A PAPER CHROMATOGRAPHIC TECHNIQUE FOR SCREENING VOLATILE CHEMICALS FOR THEIR REACTIVITY WITH THE CONSTITUENTS OF FOODS

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Fumigants, a class of volatile chemicals which are basically toxic to insects and mammals, have been used for the control of insects in food and other commodities throughout the world[1]. The use of such fumigants in the control of microflora on food-grains has also been reported[2–5]. One of the most important factors governing the choice or rejection of any particular chemical as a fumigant is the extent of its chemical reactivity with constituents of the food or commodity being subjected to treatment.

Some fumigants are not only unstable, and decompose when sorbed by a commodity, but also react chemically to form new compounds, which can be either degradation or addition products with the constituents in the fumigated materials. These products may be toxic and remain in the commodity as residues[4–7]. Under the Food and Drug Laws of various countries, the amount of residue which can be permitted in different foodstuffs has been prescribed. In order to establish the tolerances for residues, data on the fate of the fumigant reaction products have to be collected. The methods or techniques which have been employed for such studies are extremely time consuming and difficult[6–8].

WINTERINGHAM *et al.*[8] found that when methyl bromide is used as a fumigant a portion is physically adsorbed which irreversibly combines with one or more constituents of wheat. The gluten or protein fraction of the wheat accounted for 80% decomposition. These studies involved combined radioactive tracer and chromatographic techniques.

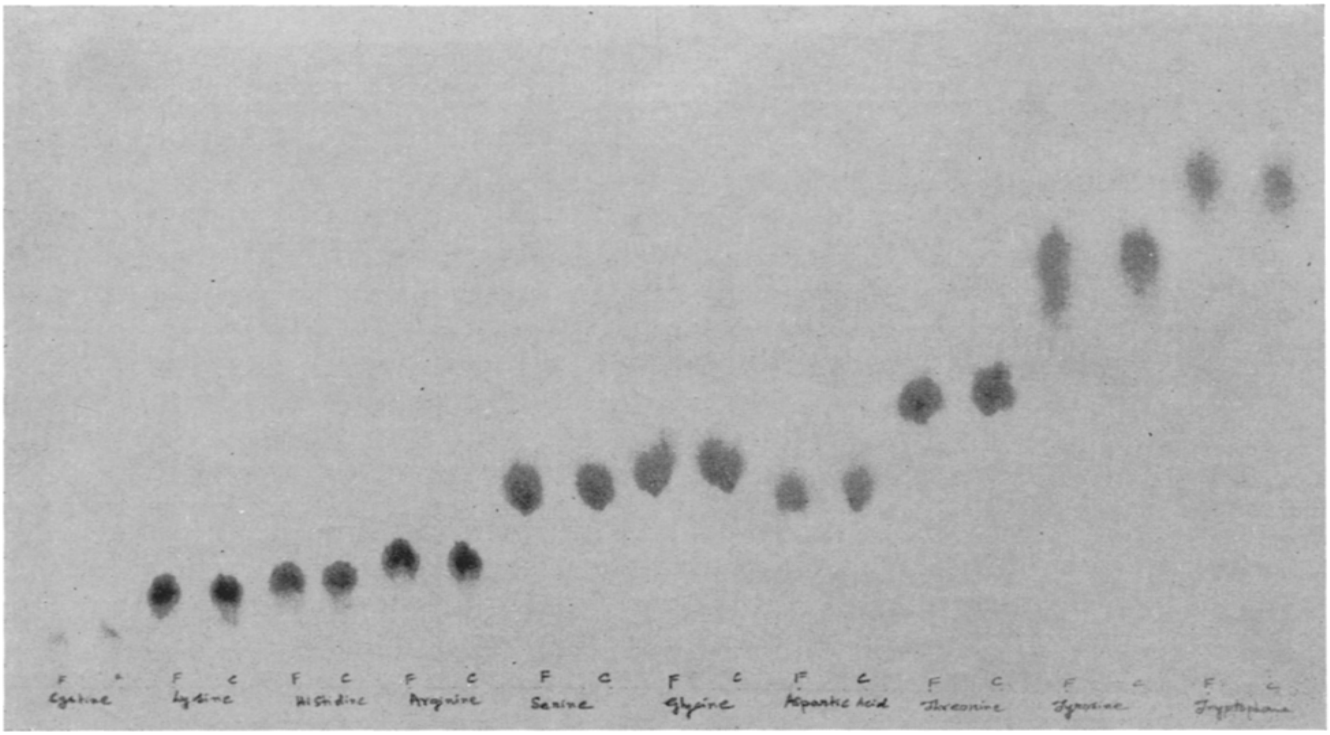
A high level of bromide residues was estimated in this laboratory[9,10] after the fumigation of cashewnuts, groundnuts and copra (dry coconut cups). Estimation of the amino acids of the fumigated and unfumigated defatted cashewnut, groundnut and coconut meals, by the paper chromatographic and colorimetric methods, showed a decrease in the concentration of methionine after fumigation[10].

