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RECENT MEDICAL RESEARCH IN CZECHOSLOVAKIA

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RECENT MEDICAL RESEARCH IN CZECHOSLOVAKIA

/Following are translations of articles on the above subject, selected from a Czech source. Source information accompanies each article/

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METHODS OF INVESTIGATING RADIOACTIVITY IN FOOD

/Following is the translation of an article by M. Kruparova and A. Wolf in Ceskoslovenska hygiena, Vol VI, No 2-3, Prague, Mar 1961, pages 129-131./

Food radioactivity is a serious danger for the health of the population. The content of radioactive substances in foodstuffs has been proved to have been increasing in recent years. The increase is accounted for by accidental events in the use of nuclear energy and by nuclear test explosions, in which a large quantity of fissile products is created. Radioactive isotopes get into soil and water by means of natural fall-out, and from there enter vegetation, animal bodies, and eventually foodstuffs.

In studying the radioactivity of foodstuffs, we see that more important are elements with longer disintegration intervals, such as Sr^{90}, with its complex of strontium nuclides, and Cs^{137}. These elements represent almost 18% of all dispersed active products, are well resorbed, and accumulate in bones and muscles. The biological interval of cesium in men is 140 days; of strontium, 7.5 years. These are reliable indicators of the radioactive contamination of foodstuffs. For this reason greatest attention is given to them, although their investigation requires very sensitive measuring instruments and technically pretentious methods.

For practical use, especially because of the danger arising from imported foodstuffs, it was necessary to establish at least some basic criteria for their evaluation from the viewpoint of radioactivity. In this country Wolf, on the basis of our experiences and partly also of information gained from the literature, considers as harmless those foodstuffs whose over-all beta activity does not exceed the computed kalium activity by more than a probable measuring error, and also those in which the content of strontium muclides does not exceed 50 SSU and the content of Sr^{90}, 10 SSU; and finally those foodstuffs which have a content of Cs^{137} lower than 20 micro-microcuries/1 kg.

For investigating properly Sr^{90}, a number of methods were worked out. They are based on repeated coagulation with nitric acid, on chromatography, or recently also on the application of cathexes.

The chemical methods of investigating Sr^{90} were modified by Nosek, who suggested a procedure which is now used at the Faculty of Medicine in Hradec Kralove. The method consists of several operations.

They are: coagulation of Sr and Ca as carbonates, their conversion into nitrates, the removal of Ba as a chromate, removal of rare minerals by ferric hydroxide, and radiometry of Sr as a carbonate. This method is technically acceptable. A certain difficulty here is the more than 1000 g by-product of ash and the laboriousness of the operation.

The workers of the Institute of Occupational Hygiene and Diseases in Bratislava tried to modify the coagulation method based on the same principle, but for smaller amounts. This method was tested by us. It appeared that certain operations in this method should be somewhat modified, especially in view of a rather small amount of ash -- 1 g only.

At our institute a simple method has been developed for the needs of the KHES /Krajske Hygienicko-Epidemiologicke Stanice -- (Kraj Hygienic-Epidemiological Stations)/. This method may be applied where current mass-production equipment is available, e.g., at the KHES stations. These devices will record only strong contamination.

This rapid orientation method includes three operations:

1. Incineration of sample.
2. Ascertainment of kalium.
3. Radiometry of sample ash.

Re 1. Investigation of the ash is made by the dry method according to current procedures at a temperature which does not exceed 600°C. The sample must be of such a composition that it contains approximately 50 mg of kalium in radiometry. If we estimate the proportion of kalium in ash between 20 and 30%, we see that we need some 300 mg of ash for direct ascertainment. The upper limit of the ash sample is given by the capacity of the measuring cell, which is approximately 500 mg. If we want to do the ascertainment of kalium and the orientation separation of the other elements individually, we must use a double quantity.

Re 2. Ascertainment of kalium is done with a flame photometer. The ash is dissolved in hydrochlorid acid, and the solution is atomized into the flame of the photometer as a fine mist. The values which are found on the galvanometer are compared with the calibration curve.

In those laboratories where a flame photometer is not available, the Kohler method of ascertaining kalium may be used. Kalium coagulates from a slightly acid solution as kalium tetraphenyl borate, which is filtered, washed, and dissolved in acetone. This acetone solution reacts with mercury chloride, by which process hydrochlorid acid is disengaged. The disengaged acid is titrated to the Tashir indicator, and the result is expressed in stechiometric relation to kalium.

