogov's antiseptic stimulators (fractions 2 and 3) are products of meat and bone meal pyrolysis that are used to treat farm animals. However, there is a lack of detailed information about their chemical composition. We aimed to study individual compositions of organic substances in the water- and oil-soluble condensates of these preparations.
Dorogov's antiseptic stimulators ASD-2F and ASD-3F (Agrovetzashchita, Russia) were used as samples of the water- and oil-soluble condensates of meat and bone meal pyrolysis. Volatile substances were identified by gas chromatography and gas chromatography-mass spectrometry, while amino acids were determined by high-performance liquid chromatography.
The initial water-soluble condensate contained ammonium salts, amides of carboxylic acids, N-heterocyclic compounds, hydantoins, amino acids, and dipeptides, with a total content of 8% of the condensate's weight. Its dehydrated concentrate had almost no ammonium salts and amides of carboxylic acids, but its contents of hydantoins, amino acids, dipeptides, and low-volatile nitrogen-containing heterocycles were 10–15 times as high as those in the initial condensate. The condensate contained 13 dipeptides and 19 amino acids with a total content of 2.5%. According to gas chromatography-mass spectrometry, the oil-soluble condensate contained over 30% of nitriles; 7–10% of higher and aromatic hydrocarbons, phenols, and amides (with esters); and 1–3% of N-heterocyclic compounds, naphthalenes, pyridines, and dipeptides. The nitrogen-containing heterocycles, as well as dipeptides, were similar to those in the water-soluble condensate.
We identified 80% of individual organic substances in the water-soluble pyrolytic condensate. Together with its concentrate, they contained more than 220 organic substances divided into 10 main groups. The oil-soluble condensate consisted of over 350 individual organic compounds. The full composition of the preparations can be further identified by three-quadrupole liquid mass spectrometry.

Keywords: Pyrolysis, chemical composition, water-soluble condensate, oil-soluble condensate, meat and bone meal, high-performance liquid chromatography, gas chromatography, gas chromatography-mass spectrometry, triple quadrupole liquid mass spectrometry

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INTRODUCTION

Today, there is a need to determine the chemical composition of liquid products that result from the joint pyrolysis of animal proteins and fats. Performed at temperatures up to 500°C, pyrolysis produces an emulsion of organic substances in an aqueous ammonium buffer solution. The separation of this emulsion results in two fractions: water-soluble and oil-soluble condensates [1–3].

A water-soluble condensate consists of water, carbon ammonium salts (mainly ammonium carbonate), and water-soluble organic substances. An oil-soluble

condensate is a stable water-in-oil emulsion containing up to 90% of organic matter [4, 5].

A number of factors account for the current interest in studying the chemical composition of these products. Firstly, the products of meat and bone meal pyrolysis have been widely used in Russian veterinary practice for more than 60 years. They are known as ASD-2F (Dorogov's antiseptic stimulator, fraction 2 – a water-soluble part) and ASD-3F (Dorogov's antiseptic stimulator, fraction 3 – a non-water-soluble part). ASD-2F is used to prevent and treat a wide range of animal and bird diseases [6, 7]. Recently, it has been produced as a dietary supplement in liquid and capsule forms [2, 8]. ASD-3F is prescribed to animals with skin and hooves pathologies [3, 9]. Secondly, foreign countries consider the oil fraction of pyrolysis products as a potential component of biofuel (pyrolytic fuel), which can be obtained from animal protein waste [10, 11]. For example, pyrolysis can be used to utilize meat and bone meal obtained from animals with spongiform encephalopathy [12]. Krolevets and Bogachev estimated the annual amount of animal meal that can be converted into fuel at hundreds of thousands of tons [13]. However, their estimates were based on the pyrolysis carried out under almost ideal laboratory conditions by condensing products at very low temperatures, which significantly limits their practical application.

In another study, Krolevets *et al.* discussed the indicators of pyrolytic fuel obtained by food waste pyrolysis in a pilot plant [14]. They found that the organic products obtained from the pyrolysis, or at least their individual fractions, can be used as additives to diesel fuel. However, the authors analyzed only gross indicators, such as C, H, N, and S contents, as well as physical-and-mechanical characteristics. Therefore, their results have a limited application. Kukonin and Grek and Yengashev *et al.* [15, 16] considered only the oil-soluble condensate to be of much use, finding the water-soluble condensate to be useful only as an aqueous ammonia fertilizer.

Nozdrin *et al.* identified more than 120 organic substances in ASD-2F, an example of the water-soluble condensate, including 4 cyclic dipeptides [17]. Gurov *et al.* identified more than 170 organic substances in the water fraction by using gas-liquid chromatography (GLC) and gas chromatography-mass spectrometry (GC-MS) on capillary columns with HP-FFAP phases (30 m, 0.25 mm, 0.25 um, PN: 19091F-433, Agilent) and 5% phenylpolymethylsiloxane [18]. The content of 26 individual substances was quantified by the absolute calibration method. The authors found that their total amount in ASD-2F reached 4% of its total mass, which was about 50% of the total mass of organic substances in the preparation. Guzev *et al.* used the simple normalization method to determine carboxylic acid amides and amines (7%), alkyl-hydantoins (30%), and dipeptides (16%), including cyclic dipeptides and their derivatives [19].

Since the chromatograms of the studied preparations had a very high density of peaks, Teshaev and Khasanova analyzed the retention time precision as an identification parameter for gas chromatography (GC) under similar experimental conditions [20]. The authors found that modern gas chromatography with high intralaboratory precision allowed for a high repeatability of the analyte's retention time, ensuring highly reliable identification.