In view of the above evidence of the reaction of a fumigant with amino acids it was of interest to examine paper chromatograms spotted with non-radioactive amino acids for reactivity with the gaseous chemicals during fumigation.

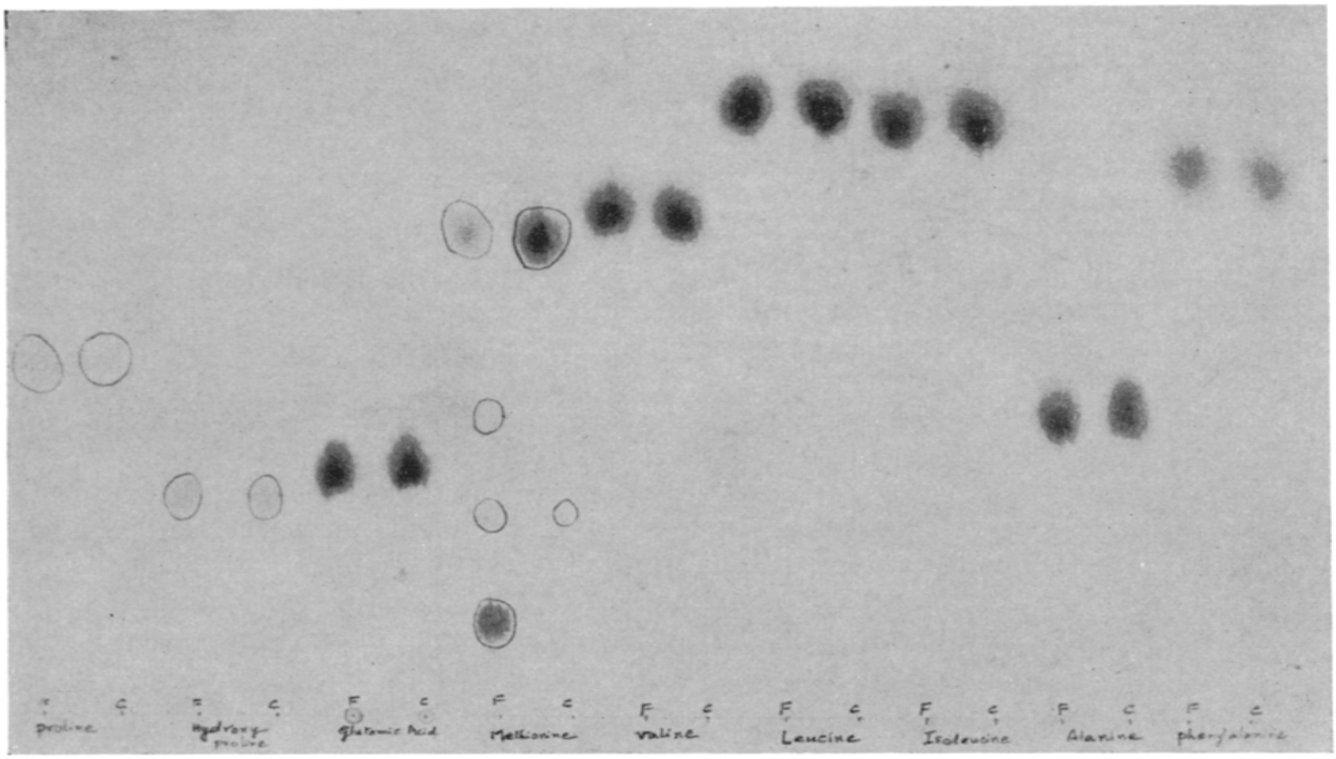
EXPERIMENTAL

Selection of an amino acid reactive with some fumigants

Ten gamma quantities of amino acids (aqueous solutions) were spotted on Whatman No. 1 filter paper (46 × 57 cm) leaving space for spotting their duplicates



(a)



(b)

Fig. 1. Reactivity of amino acids exposed for 100 h to methyl bromide in a concentration of 100 mg/l. F = fumigated; C = control. (a) From left to right: cystine, lysine, histidine, arginine, serine, glycine, aspartic acid, threonine, tyrosine, tryptophan; (b) From left to right: proline, hydroxyproline, glutamic acid, methionine, valine, leucine, isoleucine, alanine, phenylalanine.

TABLE I

REACTIVITY OF AMINO ACIDS WITH SOME FUMIGANTS: MB, EDB, MB + EDB AND EDCT* AT 100 mg/l, 25° FOR 100 h EXPOSURE

Sl. No.	*Amino acid*	*R_F values of the amino acids and additional spots if any after treatment with:*				
		MB	*EDB*	*EDB + MB*	*EDCT*	*Control*
1	Alanine	0.42	0.42	0.42	0.42	0.42
	Additional spots	Nil	Nil	Nil	Nil	Nil
2	Arginine	0.19	0.19	0.19	0.19	0.19
	Additional spots	Nil	Nil	Nil	Nil	Nil