Re 3. Radiometry of the sample. An accurately weighed sample of ash, with a weight of 300 to 500 mg, in a round plexiglass container 3 mm high, and with a circumference equal to that of the opening of the Geiger-Muller counter, was covered with cellophane

on both sides, sealed, and spread evenly. The measuring was done by contact with a decadic reductor mark Tesla-Vrchlabi GM, a beta counter with a mass of 4.7 mg/cm^2, and a background of 20-25 impulses per minute. The measuring was done for ten minutes in turns with the measuring of the background. Each minute the values of the counter were taken down. If we began with the background, we obtained 110 values of the background and 100 values for measuring of the background and the sample. These values vary because the counter records apart from the data of the sample; also, impulses of cosmic radiation and possible radioactive sources may be in the vicinity. Not even with a zero background does the sample itself emit an equal radioactive energy; thus, the values vary with the time. For that reason the work must be made more accurate with the application of the law of large numbers. The computation of the quadratic error is done by comparing the sum of the second powers of the errors with the arithmetical mean of the measured value.

In order to render the results more accurate, it is possible to eliminate the activity of kalium, to compare the measured values with the content of calcium, and to express them in the relation of mimic/1 g Ca. Calcium is thus coagulated together with strontium and strontium nuclides as oxalates or carbonates; the substance is dried and measured radiometrically. For this measuring, however, it is necessary, owing to extremely low values, to use a more sensitive reductor. Calcium is investigated by the coagulation method with permanganate.

For control purposes it is not possible to apply any of the more sensitive methods, because the procedures involved are invariably time consuming and call for large samples. In trace quantities of strontium in the samples, the results obtained when small amounts are investigated fluctuate within the sphere of analytic errors. The cathex methods appear to be more suitable.

For a fast orientation we suggest the following procedure for investigating the radioactivity of foodstuffs:

1. Ascertainment of the over-all beta activity of ash with a Geiger-Muller counter.
2. Ascertainment of the kalium content.

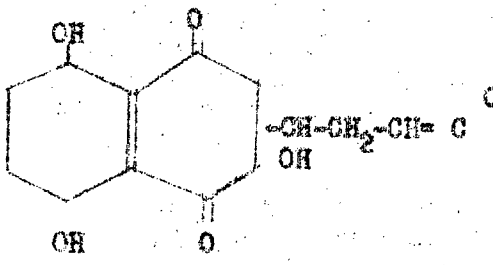
If the theoretically computed kalium activity does not exceed the actual value, then the possible measurement error of the tested object should be considered negligible.

By means of this simple method it will be possible to study the radioactivity of foodstuffs even in laboratories with average facilities; thus a good picture of the radioactive contamination of domestic and imported foodstuffs can be obtained.

ACUTE TOXICITY OF THE FOOD ADDITIVE ALKANNIN

/Following is the translation of an article by L. Hajlathova and A. Szokolay in Ceskoslovenska hygiena, Vol VI, No 2-3, Prague, Mar 1961, pages 132-134./

Alkannin belongs among the liposoluble food additives, the use of which is legally permitted. Illustration 1 shows its chemical structure and the countries in which it is used in food coloring. Since we did not find in the available literature any data on its toxicological properties, we deemed it proper to learn the necessary facts on the acute oral toxicity of this additive.



ALKANNIN

Permitted in:

Bolivia	Mexico
Costa Rica	Portugal
Czechoslovakia	Rumania
Dominican Republic	Switzerland
Egypt	Italy
Guatemala	Turkey
Chile	US
Israel	Great Britain

Illustration 1: Chemical structure of alkannin and countries in which it is used as a food additive.

For this we used mice of species H (both sexes, weight 18-20g) and Wistar rats (males, weight 170g). The additive was administered in a single dose to hungry animals in a stomach probe of olive oil which contained 15 weight units. The additive was extracted with petrolether p.a. from the ground roots of the plant Alkanna tinctoria L. Tausch. We wish to express here our thanks to the Institute of Medicinal Plants and Drugs of the School of Medicine in Budapest. The additive obtained in this way was a thick paste-like substance of a characteristic odor and containing alkannin, waxes, and fatty acids.

Immediately after the application we observed for ten minutes in the mice motor disturbances with the movements which usually precede vomiting. After a twenty-minute period with no symptoms, the motor disturbances arrived again, and at the end of the first hour they were accompanied with diarrhea and reddish-brown urine. In the following hours the motor disturbances were replaced by a slight

apathy with a lack of appetite, lasting in the surviving animals from one to four days, i.e., during the diarrhea period. The urine retained the above-mentioned color for two to six days. The visible parts of the skin and mucous membrane had a purplish tinge. The hairs had the color of wool used for cleaning silverware. In the animals that died, the apathy reached such a high degree that no responses to any stimuli were noted for two hours before death. Breathing was regular, but it slowed down ante finem. Out of the total number of animals used in the computation of the LD of 50, 55% of the males and 42.5% of the females died. The first animals perished 23 hours after the application, the last ones on the sixth day after the experiment. The females died mostly on the fourth day, the males on the fifth. Later deaths were not noted.