Serba *et al.* studied the amino acid composition of ASD-2F by high-performance liquid chromatography (HPLC) with post-column derivatization with ninhydrin [21, 22]. They reported that the preparation contained 15 free amino acids and 19 bound amino acids with total concentrations of 1 and 5%, respectively. Thus, we can assume that about 4% of the amino acids were present in the preparation as peptides that were converted into amino acids during acid hydrolysis.

Noteworthily, it is relatively easy to isolate an aqueous solution of ammonium carbon salts (distillate) from the water-soluble pyrolysis product by boiling the preparation. The resulting solution can be used as an ammonia fertilizer. Organic substances, which are the distillation residue, can be used as a pyrolytic fuel or a source of bioactive (including pharmaceutical) substances [23–27].

We aimed to study individual and group compositions of organic substances contained in both the water-soluble condensate and the oil-soluble (fuel) fraction of meat and bone meal pyrolysis products. In particular, we identified amino acids and their derivatives (including dipeptides) and quantified them in the water-soluble and oil-soluble condensates.

STUDY OBJECTS AND METHODS

ASD-2F and ASD-3F were used as samples of water-soluble and oil-soluble condensates of meat and bone meal pyrolysis products (Agrovetzashchita, Russia).

To prepare the ASD-2F sample for analysis, we pipetted 0.5 g of the substance (weighed on a laboratory scale with an accuracy of 3 decimal places) into a 15-mL plastic centrifugation tube and added 10 mL of ethanol. Since a suspension formed due to precipitation of ammonium carbon salts, the tube was centrifuged for 10 min at 4000×g, and then the supernatant was taken for analysis.

To concentrate organic substances of ASD-2F and to prepare the organic part, 10 mL of ASD-2F was thermostated in an oven at 80°C for 3 h. The concentrated organic part amounted to 6–8% of the initial mass. The losses due to the evaporation of highly volatile organic substances were estimated at about 20% of their total amounts. Thus, the total content of organic substances in the initial condensate can be reliably estimated as 7–9%. After concentration, organic substances were extracted in 10 mL of ethanol per 0.5 g of the concentrate. After complete dissolution, the sample was centrifuged under normal conditions, and then the supernatant was taken for analysis [25].

To extract organic substances from ASD-3F, we placed 1 mL of the preparation into a 15-mL plastic test tube and added 5 mL of distilled water and a solvent (methylene chloride, butyl acetate, hexane, or o-xylene). The tube was capped and stirred for 1 h. After centrifugation under the same conditions, an extract was taken for analysis – from the lower part of the tube with methylene chloride and from the upper part of the tubes with the other solvents.

The standard samples included 2-pyrollidinone, 2-piperidinone, 3-methylbutanamide, 2-amino-3-methylpyridine, 5,5-dimethylhydantoin, 5,5-ethylmethylhydantoin (Acros Organics, Belgium) with at least 95.0% of the main substance.

Gas chromatography was performed on a Khromatek-Kristall-5000.1 chromatograph with a flame ionization detector (Khromatek, Russia) and a Shimadzu GC-2010 Plus chromatograph with a GCMS-QP2020 mass selective detector (Shimadzu, Japan). Both instruments used VB-1701 30 m×0.32 mm×0.50 µm capillary columns (initial temperature: 120°C; initial isotherm retention time: 5 min; the rate of temperature rise: 10°C/min, final temperature: 230°C). Carrier gas helium was used to maintain constant pressure in front of the column at 100 kPa. The flame ionization detector operated at 250°C, with a hydrogen flow rate of 30 mL/min, an air flow rate of 300 mL/min, and an inert gas (helium) injection at 30 mL/min. The mass detector operated at an ion source temperature of 210°C, an interface temperature of 210°C, a scanning rate of 1425 amu/s, a scanned mass range from 30 to 450 m/z, a cycle's sweep rate of 0.3 s, detector voltage of 0.9 kV, and a solvent effect removal time of 1.5 min. The evaporator worked at 250°C, with a flow split ratio of 1/30, a sample volume of 1 µL, in a gas saving mode. The obtained mass spectra were interpreted using the GCMS Postrun Analysis software (GCMS Solution Version 4.4, Shimadzu, Japan). The components were identified by comparing their mass spectrum in the sample with their mass spectrum in the library (databases NIST-14 and NIST-14s). We also relied on our understanding of animal protein pyrolysis and the synthesis of new compounds from pyrolysis products.

The pH of the oil fraction was determined in the aqueous extract. For this, 2 mL of the oil fraction was placed in a 15-mL test tube and mixed with 10 mL of distilled water. The tube was stirred for 1 h and centrifuged for 10 min at 4000×g. Then, 10 mL of the aqueous solution was taken from the upper part, placed in a beaker for titration, and mixed with 100 mL of distilled water to determine the pH.

Bound and unbound amino acids were determined by high-performance liquid chromatography (HPLC) with pre-column derivatization.

We used the following reagents and solvents: CH_3OH and acetonitrile for HPLC (Panreac, Spain), FMOC (Sigma, USA), o-phthalaldehyde (≥ 99.9%) (Sigma, USA), sodium hydrogen phosphate (≥ 99.9%) (Sigma, USA), hydrochloric acid (≥ 37%), deionized water obtained on a MilliQDirect 8 system (Merck Millipore, Germany), chemically pure trichloroacetic acid (≥ 99.0%), 3-mercaptopropionic acid (≥ 99.0) (Sigma, USA), sodium hydroxide (≥ 99.0), anhydrous sodium tetraborate (≥ 99.0), and sodium tetraborate decahydrate (≥ 99.5%).