3	Aspartic acid	0.27	0.27	0.27	0.27	0.27
	Additional spots	Nil	Nil	Nil	Nil	Nil
4	Cystine	0.09	0.09	0.09	0.09	0.09
	Additional spots	Nil	Nil	Nil	Nil	Nil
5	Glutamic acid	0.36	0.36	0.36	0.36	0.36
	Additional spots	Nil	Nil	Nil	Nil	Nil
6	Glycine	0.29	0.29	0.29	0.29	0.29
	Additional spots	Nil	Nil	Nil	Nil	Nil
7	Histidine	0.17	0.17	0.17	0.17	0.17
	Additional spots	Nil	Nil	Nil	Nil	Nil
8	Hydroxyproline	0.32	0.32	0.32	0.32	0.32
	Additional spots	Nil	Nil	Nil	Nil	Nil
9	Isoleucine	0.71	0.71	0.71	0.71	0.71
	Additional spots	Nil	Nil	Nil	Nil	Nil
10	Leucine	0.72	0.72	0.72	0.72	0.72
	Additional spots	Nil	Nil	Nil	Nil	Nil
11	Lysine	0.16	0.16	0.16	0.16	0.16
	Additional spots	Nil	Nil	Nil	Nil	Nil
12	Methionine	0.58	0.58	0.58	0.58	0.58
	Additional spots: 1	0.10	Nil	0.10	Nil	Nil
	2	0.22	0.22	0.22	0.22	0.22
	3	0.40	Nil	Nil	Nil	Nil
13	Phenylalanine	0.69	0.69	0.69	0.69	0.69
	Additional spots	Nil	Nil	Nil	Nil	Nil
14	Proline	0.47	0.47	0.47	0.47	0.47
	Additional spots	Nil	Nil	Nil	Nil	Nil
15	Serine	0.28	0.28	0.28	0.28	0.28
	Additional spots	Nil	Nil	Nil	Nil	Nil
16	Threonine	0.35	0.35	0.35	0.35	0.35
	Additional spots	Nil	Nil	Nil	Nil	Nil
17	Tryptophan	0.60	0.60	0.60	0.60	0.60
	Additional spots	Nil	Nil	Nil	Nil	Nil
18	Tyrosine	0.51	0.51	0.51	0.51	0.51
	Additional spots	Nil	Nil	Nil	Nil	Nil
19	Valine	0.61	0.61	0.61	0.61	0.61
	Additional spots	Nil	Nil	Nil	Nil	Nil