In studying the effect of alkannin on erythrocytes, we found out that in a dose of 1.4 g/1 kg of mice-weight it did not affect the count of red corpuscles and the formation of Heinz bodies.

All the animals, i.e., both the spontaneously deceased and the surviving ones, were subjected to a pathologic-anatomic examination. The latter were examined on the 40th day, after having been killed with an inhalation of ether narcosis. The section discoveries were selectively corroborated histopathologically. In the animals that died spontaneously, death came as a result of a diffusion of the fat degeneration of liver, which in isolated cases was connected with the fat degeneration of the kidneys and heart. Subcutaneous fat in the abdominal cavity had a roseate color. This discovery in the surviving animals did not differ from that of the control mice.

LD 50, established by a probity analysis, amounts to 2.8 g per 1 kg of males and 3.2 g per 1 kg of females. The following table sums up the data obtained from the experiments with mice.

Table 1. Summary of the acute toxicity of alkannin in mice.

Lethal acute poisoning	LD 50 per 1 kg of mice		Note
	♂	♀	
Immediately after application, irritation of the gastro-intestinal tract, after 60 minutes followed by diarrhea. Apathy, slowing down of breathing, death.	2.838 g limit	3.298 g	H.T. up to 1.4 g/1 kg
	2.025 g	3.086 g	negative
	3.989 g	3.627 g	Diarrhea ♂ from 1.4 g/1 kg ♀ 1.8 g/1 kg

In rats we were satisfied with the statement that 1 g of alkannin per 1 kg of rats has a mild laxative effect. We also noted a change in the color of urine similar to that of the mice.

In connection with the discoveries in the test animals, we studied the possibility of the chemo-analytic proof of alkannin in

urine and fat in microscopic quantities. To the analytic methods described in the literature, we applied spectrophotometry[4]. We studied the absorption characteristics of the additive itself in solution and the urine of the test animals. The maximum of the absorption curve of urine is in the ultraviolet region close to the maximum of alkannin, but in the visible area there are conditions at 500 mμ also for a quantitative determination of the additive without extraction from urine. For that reason we prepared for that wavelength a calibration curve for the direct investigation of the additive in urine. This curve showed a linear relation which could be evaluated statistically ($y=0.022+0.1023$; $Syx=0.018$). The analytic proof or the ascertainment of the additive directly in urine was favorable when the urine was diluted with ethanol to a proportion of 1:20.

In samples larger than 0.5 ml it is more advantageous to eliminate the additive with ether.

In cases where the specimens of urine were retained on the filtration paper and were supplied for the analysis in the form of dry remainders, the additive was also extracted with ethanol.

Another method of extraction was used for specimens of abdominal fat, namely the Hatos-Rom process. According to this method, the additive was extracted with petrolether and isolated with alkaline and acid admixtures.

In the course of the described experiments, the influence of three factors was noticed: alkannin, accompanying impurities, and olive oil. The influence of the last factor may be eliminated with the respective control animals. Another group of control animals for impurities was not used because in the respective experiments we tried primarily to collect pieces of information on the acute effect of alkannin; the petrolether extraction is one of the most current ones. Consequently, even a man would receive the same accompanying substances with the additive. For the above reasons we cannot distinguish the changes caused by alkannin from those conditioned by the accompanying substances. Among the latter, probably fatty acids and the remainders of petrolether act to the disadvantage of alkannin. Later, we shall study alkannin and the accompanying substances on one hand and olive oil on the other; we followed this approach for the sake of briefness in the description.

In the control animals we did not notice in any dose any symptoms of gastro-intestinal tract irritation. We assume from this fact that alkannin in the first phase of its reaction irritates locally the mucous membrane of the gastro-intestinal tract; this would account for pre-vomiting movements, diarrhea, and motor disturbances. The gradually increasing apathy originates in this phase and the process terminates in the damage of liver after resorption. The additive is at the same time excreted in faeces and urine, and partly stored in fat. Another way of excretion, through the skin, is possible (coloring of the hairs). For a

study of the interrelations in the excretion of the additive, the calibration curve constructed by us may be used.

The livid coloring of skin and mucous membrane is probably conditioned by alkannin; this has, even at the given pH, a different color. We do not exclude the possibility of combination with an increase in the quantity of reduced hemoglobin or with the presence of methemoglobin.

On the basis of our tentative experiments, alkannin may be placed (according to Hodge and Sterner) in the category of slightly toxic substances for both kinds of experimental animals. Alkannin meets the general requirements for acute toxicity of food additives; according to our requirements, the LD 50 for rats should be more than 2 g/kg. In further experiments it is necessary to concentrate on the study of the changes resulting from a repeated introduction of the additive with respect to a possible accumulation.