To determine protein-bound amino acids, the samples were subjected to acid hydrolysis with hydrochloric acid (6 mol/L) for 24 h at 110°C. The resulting hydrolysate was transferred into a 50-cm^3 round-bottom flask and evaporated under vacuum at 60°C. The dry residue was redissolved in 1 cm^3 of the buffer (pH 2.2) and filtered through a 0.45-µm pore membrane filter into a 2-cm^3 chromatographic vial.

Unbound amino acids were extracted with a saline buffer and 20% trichloroacetic acid to precipitate proteins and peptides. The extract was stirred and kept for 1 h at a temperature from 18 to 25°C. Then, it was centrifuged for 5 min at 2000×g and the aqueous layer was filtered through a 0.45-µm pore membrane filter. Finally, the filtrate was transferred into a vial.

Chromatographic analysis was performed on a 50–150 mm long HPLC column, 2.1–4.6 mm in diameter, with a C18 reversed phase, and a particle size of 1.8–5.0 µm. We also used an Agilent 1260 Infinity LC HPLC system (Agilent Technologies, USA) with a diode array detector.

Derivatization was carried out automatically using a programmable autosampler. For this, 10 mm^3 of orthophthalaldehyde solution (for primary amino acids), 10 mm^3 of fluorenylmethyloxycarbonyl (for secondary amino acids), and 2 mm^3 of the sample's solution were injected into the chromatograph. The volume of the injected sample was 12 mm^3.

The measurements were recorded at a diode array detector wavelength of 338 nm and 262 nm. The parameters of chromatographic analysis were as follows:

Column temperature – 40°C;

Mobile phase A – acetonitrile:methanol:water – 45:45:10;

Mobile phase B – 10 mM Na_2HPO_4, 10 mM $Na_2B_4O_7$, pH 8.2;

Flow rate – 1 mL/min;

Elution mode – gradient (Table 1).

Table 1 Gradient mode parameters

Time, min	Volume of eluent A, %	Volume of eluent B, %
0	2	98
0.5	2	98
20.0	57	43
20.1	100	0
23.5	100	0
23.6	2	98
25.0	2	98

The peaks of the samples' chromatograms were identified by comparing them with the chromatogram of the calibration solution in terms of retention time and spectral ratio.

RESULTS AND DISCUSSION

Table 2 presents the physicochemical parameters of the water-soluble and oil-soluble condensates.

Figure 1 shows a general view of the aqueous condensate's gas chromatogram.

The chromatograms of gas chromatography-mass spectrometry have a large number of peaks and therefore are not informative. For this reason, we did not presented them in the article. The chemical composition of the condensates is analyzed in Tables 3–6.

Table 3 shows the chemical composition of the water-soluble condensate determined by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) and grouped in accordance with the main component. When analyzing the data, we took into account only peaks with a signal-to-noise ratio of over 10 and a signal area of at least 0.1% of the total area of the target peaks.

In addition to the actual composition of the group (Table 3, column 2), we provided the following data for each of the substances (in brackets): relative signal area, %, in the initial water condensate (by GC-MS)/ actual concentration, %, in the initial condensate (by GC)/relative signal area, %, in the concentrate (organic part) of the water condensate (by GC-MS)/actual concentration, %, in the concentrate (organic part) (by GC).

Columns 3 and 4 (Table 3) show the totals for each group, namely the data for the initial condensate (above) and the data for the concentrate (organic part) of the same condensate (below). The data in column 3 (the number of substances, n1, and their total relative concentration by peak area) were determined by GC-MS, while the data in column 4 (the number of substances, n2, and their total actual concentration) were obtained by GC.

Table 2 Physicochemical parameters of water-soluble and oil-soluble condensates

Indicator	Water-soluble condensate [7]	Oil-soluble condensate
Appearance, smell	Brown liquid with a pungent ammonia-like odor	Thick oily liquid, dark brown to black, with a characteristic smell of burnt bone
Density, g/cm^3	1.09–1.11	0.90–1.00
Water content, %	65.0–70.0	5.0–10.0
Carbon ammonium salts, %	25.0–30.0	1.0–2.0
Organic substances, %	7.0–10.0	88.0–94.0
Number of organic substances, over*	220	350

