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Investigations on the Chemical, Toxicological, and Biological Properties of the Polish Smoke Extract

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A method for producing a smoke extract has been developed at the Polish Meat Research Institute, and its details are given in the respective patents (1). Using this method on a pilot scale in 1968 there were produced such amounts of the smoke extract, which sufficed for a marketing programme on more than 2 thousands tons of smoked goods (sausages and canned meats) in some ten Polish towns with more than 100 thousands inhabitants, each.

The results of this marketing gave way to a decision concerning the construction of a full-scale plant, with an annual production covering the full demand for smoke extract on the domestic scale.

However, the construction of this full-scale plant had to be preceeded by a thorough examination of the smoke extract as to its chemical, toxicological and biological properties. This examination was indispensable from the viewpoint of Polish food laws as well as from the viewpoint of professional ethics of the modern meat industry, since both these factors require that the foodstuffs given to the consumer will be as safe to eat, as only can be assessed from the present status of medical sciences.

The presented paper is a brief report on the results gathered hitherto in examining the safety to eat of the Polish smoke extract.

CHEMICAL PROPERTIES

As it can be derived from the basic technological description given in the a.m. patents (1), the Polish smoke extract is a mixture of some tens of indi-

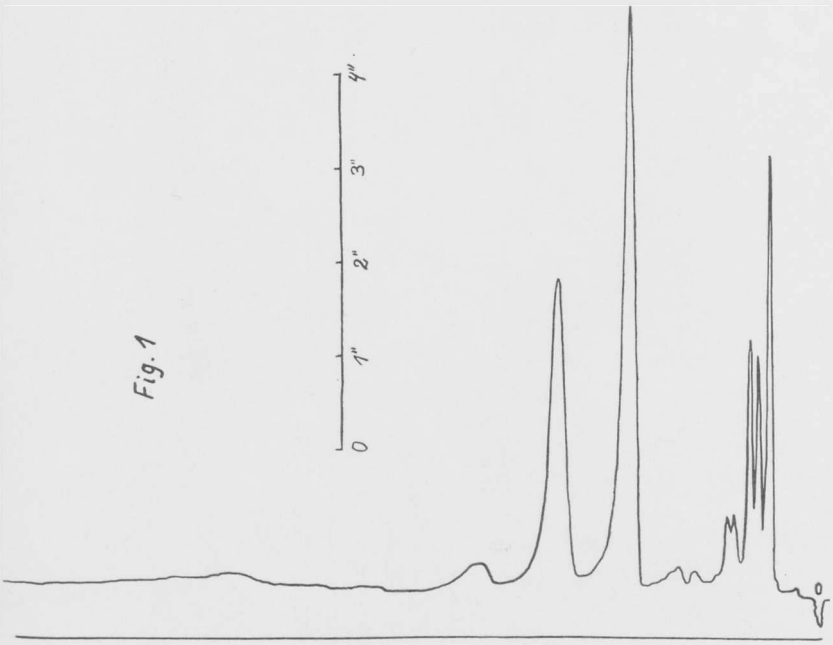

Fig. 1. *Phenolic compounds of the Polish smoke extract. Pye Argon Gaschromatograph, 0,025 mcl, Celit 100 mesh, 4', 10 % NGA, 175° C, argon flow 50 ml/min, detector voltage 1000 V. att. ×10, chart speed: 12"/h*

vidual components, among which those having at least one active phenolic function play the dominant role. Other components are low-boiling compounds, which are naturally accompanying the phenols in wood smoke.

Altogether some 56 individual compounds were found originally in the smoke extract, however, due to improvements in the separation technique this figure has been cut down to some 20 individuals at present.

Figure No. 1 represents a chromatogramme with the typical proportions of the constituents of the phenolic compounds, the smoke extract is consisting of. For comparison figure no. 2 is a record of the GLC analysis of a standard mixture of known phenols. Investigations on the chemical structure of the individual constituents of the smoke extract are advanced and will be reported separately in due course.

Apart of the power to impart the typical smoke flavor to meats, the Polish smoke extract exhibits a pronounced antioxidative activity. This is illustrated by the data gathered in table No. 1, after Miler and coll. (2).