AMPEROMETRIC ESTIMATION OF CHLORIDES IN MEAT OF ANIMALS TREATED WITH BOVINOL AND ANTRIX

/Following is the translation of an article by A. Pasek, J. Marhoulova, and V. Jedlicka in Ceskoslovenska hygiena, Vol VI, No 2-3, Prague Mar 1961, pages 144-148./

The quantitative estimation of insecticides of the chlorated carbohydrates type on the basis of DDT and HCH is the subject of study of a number of authors. Most frequent are the methods based on a partial or complete removal of hydrochloric acid and its estimation. For released chlorides, various methods of estimation may be used, e.g., the colorimetric, polarographic, titration, and other methods.

In our work we used the method of splitting a molecule of chlorated hydrochloric acid by the formerly prepared combustion method of Jedlicka and Cerna. This method was supplemented in the final phase with the amperometric method of estimation of the released Cl^{θ} on a principle described by Kolthof and Lingane.

Experimental Part

Chemicals and solutions:

Commercial preparation Antrix: mixture of 14% DDT and 8% HCH in solvent naphtha.

Commercial preparation Bovinol: mixture of 14% DDT and 8% HCH in xylene and cyclohexanol.

Test solutions of Antrix and Bovinol were obtained by diluting the commercial preparations with benzene.

Standard solution DDT was obtained by dissolving 0.1002 g of pure DDT in benzene and bringing it up to the volume of 50 ml.

Standard solution NaCl was prepared by dissolving 0.5845 g of NaCl p.a. in redistilled water and bringing it up to the volume of 1000 ml.

Titration solution 0.01 M $Hg_2(NO_3)_2$ was prepared by dissolving of 5.25 g of $Hg_2(NO_3)_2$ p.a. in water with 1 ml of 32%-HNO_3 and bringing it to a volume of 1000 ml. To preserve the constancy of the factor a drop of mercury was added to the solution. The titer was estimated by the amperometric method in the standard solution of NaCl.

2 M H_2SO_4 p.a.
1 M HNO_3 p.a.
0.01 M NaOH with 0.5%-anhydrous Na_2SO_3
1%-jelly
Anhydrous Na_2SO_4
Twice distilled benzene
Glycerine.

Apparatus:

In the operations, a complete combustion furnace for elementary analysis (with a platinum catalyzer of the pyro-reaction and absorbers of the same type, as have been already described in one of previous works) was used. The amperometric titrations were done with the application of a mercurosulphate reference electrode and a normal mercury globule electrode with a reservoir height h= 62 cm, flow speed m= 4.199 mg/sec, and globule time t= 2.11 sec. The measuring of changes of the diffusion current was secured through a photographic registration of the luminary ray, reflected from the mirror of a sensitive galvanometer. The declination of the galvanometer over the entire polarogram was 2.60 μA.

Procedure:

The homogenized biological material in the amount of some 50-250 g is extracted three times with approximately 50-250 ml of of benzene to one extract. The extractions are combined and a major part of benzene is removed by distillation in a vacuum apparatus at a temperature of approximately +45°C. Thus the extract is concentrated to a small volume, which is quantitatively transferred into a measuring retort and brought up to a volume of 25 ml or, if possible, to 10 ml. The conditions of combustion and absorption of the combustible products were in conformity with the data mentioned in the earlier cited work of ours. After the combustion is finished, the absorbers are disconnected and their contents are quantitatively transferred into a 100 ml titration retort. The content is acidified with 2 M H_2SO_4 (in methyl orange as an indicator), 2.5 ml 1 M HNO_3 are added, and the substance is heated to the boiling point for some 5 minutes, until the sodium sulphite which did not enter the reaction decomposes. Afterwards the solution is quantitatively transferred into a 20 ml measuring retort; 0.5 ml 1 M HNO_3 and 1 ml of 1%-jelly is added, and after tempering the material is mixed with redistilled water to the volume of 20 ml. The cooled and modified absorption solution is introduced into a vessel with a mercurosulphate electrode and before titration is well mixed for approximately 5 minutes with a stream of nitrogen. It is titrated with a calibration solution 1×10^{-2} M $Hg_2(NO_3)_2$ at a zero potential. It is suitable after each addition of the titration activator to mix the solution with a stream of an inert

gas. The end of titration is characteristic of a rapid increase of the diffusion stream. The expected point of equivalence becomes very prominent and is easily read in the graphic evaluation of the photographic record as it is seen in the amperogram in Graph 1 (see following page). Before evaluation, it is necessary to consider the volume correction and calculate the values obtained by measuring according to the following formula:

$$V_k = \frac{V_o + V_t}{V_o},$$

in which V_k = corrected volume in ml V_o = original volume in ml, and V_t = volume of the quantity of the titration solution added to the tested material (in ml).