* MB = methyl bromide; EDB = ethylene dibromide; MB + EDB = a mixture of methyl bromide and ethylene dibromide; EDCT = ethylene dichloride–carbon tetrachloride.

after the fumigation of the spots. These spotted papers were subjected to the action of vapours of the fumigants at 25 ± 1° in a concentration of 100 mg/l in glass tubes for 100 h by the procedure described below.

The spotted papers were placed inside glass tubes (150 cm length, 5 cm diameter) fitted with glass stop cocks, terminating on the inside in small extraction thimbles which acted as evaporators for the liquid fumigants. To admit the fumigant, the tube

TABLE II

EFFECT OF DIFFERENT FUMIGANTS ON METHIONINE

Sl. No.	*Fumigants*	*Chemical formulae*	*No. of additional spots*	R_F *values*				*% Recovery of methionine*	*Extent of reaction (%)*
				1	*2*	*3*	*4*		
1	Acetone	CH_3COCH_3	—	—	0.22	—	—	95	5
2	Acrylonitrile	CH_2CHCN	—	—	0.22	—	—	98	2
3	Ammonia	NH_3	—	—	0.22	—	—	98	2
4	Aniline	$C_6H_5NH_2$	—	—	0.22	—	—	98	2
5	Benzene	C_6H_6	—	—	0.22	—	—	95	5
6	β-Propiolactone	$CH_2CH_2C{=}O$ (O)	2	0.09	0.22	—	0.89	62	38
7	Carbon disulphide	CS_2	—	—	0.22	—	—	98	2
8	Carbon tetrachloride	CCl_4	—	—	0.22	—	—	93	7
9	Chlorobenzene	C_6H_5Cl	—	—	0.22	—	—	89	11
10	Chloroform	$CHCl_3$	—	—	0.22	—	—	98	2
11	Chloropicrin	CCl_3NO_2	—	—	0.22	—	—	88	12
12	Ethyl acetate	$CH_3COOC_2H_5$	—	—	0.22	—	—	98	2
13	Ethyl acetoacetate	$CH_3COCH_2COOC_2H_5$	—	—	0.22	—	—	98	2
14	Ethyl bromide	C_2H_5Br	1	0.10	0.22	—	—	81	19
15	Ethyl formate	$HCOOC_2H_5$	—	—	0.22	—	—	98	2
16	Ethylene chlorhydrin	CH_2ClCH_2OH	—	—	0.22	—	—	98	2
17	Ethylene dibromide	$C_2H_4Br_2$	—	—	0.22	—	—	93	7
18	Ethylene dichloride	$C_2H_4Cl_2$	—	—	0.22	—	—	95	5
19	Ethylene dichloride–carbon tetrachloride (EDCT) (3:1, v/v)		—	—	0.22	—	—	93	7
20	Ethyl methyl ketone	$CH_3COC_2H_5$	—	—	0.22	—	—	95	5
21	Lindane (γ-BHC)	$C_6H_6Cl_6$	—	—	0.22	—	—	98	2
22	Menthol	$CH_3(C_3H_7)C_6H_9{-}OH$	—	—	0.22	—	—	93	7
23	Methyl alcohol	CH_3OH	—	—	0.22	—	—	98	2
24	Methyl bromide	CH_3Br	2	0.10	0.22	0.40	—	48	52
25	Methyl bromide + ethylene dibromide (EDB + MB) (1:1, w/w)	$CH_3Br + C_2H_4Br_2$	1	0.10	0.22	—	—	71	29
26	Methyl cyanide	CH_3CN	—	—	0.22	—	—	98	2
27	Methyl iodide	CH_3I	1	0.11	0.22	—	—	88	12
28	Hexane (normal)	$CH_3(CH_2)_4CH_3$	—	—	0.22	—	—	96	4
29	Naphthalene	$C_{10}H_8$	—	—	0.22	—	—	93	7
30	Nitrobenzene	$C_6H_5NO_2$	—	—	0.22	—	—	88	12
31	Nitromethane	CH_3NO_2	—	—	0.22	—	—	95	5
32	*p*-Dichlorobenzene	$C_6H_4Cl_2$	—	—	0.22	—	—	98	2
33	Phosphine	PH_3	—	—	0.22	—	—	97	3
34	Pyridine	C_5H_5N	—	—	0.22	—	—	98	2
35	Quinoline	$C_6H_4N{=}CHCH{=}CH$	—	—	0.22	—	—	94	6
36	Thymol	$CH_3(C_3H_7)C_6H_3OH$	—	—	0.22	—	—	93	7
37	Trichloroethylene	$CHCl{=}CCl_2$	—	—	0.22	—	—	98	2
38	Vapona (DDVP), O,O-dimethyl-2,2-dichlorovinyl phosphate	$CH_3O\diagdown\!\!\diagup P(=O)\diagup\!\!\diagdown OCHCCl_2$ (CH_3O)	1	0.10	0.22	—	—	83	17
39	Xylene	$C_6H_4(CH_3)_2$	—	—	0.22	—	—	89	11
40	Control (unfumigated)	—	—	—	0.22	—	—	98	2