* Low volatile organic substances are hard to determine by gas chromatography

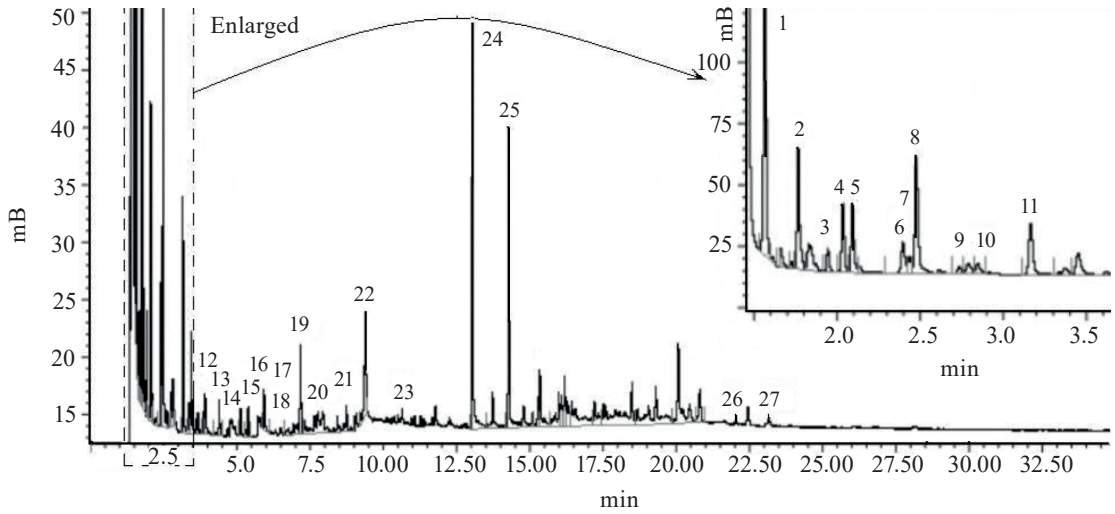

Figure 1 Gas chromatogram of the water condensate: 1 – acetic acid (1.545); 2 – propionic acid (1.743); 3 – acrylic acid (1.804); 4 – butanoic acid (2.068); 5 – dimethylformamide (2.217); 6 – isovaleric acid (2.375); 7 – N-methylformamide (2.419); 8 – acetamide (2.464); 9 – valeric acid (2.713); 10 – N,N-dimethylacetamide (2.786); 11 – propionamide (3.151); 12 – caproic acid (3.861); 13 – butyramide (4.395); 14 – 2-Aminopyridine (4.579); 15 – phenol (5.112); 16 – 3-Methylbutanamide (5.395); 17 – 2-Amino-3-Methylpyridine (6.267); 18 – valeramide (6.529); 19 – 2-Pyrrolidinone (7.175); 20 – caprylic acid (7.780); 21 – urotropin (8.259); 22 – 2-Piperidinone (9.375); 23 – picolinamide (10.305); 24 – 5,5-Dimethylhydantoin (13.047); 25 – 5-Ethyl, 5-Methylhydantoin (14.277); 26 – dipeptide Cyclo (Leu-Pro) (22.107); 27 – dipeptide Leu-Pro (23.037)

Table 3 Chemical composition of the water-soluble condensate by group

Chemical composition by group	Total substances in the group/concentration	
	GC/MS n1/S1, units/%	GC n2/S2, units/%
Carboxylic acids	15/14.7	7/1.42
Acetic (7.03/0.88/1.5/0.23)	8/2.02	7/1.02
Propanoic (1.4/0.22/0.2/0.11)		
2-Propenoic, 2-methyl-Propanoic (0.37/.../…/…)		
Butanoic (1.82/0.11/0.22/0.13)		
3-methyl-Butanoic (0.64/../…/….)		
2-methyl-Butanoic(0.34/0.06/0.1/0.11)		
Pentanoic, 3-methyl- (0.27/0.01/…/….)		
Pentanoic, 4-methyl- (1.12/0.22/…/…)		
5-Hexanoic (0.43/0.13/0.38/0.06)		
4-(Methylamino) butyric (0.19/.../…/…)		
Heptanoic (0.21/../…/….)		
6-Heptenoic (0.41/.../…/….)		
Octanoic (0.27/0.01/0.1/0.32)		
Amides	15/14.4	7/0.65
Formamide (0.17/…/…/)	12/4.31	6/7.67
Acetamide (7.34/0.41/1.47/2.16)		
N,N-Dimethylacetamide (0.31/0.03/0.1/0.15)		
Acetamide, N-methyl- (0.47/…/…/…)		
Propanamide (2.71/0.14/0.71/0.88)		
Propanamide, N-methyl (0.18/…/…/…)		
Propanamide, 2-methyl (0.31/…/…/…)		
Butanamide (0.83/0.03/0.33/0.24)		
Pentanamide (0.1/.../…/…)		
Enanthamide (0.27/../0.21/….)		
2-Propanamid, N, N, 2-trimethyl- (0.25/.../0.14/…)		
Picolinamid (0.21/0.02/0.21/0.07)		
7-Noneamide (0.1/.../…/…)		
N,N-Diethylpropioonamide (0.11/.../0.23/…)		
Imidazoles	10/23.1	2/0.96
1-H-Imidazole, 2-ethenyl (0.52/…/0.54/…)	10/34.1	2/18.65
2,4-Imidazolidinone (retention time 6.929–16.218)		
3,5,5-trimethyl (0.1/…/0.14/…)		
1-methyl-5-piperidin-yl (0.12/…/0.13/…)		
5-methyl- (0.2/…/0.26/…)		
5,5-dimethyl (12.2/0.66/17.06/13.08)		
5-ethyl (0.9/…/0.83/…)		
5-ethyl-5-methyl (6.84/0.31/9.4/5.57)		
5,5-diethyl (0.54/…/0.83/…		
5-isopropyl- (1.24/.../1.71/…)		
5-methyl-5-isopropyl- (0.4/.../2.36/…)		
Pyrroles	7/4.14	1/0.8
Pyrrole (0.38/…/0.33/…)	7/4.08	1/1.24
2-carbonitrile (0.11/…/0.13/…)		
2-oxamide (0.78/…/1.04/…)		
1-methyl-2-(pyrrolidinyl)- (0.26/…/0.26/…)		
1-methyl-2-(pyrrolidinyl)- (0.26/.../0.34/…)		
2-Pyrrolidinone (1.78/0.8/1.6/1.24)		
Pyrrolidine, 1-acetyl (0.56/…/0.28/…)		
3,4-Dimethyl-3-pyrrolin-2-one (0.1/…/0.41/…)		
2-Pyrrolidinone, 4,4-dimethyl-5-methylidene- (0.17/…/0.25)		
Pyridines	6/1.16	–
2-Aminopyridine (0.37/…/0.33/…)	6/0.95	
2-Amino-4-methylpyrimidine (0.2/…/…/…)		
2-Pyridinamine,3-methyl (0.19/…/…/…)		
2-Pyridinamine,5-methyl (0.13/…/0.24/…)		
2(1H)-Pyrididinone, 3,6-dimethyl- (0.1/…/0.14/…)		
3H-Imidazo[4,5-b] pyridine-2-ethanamine, 3-n (0.17/…/0.24/…)		
Lactams	1/5.4	1/0.18
2-Piperidinone (5.4/0.18/5.0/2.38)	1/5.0	1/2.38