Results:

The results shown in Table 1 (see following page) may serve as a corroboration of the method of estimation of Cl' in various halogen compounds, both inorganic and organic. The results also indicate that the commercial preparations Antrix and especially Bovinol have, when compared with the standards, only a technical purity and therefore contain different quantities of the active substance. After obtaining of the mentioned favorable results, another check was made of the suitability of the methods for estimation of very small quantities of Cl' in various types of biological material, namely silage leaves, beef, milk, and urine. The above types of biological material represent a closed cycle of a possible rotation of the residual insecticide from the contaminated fodder to the excrements of the ruminant-consumer. The results of an analysis of the biological material, contaminated with standard NaCl, are given in Table 2 (see page 12). After obtaining the mentioned results, a similar biological material was contaminated either with the Antrix or Bovinol preparation. The results of the analysis document on one hand the data of Table 3 (see page 13) and on the other Graph 2 (see following page), with an indication of diffusion of the determined values of four independent measurings. After these checks of reliability and reproductibility of the respective method, analyses were made of the specimens of the meat of animals veterinary-treated with Antrix and Bovinol. In no case was there found any residuum of the contact insecticide which would be below the lower limit of sensitiveness of the method used, as is unmistakably proved by the results given in Graph 3.

Graph 1

Amperogram of three Specimens of Milk

1. Unobjectionable milk
2. Milk contaminated with Antrix
3. Milk contaminated with Bovinol

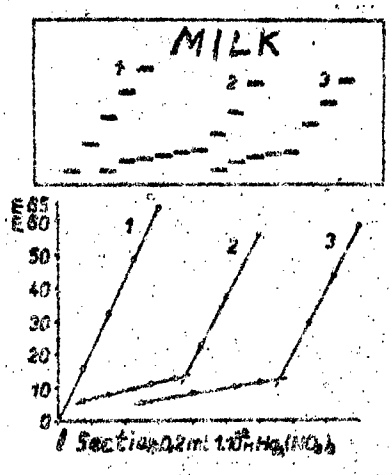
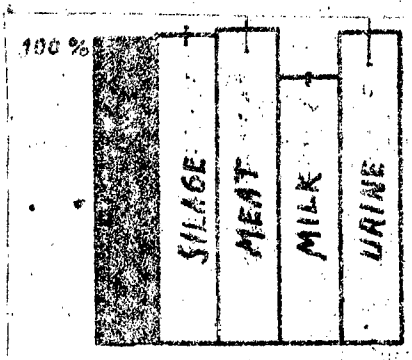
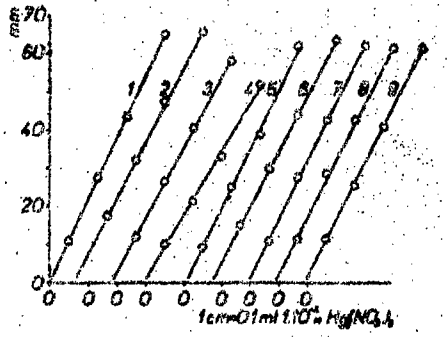


Table 1. Estimate of Cl' in Various Halogen Compounds

	Amount of Cl' in %		Expressed in %	Mean value in %
	Added	Found		
Standard NaCl	177,3 354,6	178,4 347,2	100,6 97,9	99,2
Standard DDT	500,0 500,0	498,5 504,8	99,7 100,3	100,3
Antrix	256,6 512,2 512,2	256,3 510,1 543,1	99,8 99,4 105,8	101,3
Bovinol	256,6 512,2 512,2	283,2 573,0 579,9	110,4 112,6 113,0	112,0



Graph 2. Estimate of Antrix and Bovinol in Biological Material



Graph 3. Representation of the Results of Analyses of nine Specimens of Meat of Animals treated with Antrix or Bovinol

Table 2. Analysis of Biological Material Contaminated in a Model Way With a NaCl Standard
/Columns:/ A -- Analyzed Type of Biological Material; B -- Quantity of Cl' in g ; B_1 -- Added; B_2 -- Found; C -- Expressed in %; D -- Mean value in %./
/Lines:/ I -- Silage; II -- Meat; III -- Milk; IV -- Urine.

Table 2. Analysis of Biological Material Contaminated in a model Way with a NaCl Standard

Type of Biological Material	Quantity of Cl' in g		Expressed in %	Mean Value in %
	Added	Found		
Silage	177.3	190.1	107.2	110.5
		201.7	113.8	
Meat	177.3	197.8	111.6	110.5
		194.0	109.4	
Milk	177.3	190.1	107.2	107.2
		190.1	107.2	
Urine	177.3	193.9	109.4	108.8
		192.0	108.3	

Discussion of the Results

One of the reasons motivating our contribution was the research work of the veterinary service workers of the okres Zilina and the workers of the J. Dimitrov Works in Bratislava; their object was to discover effective prophylactic measures against cattle gadflies through an application of suitable preparations of domestic production. The examined preparations, Antrix and Bovinol, are contact insecticides of domestic origin and made from dichlordiphenyltrichlorethan and hexachlorcyclchexan.