was evacuated to a pressure of 2 in. absolute and the liquid fumigants were pipetted into the chambers by connecting the pipette to the stop-cock and opening the stop-cock to draw in the fumigant, at the same time restoring the atmospheric pressure. While administering methyl bromide essentially the same technique was adopted, but the fumigant was administered as a gas from a calibrated 2-way "Strand" type flask filled to atmospheric pressure with methyl bromide and displaced into the fumigation chamber by running calcium chloride saturated water into the flask[11]. When ethylene dibromide–methyl bromide mixture was used the micro-pipette and the "strand" flask were connected in series while dosing, the latter being disconnected while restoring atmospheric pressure[11].

In the experiment on the selection of a reactive amino acid for further studies only four commonly used fumigants were used. These were ethylene dibromide, methyl bromide, a mixture of ethylene dibromide and methyl bromide and a mixture of ethylene dichloride and carbon tetrachloride. After exposure to the fumigants and aeration of vapours and spotting the corresponding unfumigated amino acids alongside the treated ones, the chromatograms were irrigated with a butanol–acetic acid–water (4:1:5) solvent system by the descending chromatographic technique.[12] The spots were developed and examined for their R_F values, comparison being made with the corresponding unfumigated amino acids. On developing the paper chromatogram it was observed that out of the nineteen amino acids tested, additional spots were only observed on the paper chromatogram in the fumigated samples in the case of methionine (see Table I). Out of the fumigants tried in the experiment, indication of the presence of reaction products of methionine was most obvious in the chromatograms which were exposed to methyl bromide (see also Fig. 1). This led to the possibility of developing a new and rapid screening technique for the chemical reactivity or inertness of the fumigants with the constituents of foods.

Screening for reactivity of fumigants with methionine

A large number of chemicals (Table II) which are either conventional fumigants or have some prospect of being used as fumigants for disinfestation of foods were screened for their reactivity by exposing the chromatographic papers (28 × 8 cm) spotted with methionine (5 to 10 γ) in aluminium alloy pressure cookers (4.75 l) fitted with glass stop-cocks, terminating on the inside into small extraction thimbles which acted as evaporators for the liquid fumigants (see Fig. 2). The procedure for the application of the liquid and gaseous fumigants was the same as that followed in the previous experiment. In case of solids, weighed quantities of the crystals were placed inside the chamber and the lids were immediately closed. For the generation of phosphine the commercial preparation "Phostoxin" tablet (M/s Degesch) was used. All the chemicals were applied to give a nominal concentration of 100 mg/l, except in the case of β-propiolactone, which was applied at a concentration of 50 mg/l.