Continuation of Table 3

Chemical composition by group	Total substances in the group/concentration	
	GC/MS n1/S1, units/%	GC n2/S2, units/%
Indoles, pyrimidines	6/1.09	–
Indolizine,octahydro (0.1/…/0.1/…)	6/1.88	
2,4(1H,3H)-Pirimididinedione, dihydro-3-methyl (0.22/…/0.43/…)		
1-H-Indazol-5-amine, 3-methyl (0.19/…/0.35/…)		
Thymine (0.11/…/0.13/…)		
5,6-dimethyuracil (0.31/…/0.47/…)		
6-Azathymine (0.16/…/0.2/…)		
Alcohols and O-heterocycles	8/4.13	–
1-Propanol, 2-amino-2-methyl- (0.43/…/…/…)	5/4.37	
2-Hexanone oxime (0.17/…/…/…)		
2,5-Furandione, 3,4-dimethyl- (0.11/…/…/…)		
Oxetane, 2,3,4-trimethyl- (0.58/…/0.4/…)		
2,5-Furandione, dihydro-3-methyl (0.74/…/1.13/…)		
Oxetane, 3,3-dimethyl- (0.23/…/0.32/…)		
Furan, tetrahydro-3,4-dimethyl- (0.18/.../0.16/…)		
Benzoxazole, 2-methyl- (1.69/.../2.36/…)		
Total substances (out of 220) in the groups/concentration	69/68.4	18/4.01
	53/57.3	17/31.0

GC – gas chromatography; MS – mass spectrometry

Substances in bold type are of interest as possible marker indicators that can be used to develop production quality control.

As an example, acetic (7.03/0.88/1.5/0.23) in the carboxylic acids group (Table 3) can be interpreted as follows: acetic acid can be used as a standard substance; its relative content in the initial condensate is 7.03% (by GC-MS), its actual absolute content is 0.88% (by GC); its relative content in the concentrate is 1.5% (by GC-MS); and its actual content is 0.23% (by GC). The omission dots in brackets can be interpreted as follows: the substance was identified and its relative concentration was determined by GC-MS, but it was not analyzed by GC. In addition, some substances can be evaporated during concentration, for example, 2-methyl-propionic acid (2-methyl-Propanoic) is absent in the concentrate.

As can be seen in column 3 (Table 3), we identified 15 carboxylic acids in the water condensate and 8 carboxylic acids in the concentrate with a total relative concentration of 14.7 and 2.02%, respectively (by GC-MS). According to column 4 (Table 3), GLC identified 7 carboxylic acids in the water condensate and the same acids in the concentrate but with a lower concentration (1.02%) compared to the condensate (1.42%).

The organic part of the initial water condensate and its concentrate (Table 3) contained mainly nitrogen-containing substances. In the initial condensate, carboxylic acids (mainly fatty acids and ammonium salts) had a significant concentration of 15 (by GC-MS) and 1.4% (by GC), while in the concentrate, they were under 2 (by GC-MS) and 1% (by GC). A similar picture was observed for amides, which numbered 15 and 12 in the initial condensate and its concentrate, respectively (by GC-MS). Their contents in the group were 14.4 and 2.3% for the initial condensate and its concentrate, respectively. Additionally, the GC method identified 7 substances for the initial condensate and 6 substances for its concentrate at their absolute group concentrations of 0.65 and 7.7%, respectively.

Imidazoles (hydantoins) constitute one of the main groups of organic substances in condensates. They numbered 10 in both samples, with their content increasing from 23% in the initial condensate to 34% in its concentrate (by GC-MS). According to GC, their concentration increased even more significantly: from 1% in the condensate to 19% in its concentrate. The most important substances in this group are 5,5-dimethylhydantoin (0.7 and 13%) and 5-ethyl,5-methylhydantoin (0.31 and 5.6%) in the condensate and its concentrate by GC-MS and GC, respectively.

Of particular interest are pyrroles and lactams mainly represented by 2-pyrrolidinone and 2-piperidinone, whose concentration did not change significantly even after concentration. According to GC-MS, the group of pyrroles consisted of 7 substances with a total content of about 4%. The concentration 2-pyrrolidinone in the initial condensate was 1.78 and 0.8% by GC-MS and GC, respectively, while in the concentrate it amounted to 1.6 and 1.24% by GC-MS and GC, respectively. The group of lactams, however, was represented by only one substance, 2-piperidinone (δ-valerolactam), according to GC-MS. Its concentration in the initial condensate was 5.4 and 0.18% by GC-MS and GC, respectively, whereas in the concentrate it reached 5.0 and 2.38% by GC-MS and GC, respectively.