Our task was to suggest a suitable method of chemical estimation of residual quantities of the applied preparations in order to obtain reliable analytic data for a hygienic examination of the meat extracted from the treated animals.

The experimental solution of the problem of detecting the mentioned preparations in biological material falls into two stages:

1. The extraction of very small parts of the respective insecticide from the heterogenous mass of biological substratum with the subsequent splitting of the molecule of the isolated insecticide into reactive components.

Table 2. Analysis of Biological Material Contaminated in a model Way with a NaCl Standard

Type of Biological Material	Quantity of Cl' in γg		Expressed in %	Mean Value in %
	Added	Found		
Silage	177.3	190.1 201.7	107.2 113.8	110.5
Meat	177.3	197.8 194.0	111.6 109.4	110.5
Milk	177.3	190.1 190.1	107.2 107.2	107.2
Urine	177.3	193.9 192.0	109.4 108.3	108.8

Table 3. Estmate of Antrix and Bovinol in Model Specimens of Biological Material

Type of Biological Material	Added Amounts of			Found Amounts of			In %	Mean Value in %
	Antrix in mg	Bovinol in mg	Equals γg Cl'	Antr. in mg	Bovin in mg	Equals Cl' g		
Silage	1.628	-	208.9	1.60	-	205.6	98.4	101.5
				1.68	-	215.3	103.1	
	4.071	-	522.3	4.11	-	527.6	101.1	
				4.22	-	541.1	103.6	
Meat	-	-	208.9	1.71	-	219.2	104.9	103.3
	-	-		1.69	-	217.2	104.0	
	-	-	522.3	3.90	-	500.4	95.8	
				4.41	-	566.4	108.4	
Milk	-	1.795	230.3	-	1.49	191.3	88.1	87.5
	-	4.488	575.8	-	1.57	201.7	87.6	
				-	3.90	500.4	86.9	
				-	3.91	502.4	87.3	
Urine	-	1.795	230.3	-	1.86	238.6	103.6	102.5
				-	1.65	211.4	91.8	
	-	4.488	575.8	-	4.82	618.7	107.4	
					4.81	616.8	107.1	

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2. A suitable (if possible, objective and unequivocal) method of estimation of the looked-for residuum, which we solved by applying the amperometric principle of estimation of chlorides with photographic registration.

The amperometric method may be applied wherever the formation of practically insoluble or complex compounds may be expected, under the condition that at least one of the reacting substances renders a polarographic diffusion stream. The changes of the diffusion stream (which are in correlation with the concentration changes of the depolarizor) present in the solution may be very easily registered on a permanent photographic record. Before evaluation, the obtained values must be corrected by the volume coefficient.

The point of equivalence, unlike the potentiometric method of estimation, is very prominent and can be easily seen in the graphic evaluation of the photographic record.

EXPERIMENTAL CONTRIBUTION TO THE PROBLEM OF SOME PHYTONCIDES

/Following is the translation of an article by St. Hruby and L. Pelech in Ceskoslovenska hygiena, Vol VI, No 2-3, Prague, Mar 1961, pages 162-166./

Tokin defined phytoncides as substances of vegetable origin which possess antibacterial and antimycotic properties.

Dagis and associates, Tokin, Lesnikov, Osborn, and others made many experiments by which they prove that every vegetable tissue is phytocidal. The effectiveness, however, is different, owing to various influences. There may be differences between individual varieties of plants, etc. Tokin recommends phytoncides as preservative, or rather co-preservative, substances in the food industry. Kyzlink mentions strong phytocidal properties of certain macerated spices, especially mustard seed, cinnamon, coriander, and clove; he believes that by adding them to foodstuffs a relatively sizable antimicrobial protection is obtained. Also Chipault and associates, Junack, Kelch, and other authors ascribe to spices phytocidal properties which extend the life of meat and meat products. Rogacevova and associates studied the reduction of microflora by means of phytocidal factors of the normal components of the preserved foodstuffs, especially vegetable mixtures and spices, and demonstrated their considerable effectiveness. In addition to this, she discovered that phytoncides of certain less effective vegetables and spices are activated while being heated for sterilization purposes (and probably thereby they are released) and subsequently suppress the microorganisms even more. The antibiotic effects of volatile oils are described by Kabelik, Fimbinger, Burian, Sedlacek, and others.

In our department we studied the microbial flora of spices and also the antimicrobial and antimycotic effects of certain types of spices, such as clove, cinnamon, paprika, and mustard seed and their volatile oils or extracts.