Unreacted methionine on the chromatograms was estimated by eluting the ninhydrin colour of the spots in 75 % alcohol and measuring the intensities in a Klett Summerson colorimeter[13]. R_F values of new reaction products resulting from the action of the fumigant with methionine and the extent of reaction after 100 h of exposure to a 100 mg/l concentration at 25 ± 1° were noted.

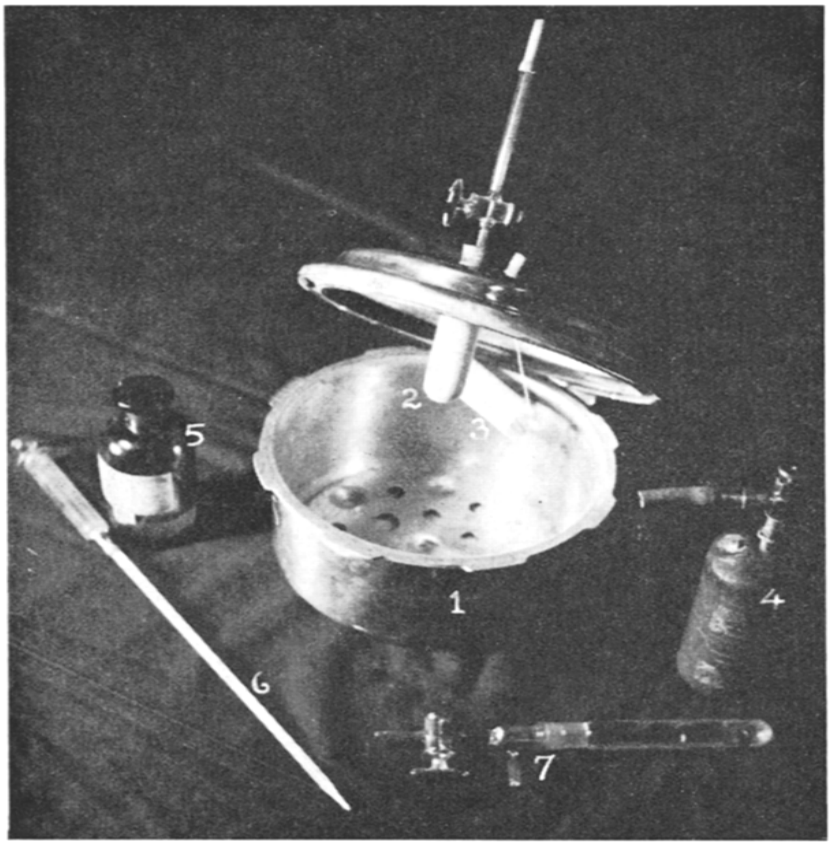
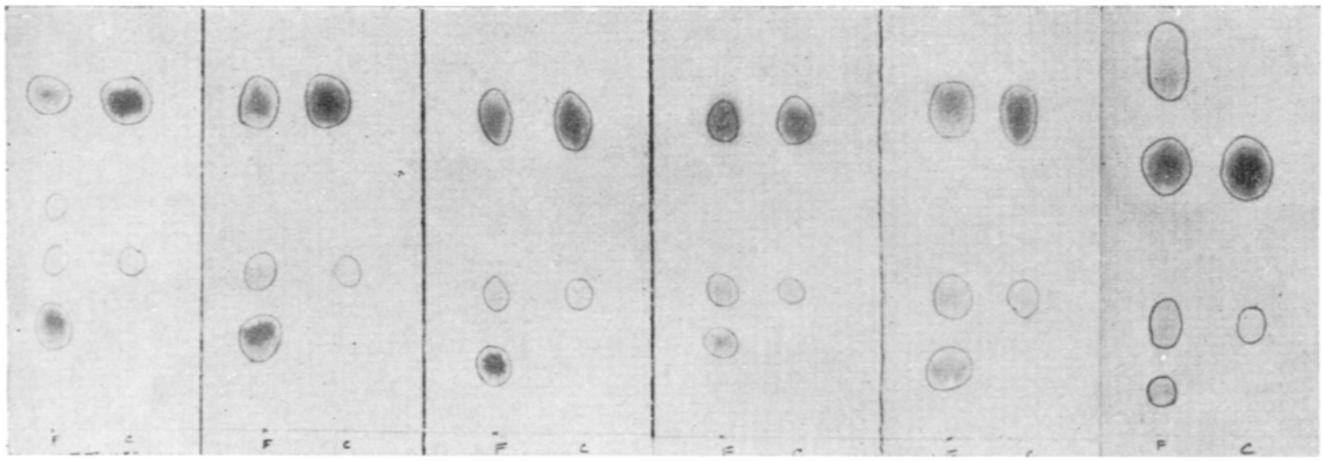