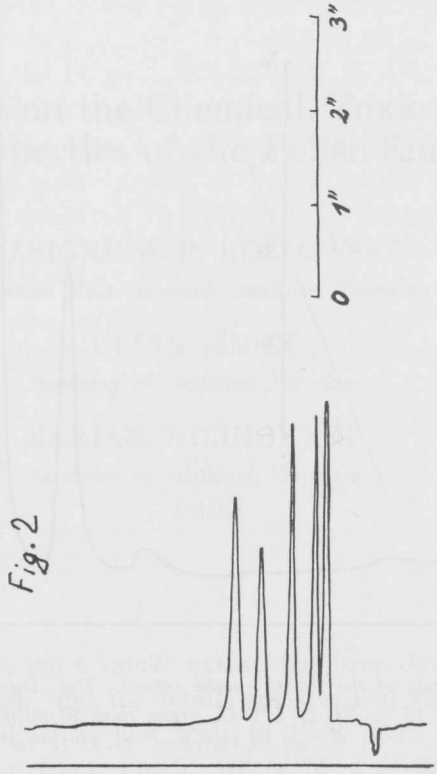

Fig. 2. *Standard mixture of phenols: 1 — guaiacol, 2 — phenol, 3 — p-cresol, 4 — n-propylphenol, 5 — 3,4-xylenol, separation conditions as in fig. 1.*

Table 1. *Prolongation of the storage time of lard under the influence of the Polish smoke extract (values represent the ratios of the keeping time for samples with added smoke extract to the keeping time of control samples)*

Temperature of storage (° C)	*prolongation of storage time at the extract concentrations*		
	50 ppm	*100 ppm*	*150 ppm*
4	2,46	30,3	99,6
10	2,35	27,9	97,1
18	2,36	25,2	93,9

Furthermore, the extract has been found to retard bacterial growth in meat mixes, as can be seen from table No. 2, cited after Rojowska (3).

Table 2. *Prolongation of the keeping time and reduction of residual bacteria counts in scalded sausages under the influence of the Polish smoke extract*

Amount of added smoke extract (ppm)	*prolongation of keeping time*[1] *(times)*	*reduction of residual bacteria counts*[2] *(times)*
10	1,08	3,18
20	1,18	3,89
30	1,30	4,44
40	1,45	4,92
50	1.63	5.35
60	1.87	5.74
70	2.19	6.12
80	2.64	6.47
90	3.31	6.82
100	4.46	7.14
110	6.81	7.45
120	14.42	7.77

[1]) Expressed as ratio of keeping time for samples with added smoke extract to the keeping time for control samples.

[2]) Expressed as ratio of residual bacteria counts in control samples to the residual bacteria counts in samples with added smoke extract.

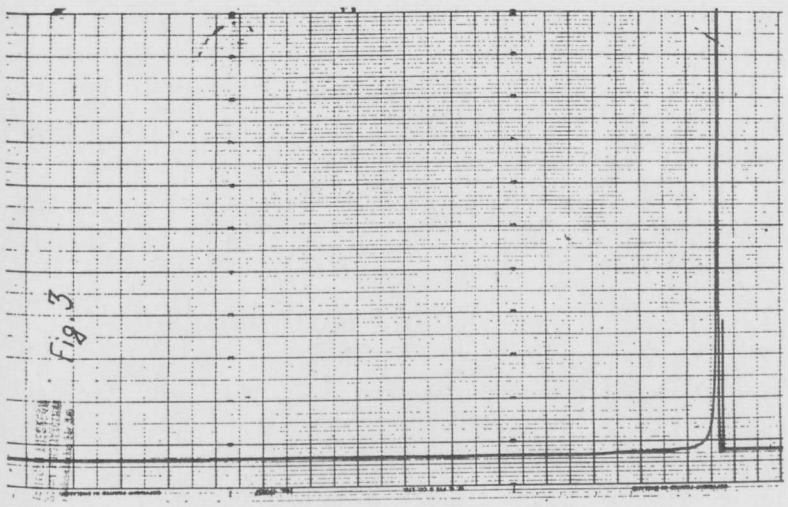

Fig. 3. *Non-phenolic compounds of the Polish smoke extract GLC Pye M. 105/15, 60.0 mcl, Celit 60—72 mesh, 7', 3 % Apiezone L, inlet pressure (argon) 12 psi, FID, 183° C isotherm., att. 5×10^3, chart speed 10"/h.*