The tests were made in solid and liquid media. Tested were B. subtilis, St. pyogenes aureus Ox., E. coli, and molds of the genus Rhysopus. In studying the effects of the volatile oils we worked with a 75% Ol. Cinnamoni, 82% Ol. Caryophyllorum, 95% Ol. Sinapis, and also with alcohol extracts of pepper and paprika. We investigated the effectiveness of various concentrations, namely from 1:1 to 1:100. The bactericidal effects were tested

with the paper-strip method. In work with liquid media, to bouillon we added suspensions of the test cultures and spices, volatile oils, or extracts. When studying the fungicidal effects of Ol. Caryophyllorum, we used for testing also Aspergillus nuger, Candida albicans, Absidia, Trychofyton gypseum, and Trychofyton purpureum; we compared its effectiveness with the effectiveness of certain common antimycotic preparations, such as Visorit, Fungicidin, Nitrofungin, benzoic acid, sorbic acid, etc.

In the result evaluation we discovered that the volatile oils and extracts had bactericidal and fungicidal effects, but it became apparent that there are considerable differences as far as the type is concerned; besides, even within the same type various effects upon various microorganisms were noted. The action of the volatile oils upon the individual groups of the tested microorganisms are shown in outline in Graphs 1-4. We notice that the maximum inhibition effect produced Ol. Sinapis, which in higher concentrations completely inhibited the growth of all the tested microorganisms. In lower concentrations the effects were considerably weaker and in the concentration 1:100 only very faint. The effects of the volatile oils of clove and cinnamon are considerably weaker than those of mustard seed, but the decrease of their effectiveness was much smaller. At the same time, Ol. Cinnamoni possesses better properties against St. pyogenes aureus Ox. and B. subtilis, whereas Ol. Caryophyllorum is more effective against E. coli and molds. Still weaker were the effects of the extracts of pepper and paprika. Undiluted extracts were somewhat effective against St. pyogenes aureus Ox. and E. coli, but with reduced concentration their effectiveness lessened rapidly. In experiments with spices proper, the bactericidal and fungicidal effects were not as uniform. The effects of clove and cinnamon, on one hand, were evident (cinnamon had a limited effect against molds); paprika, pepper, and mustard seed, on the other hand (except for a reduced growth of molds), were ineffective. And it is to be said that the test was not diluted more than 1:10, so that the concentration of the spices was very high.

With respect to a very strong suppression of the growth of molds with the application of Ol. Caryophyllorum, we compared its effectiveness with the effects of several common medical and food fungicides. The results are shown in Table 1, in which are recorded mean values of the widths of the sterile zones in milimeters. We found out that a mere 10% Ol. Caryophyllorum is, in comparison with other fungicidal products, much more effective. The differences are especially large in comparison with the effects of Vizorit, Nitrofungin, and Fungicidin on pathogenic molds.

Table 1. Mean widths of sterile zones in mm of Ol. caryophyllorum in comparison with various fungicides

	Asp. n.	Abs.	Cand. a.	Trich. g.	Trych. p.
Eugenol 10%	15	15	14	35	40
Sorbic acid 14.5%	2	11	9	21	21

	Asp. n.	Abs.	Cand. a.	Trich. g.	Trych. p.
Benzoic acid 50%	8.5	10	5.5	13	18
Visorit	6	6	1.5	3.5	1.5
Nitrofungin	9	10	5	3	6
Fungicidin	4	1	7	1.5	2

If we study the bactericidal and fungicidal effects of the spices and their volatile oils, which we subjected to investigation, we discover that the inhibition effects of the volatile oils are noticeable, but in a concentration of 1:100 they are very small and in the case of pepper and paprika even non-existent. In spices proper, there are present basically the same effects, but two important circumstances should be considered:

1. The concentration of the volatile oils in spices is relatively low and the inhibition effects in concentrations lower than 10% decrease considerably.

2. The amounts of spices which reach the foodstuffs are very small, so that no inhibition effect of the volatile oils upon microorganisms can be expected, for their concentrations are insignificant.

Table 2. Differences in the number of proteolytic sporulates in tested meat products

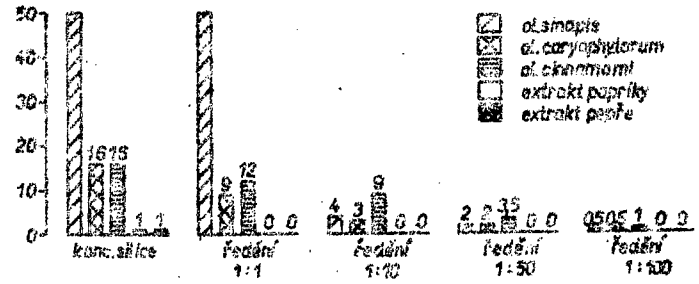
Product	Number of microorganisms in 1 g	
	Natural spices	Extracts
Kabanos	14.670	173
Moravian sausage	11.575	1.435
Bacon sausage	4.760	720
Diabetic frankfurters	3.000	232
Smoked sausage	3.530	415
Debrecen sausage	14.250	1.775
Polish sausage	6.035	1.825
Ham salami	3.750	500
Parisian salami	1.100	500
Prague salami	4.500	805