Such groups as pyridines, indoles, pyrimidines, O-heterocycles, and alcohols were only analyzed by

Table 4 Amino acid derivatives and dipeptides in ASD-2F by HPLC

Group of substances	Type of condensate sample		
	Initial condensate with derivatization, S, %	Initial condensate, S, %	Condensate concentrate (organic part), S, %
Free amino acid derivatives			
l-Lysine, methyl ester	0.12	–	–
Glycine, N-acetyl-	1.20	–	–
Alanine, 2-methyl-	0.88	–	–
L-leucine, N-acetyl, methyl ester	0.22	0.98	2.40
L-alanine, N-acetyl-	–	0.14	0.34
D-Alanine, N-allyloxycarbonyl-, dodecyl ester	–	0.26	0.41
L-Proline, 5-oxo-	1.14	–	–
L-proline, 1-acetyl-	–	0.37	0.52
L-Proline,5-oxo-, methyl-	–	0.36	0.73
Pyrrole-2-carboxylic acid	0.42	–	–
TOTAL (number/signal area)	6/3.98	5/2.11	5/1.48
Dipeptides			
Gly-Gly	0.56	–	–
Gly-Pro	–	0.90	1.24
Cyclo (-Gly-Pro)	–	3.27	4.41
Cyclo (-Ala-Ala)	–	0.20	0.42
Ala-Leu	–	0.41	0.74
Ala-Val	–	0.47	0.67
Cyclo (-Ala-Val)	–	0.45	0.66
Cyclo (-Ala-Pro) N-acetyl-	–	1.67	2.45
Cyclo (-Ala-Trp)	–	0.76	1.20
Val-Val	0.84	–	–
Cyclo (-Val-Val)	–	0.12	0.19
Cyclo (-Leu-Pro)	–	2.22	3.11
Cyclo (-Pro-Pro),Diethyl ester	–	2.03	2.73
Ile-Pro	–	1.03	1.29
Cyclo (-Ile-Pro)	–	0.15	0.30
TOTAL (number/signal area)	2/1.40	13/13.76	13/22.33

GC-MS. In total, we identified more than 20 individual organic substances (Table 3).

Table 4, which continues Table 3, shows the contents of amino acids and dipeptides in the initial water condensate and its concentrate by GC-MS. In addition, it presents the GC-MS data on silyl derivatives of the condensate obtained after derivatization.

Amino acid derivatives and dipeptides make up a significant part of the organic substances of the water condensate (Table 4).

As can be seen in Table 4, the GC-MS method without derivatization identified 5 amino acid derivatives with a total concentration of 1.5 to 2.1% in the water condensate and its concentrate, respectively. Alanine and proline derivatives dominated among the amino acids. Also, we identified 13 cyclic and linear dipeptides with a total relative concentration of 13.8 and 22.3% in the water condensate and its concentrate, respectively. Noteworthily, the dipeptides were mostly represented by alanine (6 dipeptides) and proline (6 dipeptides), and to a lesser extent by valine (3 dipeptides), glycine (3 dipeptides), leucine (2 dipeptides), isoleucine (2 dipeptides), and tryptophan (1 dipeptide).

Table 5 shows the contents of free and bound amino acids in the water condensate determined by HPLC. As we can see, 95% of all amino acids was presented by lysine, phenylalanine, leucine, argininepro line, and hydroxyproine, whose total content exceeded 2600 mg/100 mL (2.4% in the water condensate).

The profile of bound amino acids in the water condensate differed significantly from the profile of free amino acids. This might be due to the effect of hydrolysis or the type and concentration of dipeptides in the initial condensate. Our analysis confirmed that the condensate's organic substances contained significant amounts of dipeptides based on glycine, alanine, valine, histidine, proline, hydroxyproline, cysteine, and methionine.

According to Table 5, the total concentration of the organic substances identified in the study exceeded 90% of all the substances found during the chromatographic analysis of the water condensate and its concentrate.

The analysis of the oil-soluble condensate. The oil-soluble condensate (pyrolytic fuel, ASD-3F) is a mixture of organic pyrolysis products with low solubility in water which, after separation, form a fraction clearly separable from the water condensate. We used various organic solvents to extract organic substances from the oil fraction. According to our results, at least 60% (by mass) of substances could be extracted with butyl acetate, and about 70%, with hexane and ortho-xylene. The most efficient solvent was methylene chloride, which enabled the extraction of 80% of organic substances from the oil fraction. In total, we identified more than 320 organic substances by GC-MS, of which 118 substances had a content (signal area) of over 0.1%. They were grouped in the same way as the data on the water condensate (Table 6).