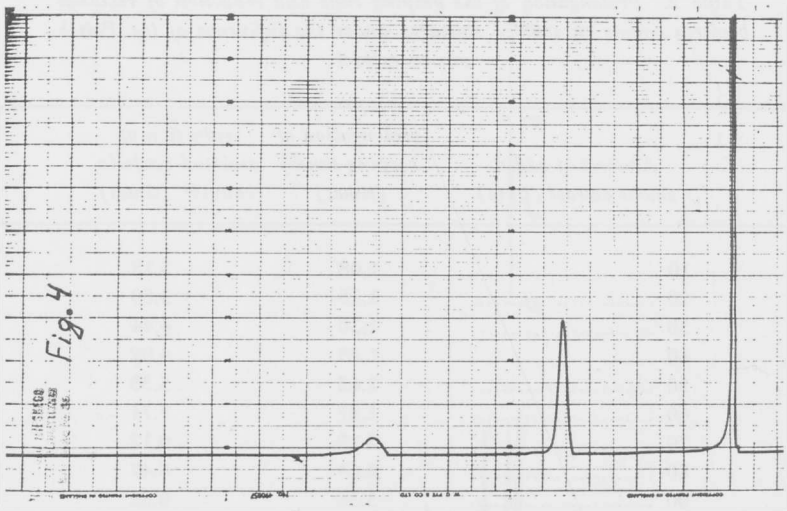

Fig. 4. *Non-phenolic compounds as in fig. 3, fortified with benzo(a)pyrene and dibenzo (a,h) anthracene, separation conditions as in fig 3.*

The most important feature, however, which the extract has to possess, is the lack of any known carcinogens. In this field the Polish Meat Research Institute has experience dating back since a couple of years, and is in close cooperation with the Trace Substance Commision of I.U.P.A.C. — Figures No. 3 and 4 are an illustration for the purity of the extract in this respect. Figure No. 3 represents a chromatogramme of the smoke extract alone, whereas figure No. 4 is a record of the same sample fortified with known amounts of benzo (a) pyrene and dibenzo (a, h) anthracene. The applied analytical procedure enabled to measure the amounts of benzo (a) pyrene at the level of 0.073 mcg, and the presence of dibenzo (a, h) anthracene at the level of 0,15 mcg. The chromatogrammes are a clear proof for the lack of said hydrocarbons and related substances in the smoke extract.

TOXICOLOGICAL PROPERTIES

The toxicological properties of the smoke extract were determined according to the FAO/WHO requirements (4, 5) on the following animal species:

a) acute toxicity tests — on mice, rats, cats, and pigs,

b) chronic toxicity tests — on rats, cats, and pigs.

As a result of the acute toxicity tests the following LD_{50} doses were established:

— mice: 2,750 mg/kg of body weight

— rats: 3,750 mg/kg of body weight
— cats: 192 mg/kg of body weight
— pigs: above 5,000 mg/kg of body weight.

Chronic toxicity tests were run at the following daily intakes of the smoke extract:

— rats: 5 %, 10 %, and 15 % of the LD_{50} dose
— cats: 5 %, and 15 % of the LD_{50} dose
— pigs: 100 mg/kg body weight, and 300 mg/kg body weight.

The smoke extract was administered »per os».

After 3 months of feeding the animals were sacrified, and their inner organs (hearts, livers, kidneys, spleen) were subjected to histopathological examination. During all the feeding period the animals were thorougly observed (appearance, behaviour) and the weight increase was controlled systematically. In the case of pigs systematic analyses of blood and urine were made, too. Control groups of animals of the same species were fed with the same diet, however without any addition of the smoke extract. The ante- and post-mortem treatment of these animals was identic with that of the control groups.

These prolonged feeding tests showed that there were no significant differences in the results of examinations between the experimental and the control groups of animals. This seems to be a proof for the statement that the consumption of the smoke extract in doses up to 15 % of the LD_{50} dose does not cause any intoxication of the organisms in the investigated species at prolonged feeding periods.

BIOLOGICAL INVESTIGATIONS

The performed biological examinations had to answer two questions, namely:

a) whether the consumption of the smoke extract bears any risk of inducing carcinomas,
b) whether the consumption of the smoke extract gives rise to any mutagenic effects, especially teratogenic effects.