Fig. 2. Fumigation assembly consisting of: (1) fumigation chamber; (2) fumigant inlet; (3) amino acid spotted paper roll suspended from centre; (4) methyl bromide container; (5) ethylene dibromide; (6) micropipette for liquids; (7) "strand" type gas applicator.

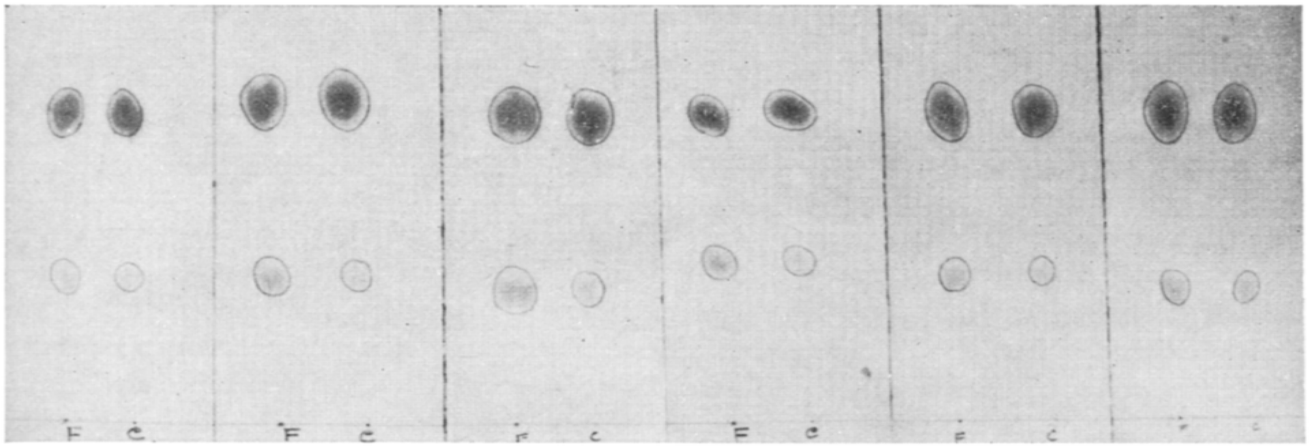
RESULTS AND DISCUSSION

Of the nineteen amino acids examined, methionine was found to be the most reactive amino acid with the fumigants used for screening (see Table I and Fig. 1). It was also noted that even the unfumigated spot of methionine gave rise to an additional spot on the paper chromatogram, which was present in all the experimental chromatograms. This common additional spot had an R_F value of 0.22 in all the chromatograms. Results in Table II revealed that depending on the reactivity of the fumigant with methionine, additional spots were present.

Fumigation with methyl bromide and β-propiolactone resulted in two spots of reacted products of methionine in addition to the common spots at R_F value of 0.58 (unreacted methionine) and R_F 0.22 (oxidised methionine). Vapona, ethyl bromide and methyl iodide reacted with the methionine spot and resulted in one additional spot indicating their reactivity with methionine even at room temperature (see Fig. 3a). The rest of the fumigants did not show discernible new spots of reaction products. Unreacted methionine was estimated from all chromatograms. The recovery data and the intensity of the spots indicated that chloropicrin, nitrobenzene, chlorobenzene, xylene, ethylene dichloride–carbon tetrachloride also reacted slightly with methionine. They also resulted in an increase in intensity of the common additional spot of R_F value 0.22 (see Fig. 3b).