A seemingly paradoxical result was demonstrated in our work with mustard seeds in liquid media, where the increase of tested microorganisms developed practically in the same way as in the controls, even though Ol. Sinapis had the strongest bactericidal effects. The explanation is very easy if we realize that mustard seeds contain only 0.6% of volatile oil and that this concentration was further diluted with bouillon ten times. This is why we do not share the opinions of Tokin, Rogacev, and other authors, who recommend the use of spices for their phytocidal properties as a preservative or co-preservative of food. On the contrary, certain types

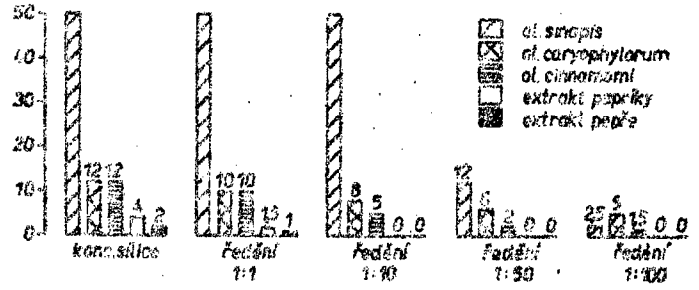
of spices, such as paprika, pepper, etc., are themselves carriers of such a large number of proteolytic sporulates that the addition of even small quantities to food means a considerable deterioration of its quality from the viewpoint of microbiology. Table 2 gives the differences in the number of proteolytic sporulates in tested meat products, in the production of which either normal natural spices or sterile spice extracts were used. The number of sporulates was much lower in the products of the latter group. Naturally, the storage time of meat products in which spice extracts were used will be longer. The bactericidal and fungicidal effects of volatile oils or extracts, however, could be used on a much larger scale than at present. For example, at the Dermatological Clinic of the School of Medicine of the Hradec Kralove University experiments are in progress aimed at the possibility of applying certain phytoncides in the treatment of mycoses. So far, the results showed that these substances, when applied in correct concentration, do not have any irritating effects and that it is possible to obtain good therapeutic results. Fifteen patients have been treated, and it appears that the time of treatment is quite small when compared with other commonly used therapeutic methods.

Conclusion

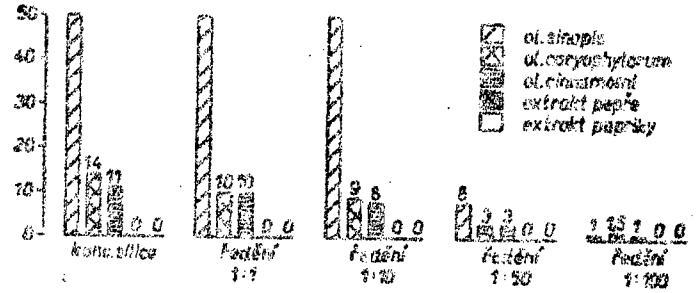
The phytoncidal effects of spices vary according to the type and especially the content of volatile oils. The amount of spices which reach the foodstuffs is very small, so that the normal low concentration of volatile oils in the spices is diluted to such an extent that practically no inhibition effect of volatile oil against the microorganisms is noted. For that reason the addition of spices to foodstuffs cannot be applied as a preservative or co-preservative measure. Certain volatile oils and extracts, however, may be used directly, both in the food industry and in medicine for treatment of certain mycotic diseases.



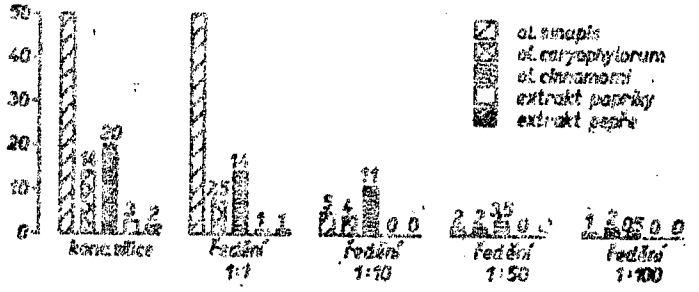
Graf 1. B. subtilis



Graf 2. E. coli



Graf 3. Rhisopus nig.



Graf 4. Staphylococcus pyogenes aureus Oxford

Graphs 1, 2, 3, 4

Legend:

konc. silice = Strength of volatile oil

ředění = Concentration

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