The carcinogenic properties of the smoke extract were investigated on mice and rats, and in fact these experiments are still lasting. The experiments were started in March 1967 and the following breeds were taken:

a) White Wistar rats in the following three groups:
   experimental group — 65 animals
   control group I — 57 animals
   control group II — 60 animals
b) mice of the F_1 (RIII × C57BI) breed in the following three groups:

experimental group — 91 animals
control group I — 100 animals
control group II — 60 animals

The experimental groups of animals were fed for 6 days weekly with the following diets only:

rats — with sausages made from beef without any additives other than 0.1 % common salt, and 0.0111 % smoke extract. The sausages were dried unless they lost 70 % of their initial weight. The concentration of the smoke extract in the final product was 0.037 %.

mice — with a granulated standard fodder »LSM», fortified with 0.037 % of smoke extract.

Once weekly these diets were changed to vegetables.

Control groups I were fed with the same diets as the experimental groups, however, without any admixture of smoke extract.

Control groups II were fed with the granulated standard fodder »LSM» only.

The control of the daily intake of the granulated standard fodder »LSM» very early turned out to be unprecise, because this fodder crumbled easily and was spilt all around the bottom of the cages. However, on the basis of the daily supply of the cages with this fodder a rough estimate of the daily intake was made on the level of some 3 grammes per mouse, i.e. some 22,2 mg/kg of body weight.

Table 3. *Daily intake of sausage in experimental groups of rats (per capita)*

Group code		*Daily per capita intake of sausage (grammes per kg of body weight)*
1. Males:	D—4	45,80
	D—5	63.14
	D—6	61.49
	mean:	56.81
2. Females:	D—1	44.86
	D—2	45.31
	D—3	54.02
	D—19	44.82
	mean:	47.25

The daily intake of the sausages by rats could be very precisely controlled, and table No. 3 gives the average values for the respective subgroups of the

experimental group of rats. From these data follows that the male rats ate daily 56,81 grammes of sausages per 1 kg of body weight, which corresponds to 20,81 mg of smoke extract/kg of body weight. The females ate 47,25 g/kg of body weight, which corresponds to 16,48 mg of smoke extract/kg of body weight.

If we take into account the average per capita consumption of sausages and smoked meats in Poland* and assume that the mean body weight of the statistical consumer is 50 kg and the upper limit for the concentration of smoke extract is 150 mg/kg of product, then the daily intake of the average consumer is 0.18 mg of smoke extract per 1 kg of body weight. Comparing this value with those for rats and mice in the experiments on carcinogenic properties of the smoke extract, we see that these experiments were run at daily doses exceeding by 100 times the experted maximal daily intake of the extract by human consumers.

Although 2 years elapsed already from starting the above described experiments, many of the animals are still alive, and therefore the experiments are continued. However, the frequency of observed neoplasms in the experimental group was equal to that in the control groups (2 cases in each). A complete report will be published after termination of the experiments.

Investigations on mutagenic activity of the smoke extract are run on white Wistar rats, and actually we are on the step F-4 (fourth generation) without any observed visually, by means of X-rays, and histopathologically deviation from normality. Also these experiments are continuing and a full report will be printed after exhausting the research programme (up to F-6).

CONCLUSIONS

On basis of the experiments mentioned above, and which in the biological programme are still lasting, the authors can say:

1) The Polish smoke extract, apart from the fact of imparting the typical smoke flavour to meats, exhibits also a pronounced antioxidative activity and interesting bacteriostatic properties.

2) The smoke extract does not contain detectable amounts of carcinogenic hydrocarbons, and does not induce carcinoma in mice and rats fed regularly with doses exceeding by 100 times the expected upper daily intake of smoke extract by human beings.

3) The determined LD_{50} doses in four species of mammalians indicate that when applying the 100-fold safety margin, especially to those found for pigs, the smoke extract does not bear any risk of acute toxicity for people at daily intakes at least up to 50 mg per 1 kg of body weight. There is also no danger of chronic toxicity in prolonged consumption of the smoke extract, at least with daily intakes up to 15 % of the established LD_{50} doses.

4) Experiments run already on the fourth generation of white Wistar rats and indicate that the smoke extract most probably is free of any mutagenic activity.

*) From the statistical yearbook 1968 (6) follows that the per capita consumption of sausages and smoked meats in Poland in 1967 was approx. 0.03 kg. Assuming that 50 % of the population do not eat smoked goods, we have a daily consumption per capita of 0.06 kg.

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