(a)



(b)

Fig. 3. Effect of some fumigants on methionine. F = fumigated; C = control. (a) From left to right: methyl bromide, methyl bromide + ethylene dibromide, methyl iodide, ethyl bromide, vapona, β-propiolactone; (b) From left to right: chlorobenzene, nitrobenzene, chloropicrin, xylene, carbon tetrachloride, ethylene dichloride.

Since the additional spots which were ninhydrin positive only could be seen on the paper chromatogram, it is possible that the other reaction products of methionine, those which are ninhydrin negative, could not be detected by the procedure. The data on the recovery of methionine and also the extent of reactivity as presented in Table II give some indication about the total reaction brought about by the fumigant with the methionine spots. Further investigations will therefore be needed to obtain data on the different reaction products which are ninhydrin positive as well as those which are negative. However, the recovery data of the unreacted methionine from the chromatogram, after fumigation of the spots, could indicate the extent of reaction. Scanning the intensity and area of the spots would give direct measures of the extent of losses due to the reactivity of the fumigants.

Results presented in Tables I and II and Figs. 1 and 3 indicated that the reactivity with methionine could be obtained with extreme rapidity and reliability for fumigants for which no information is previously available. Out of the volatile

chemicals screened for their reactivity with methionine, the following showed a high degree of reactivity: methyl bromide, methyl iodide, ethyl bromide, β-propiolactone and vapona (Fig. 3a).

This new *in situ* reaction of chemical constituents of foods on the spotted chromatographic paper with the chemical vapours screened for use as fumigants for insect control in foodstuffs, seems to offer a simple, reliable and extremely sensitive technique for investigating the possibility of their safe use. As non-reactivity with the component of food is a very desirable property for a chemical to be applied in fumigation of foodstuffs, the candidate chemicals can be selected on the basis of the direct evidence as obtained by this new technique. The essential constituents such as proteins, sugars, vitamins, fats and minerals should not be reacted upon by the fumigants so as to render them either unavailable as nutrients or make them toxic. Hitherto the techniques which are employed for the reactivity of fumigants are indirect and extremely laborious. Due to this fact, very little is known even about the fate and nature of residues of even the commonly used fumigants. With this technique the newly introduced fumigants such as vapona (DDVP), methyl iodide, β-propiolactone and many others could be studied for their reactivity with methionine even in microquantities of as low as 5 γ.

The chemical constituents of foods which can be detected and estimated by a paper chromatographic technique can be subjected to the action of fumigant vapour and examined for their reactivity by the technique described in this communication. This seems to have opened a vast possibility for rapid screening of candidate fumigants for their safety for use on foods. Many plant products, drugs, and foods require disinfestation treatment for safe storage but before they are subjected to the action of these volatile chemicals, quick and reliable information about their non-reactivity with any particular constituents for which the materials are valued should be obtained. In such cases this type of technique seems to offer great scope.

Similar studies using this technique are in progress with vitamins, sugars and fatty acids, for their susceptibility to reaction with fumigants.

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SUMMARY

A new technique is reported on the rapid screening of fumigants for their reactivity or non-reactivity with chemical constituents of foods particularly those which can be studied by paper chromatography. In the present study, extremely low quantities of amino acids spotted on paper and exposed to the volatile chemicals indicated high reactivity of methyl bromide, methyl iodide, ethyl bromide, vapona and β-propiolactone with methionine, after development of the chromatogram. This chromatographic technique offers good scope for picking out the non-reactive fumigants with ease, rapidity and reliability. It can be used as a tool for predicting the

reactivity of the chemical with the constituents of the food, and the residues which a fumigant would leave in fumigated foodstuffs during exposure.

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