Table 5 Content of amino acids in the water condensate

Name of amino acid	Free amino acids, mg/100 mL	Bound amino acids, mg/100 mL
Glycine	8.54 ± 1.28	281.11 ± 19.68
Alanine	4.99 ± 0.75	528.07 ± 36.96
Valine	2.69 ± 0.40	175.05 ± 12.25
Leucine	298.00 ± 44.71	80.032 ± 5.600
Lysine	1067.00 ± 160.07	667.47 ± 46.72
Arginine	248.80 ± 37.32	–
Histidine	33.88 ± 5.08	214.21 ± 14.99
Aspartic acid	8.54 ± 2.98	11.19 ± 0.78
Glutamic acid	13.34 ± 2.00	95.26 ± 6.67
Isoleucine	3.90 ± 0.59	24.22 ± 1.70
Proline	109.30 ± 16.39	530.13 ± 37.11
Phenylalanine	689.30 ± 103.39	38.14 ± 2.67
Methionine	1.75 ± 0.26	658.13 ± 46.07
Serene	25.43 ± 3.81	3.05 ± 0.21
Threonine	12.40 ± 1.86	–
Cysteine	14.22 ± 2.13	214.19 ± 14.99
Tyrosine	5.93 ± 0.89	2.05 ± 0.14
Hydroxyproline	185.50 ± 9.30	1855.69 ± 129.89
Hydrolysin	11.10 ± 0.76	11.37 ± 0.79
TOTAL	2744.60 ± 192.00	5389.36 ± 377.25

Table 6 Chemical composition of the oil-soluble (fuel) fraction of pyrolysis products by group

Substances by group (retention time, min)	Total substances in the group/Total signal area
Aromatic hydrocarbons	20/7.69
1-Octene,3.7-dimehyl- (0.12)	
Toluene (1.37)	
2-Decene, 6-mehyl-,(Z) (0.36)	
Ethylbenzene (0.46)	
p-Xylene (0.24)	
o-Xylene (0.33)	
Styrene (1.06)	
Benzene, propyl- (0.31)	
Benzene, 1-ethyl-2-methyl- (0.24)	
Benzene, 1-ethyl-3-methyl- (0.29)	
Mesitylene (0.14)	
Benzene, 1-ethenyl-2-methyl- (0.56)	
Benzene, 2-propenyl- (0.19)	
Benzene, n-butyl- (0.19)	
Benzene, pentyl- (0.34)	
Benzene, (1-methyl-2-cyclopropen-1-yl)- (0.29)	
Benzene, hexyl- (0.12)	
Butylated Hydroxytoluene (0.17)	
Indene (0.78)	
1H-Indene,1,1-dimethyl (0.13)	

Continuation of Table 6

Substances by group (retention time, min)	Total substances in the group/Total signal area
Higher hydrocarbons	15/10.75
1-Decene (0.73)	
1-Undecene (1.06	
1-Tridecene (1.16)	
Tridecane (0.54)	
Cyclododecane (1.17)	
Tetradecane (0.6)	
Pentadecane (1.27)	
n-Tridecan-1-ol (0.66)	
Hexadecane (0.68)	
n-Pentadecanol (0.77)	
3-Heptadecene, (Z)- (0.63)	
Heptadecane (0.70)	
Octadecane (0.23)	
1-Octadecene (0.22)	
Nonadecane (0.28)	
Phenols	10/7.94
Phenol (2.23)	
o-Cresol (0.8)	
Phenol, 2,6-dimethyl (0.15)	
p-Cresol (3.0)	
Phenol, 2-ethyl-(0.18)	
Phenol, 2,4-dimethyl- (0.7)	
Phenol, 2-ethyl-5-methyl- (0.13)	
Phenol, 2,3,6-trimethyl- (0.2)	
Phenol, 2-ethyl-4-methyl- (0.4)	
Biphenyl (0.15)	
Amides, esters	11/10.14
Enanthamide (0.13)	
Hexanamide (0.2)	
Phenylamide, 4-methyl- (0.52)	
Phenylpropanamide (0.16)	
Heptadecanoic acid,methyl ester (0.17)	
Hexadecanamide (3.42)	
Miristamide, N-methyl- (0.92)	
Octadecanamide (2.07)	
Propanamide, cyclopentyl-N-methyl- (1.05)	
Miristamide, N-methyl- (0.3)	
Nitriles	25/31.27
Butanenitrile, 3-methyl- (0.17)	
Heptanonitrile (0.2)	
6-Hepten-1-nitrile (0.32)	
Benzonitrile, 3-methyl- (0.12)	
Octanenitrile (0.24)	
7-Octene-1-nitrile (0.38)	
Benzonitrile, 2-methyl- (0.16)	
2,4-Pentadienenitrile, 2-amino-4-methyl (0.19)	
Nonanenitrile (0.27)	
Benzyl nitrile (0.63)	
9-Decene-1-nitrile (0.17)	
Benzenepropanenitrile (0.75)	
1H-Pyrrole-2-carbonitrile (0.44)	
Undecanenitrile (0.28)	
Benzonitrile, 2,4,6-trimethyl- (0.38)	
1,5-Dimethyl-2-pyrrolecarbonitrile (0.11)	
Tetradecanenitrile (0.32)	
10-Undecenenitrile (0.13)	
Propanenitryle, 3,3-thyobis- (0.49); Pentadecanenitrile (0.1)	
Oleanitrile (9.95)	
Heptadecanenitrile (9.39)	
Octadecynenitrile (5.18)	
Nonadecanenitrile (0.18)	

Continuation of Table 6

Substances by group (retention time, min)	Total substances in the group/Total signal area
Pyridines	6/1.00
Pyridine (0.18)	
Pyridine, 2,6-dimethyl- (0.11)	
Pyridine, 2-ethyl- (0.24)	
Pyridine, 2,4-dimethyl- (0.25)	
Pyridine, 1,2,3,6-tetrahydro-1-(phenylmethyl)- (0.22)	
Naphthalenes	6/2.37
Naphthalene, -tetrahydro (0.11)	
Naphthalene, -dihydro (0.11)	
Naphthalene (0.87)	
Naphthalene, 2-methyl- (0.38)	
Naphthalene, 1-methyl- (0.75)	
Isopropenylnaphthalene (0.15)	
N-heterocycles	16/9.04
Pyrrole (2.03)	
Pyrazine, 2,6-dimethyl- (0.15)	
1-H-Pyrrole-2,5-dione, 3-ethyl-4-methyl- (0.11)	
Indole (2.97)	
Indole, 3-methyl- (1.01)	
1H-Indole, 4-methyl- (0.13)	
1H-Indole, 2-methyl- (0.5)	
1H-Indole, 2,3-dimethyl- (0.46)	
1H-1,3-Benzimidazol-4-amine, 5-methyl- (0.2)	
Benzofuro[3.2-d]pyrimidin-4(3H)-one (0.34)	
1H-Benzimidazole, 2,5-dimethyl- (0.11)	
5H-Indeno[1,2-b]pyridine (0.19)	
9H-Pyrido[3,4-b]indole,1-methyl- (0.2)	
2,4-Imidazolidinone-5,5-dimethyl (0.12)	
2,4-Imidazolidinone-5-methyl-5-ethyl- (0.4)	
2,4-Imidazolidinone-5-methyl-5-(2-methyl)- (0.12)	
Dipeptides	8/1.56
Cyclo (-Val-Val) (0.15)	
Cyclo (-Ala-Trp) (0.16)	
Cyclo (-Gly-Pro) (0.1)	
Leu-Pro (0.12)	
Cyclo (-Leu-Pro) (0.32)	
Cyclo (-Pro-Pro), Diethyl ester (0.23)	
Ile-Pro (0.13)	
Cyclo (-Ile-Pro) (0.36)	
Total (out of 350 substances, 100%)	118/81.5

According to Table 6, the group of nitriles contained the largest number of substances (25) with the highest total content (31%). High concentrations were found for the nitriles of oleic acid ($C_{18}H_{31}N$, about 10%), margaric acid ($C_{17}H_{33}N$, 9.4%), and stearic acid ($C_{18}H_{35}N$, 5.2%), which accounted for over 25% of all organic substances.

Almost equivalent in concentration were the groups of amides and esters (11 substances, 10%), N-heterocycles (16 substances, 9%), and higher hydrocarbons (15 substances, more than 10%). The main substances in the group of amides and esters were palmitic acid amide (3.4%) and stearic acid amide, together accounting for half of all the substances in the group. Indole had the highest concentration in the N-heterocycle group (about 3%), accounting for almost a third of all organic substances in the group. The group of higher hydrocarbons did not have any dominant substances, with most substances making up 0.5–0.7%.

The groups of aromatic hydrocarbons and phenols had a similar total content (about 8%), but differed sharply in the number of substances (20 and 10, respectively). Aromatic hydrocarbons were mainly represented by aliphatic derivatives of benzene, as well as styrene. Only toluene and styrene had concentrations above 1%, with the other substances having lower contents. In the group of phenols dominated phenol itself (2.2%), cresols (2 substances, 3.8%), and xylenol (0.7%).

The naphthalene group contained 6 substances and had a total concentration of 2.4%, where naphthalene and methylnaphthalene made up 1.6%.

The oil-soluble condensate contained relatively few pyridines and dipeptides, accounting for 3% in total. However, the dipeptides in this condensate appeared to be similar to those found in the water condensate of the pyrolysis products.

CONCLUSION

We studied the chemical composition of the water- and oil-soluble condensates of meat and bone meal pyrolysis by gas chromatography (GC), gas chromatography-mass spectrometry (GC-MS), and high-performance liquid chromatography (HPLC). These methods identified up to 80% of individual organic substances in the water-soluble condensate.

The water-soluble condensate and its dehydrated and desalted concentrate contained over 220 organic substances divided into 10 main groups. The condensate was mostly represented by ammonium salts and carboxylic acid amides, N-heterocyclic compounds, hydantoins, amino acids, and dipeptides, with a total content of 8% of the condensate's weight. We used gas-liquid chromatography and analytical standards to determine the contents of 27 substances in the water condensate. Their total concentration was 4% of the condensate's organic substances, or over 50% of the relative content.

HPLC identified 19 free amino acids and peptides in the water condensate, with a total content of 2.5% each. We found that the organic substances identified during our study exceeded 90% of all the substances determined chromatographically in the initial water condensate. Its dehydrated concentrate had almost no ammonium salts or carboxylic acid amides. However, the contents of hydantoins, amino acids (10), dipeptides (13), and low-volatile nitrogen-containing heterocycles were 10–15 times as high as in the initial condensate, accounting for 31% of the total content of organic substances.

The oil-soluble condensate (pyrolytic fuel) contained over 350 individual organic substances divided into 9 main groups, which were determined by GC-MS. More than 40% of the substances were nitriles and amides of fatty acids, which were part of original animal fats, as well as aromatic and higher hydrocarbons, N-heterocyclic compounds, and aliphatic derivatives of phenols. We found that the nitrogen-containing heterocycles and a small amount of dipeptides (up to 10%) were similar to those in the water-soluble condensate.

CONTRIBUTION

The authors were equally involved in writing the manuscript and are equally responsible for plagiarism.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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