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Effect of Cooking and Storage on Phospholipids in Broiler

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Phospholipids from thigh and breast muscles stored at 4° and -10°C were fractionated by thin layer chromatography. Breast muscle had more phospholipid than thigh muscle. Phosphatidyl choline and phosphatidyl ethanolamine were the predominant phospholipids. Presence of lysophosphatidyl ethanolamine fraction was noted in lipid of broiler. Cooking and storage decreased all the phospholipids with simultaneous increase of free fatty acids and TBA values. The rate of decrease in phospholipid was more at 4°C than at -10°C of storage.

Phospholipids play an important role in controlling the quality of poultry meat. Cooking of broiler in oil and storage at various temperatures are known to cause the breakdown of phospholipids[1-3]. Differences in total phospholipid content of thigh and breast muscles have been reported[4], but detailed phospholipid composition has not been studied separately in the two muscles. In the present investigation, the fractions of phospholipids from thigh and breast muscles of broiler were estimated and the changes occurring in phospholipids and thiobarbituric acid (TBA) values due to cooking with steam under pressure and storage at 4° and -10°C for various periods were studied.

Materials and Methods

Experimental method followed was as described in an earlier paper.[5] Samples were taken in triplicate for estimation of phospholipids, free fatty acids (FFA) and TBA values. Thin layer chromatography (TLC) of the samples was carried out on Silica gel G plates (250μ thickness) using the solvent system — chloroform : methanol : 7M ammonium hydroxide in the ratio of 115:45:7.5 (V/V/V)[6]. The phospholipid spots were identified by comparing their R_f values with authentic standard co-chromatographed with each run. The procedure of Bartlett[7] as modified by Marinetti[8] was used for the estimation of phospholipid phosphorus. FFA were estimated colorimetrically according to the method of Duncombe[9]. For estimation of TBA value, methods of Tarlandgis *et al.*[10] and Holland[11] were followed.

Results and Discussion

Values of total lipid and phospholipid contents in the thigh and breast muscles are presented in Table 1. Phospholipd content in breast muscle was more than in the thigh muscle. The variance between muscles was significant ($P < 0.01$). Phospholipid contents when expressed as percentage of total lipid, were 60 and 45 in breast and thigh muscles respectively. Similar values for total phospholipids in fresh muscles of broiler were reported[3,4,12]. Phospholipid content in breast muscle was higher as compared to that in thigh muscle[4,12]. A greater proportion of phospholipd in breast as compared to thigh muscle suggested that the level of phospholipid is inversely related to the level of total lipid[13].

Cooking decreased the total phospholipid content in both breast and thigh muscles (Table 1). This was in agreement with the previous reports in poultry[2] and beef[14]. The loss of phospholipids on cooking could be due to their hydrolysis as suggested by Lee and Dawson[2].

During storage at 4° and -10°C, the phospholipid contents of thigh and breast tissues decreased and the FFA increased (Table 2). Lypolysis of phospholipids and formation of FFA during frozen storage was reported in poultry[1], beef[15] and fish[16,17]. Fishwick[18] observed that lipases and phospholipases were active in turkey muscle stored at -10° and -20°C. Cooking of cod fillet for 30 min at 100°C did not result in any appreciable loss of phospholipase activity[19]. In the present study, meat was cooked at a higher temperature (122°C) and pressure (1.1 kg/cm^2) for 8 min. Whether the loss of phospholipids on storage in cooked sample in the present experiment was due to action of residual phospholipases or to the non-enzymatic oxidation of lipid, is not certain since no enzyme assay was carried out.

The total phospholipids of thigh and breast muscles were fractionated on TLC into phosphatidyl inositol (PI),

TABLE 1. TOTAL LIPIDS* AND PHOSPHOLIPIDS* OF RAW AND COOKED BROILER MEATS DURING STORAGE

Storage temp. (°C)	Storage period (days)	Thigh				Breast			
		Raw		Cooked		Raw		Cooked	
		Total lipid	Phospholipid	Total lipid	Phospholipid	Total lipid	Phospholipid	Total lipid	Phospholipid
4	0	20.71 ±0.43	9.47 ±0.20	21.82 ±0.20	8.85 ±0.06	17.98 ±0.49	10.96 ±0.05	20.51 ±0.24	10.31 ±0.29
,,	3	21.14 ±0.40	8.81 ±0.30	22.26 ±0.52	8.07 ±0.43	18.62 ±0.23	11.13 ±0.24	20.38 ±0.48	8.93 ±0.41
,,	6	21.44 ±0.85	7.57 ±0.49	22.05 ±0.57	6.90 ±0.20	19.20 ±0.66	9.99 ±0.13	20.74 ±1.00	8.02 ±0.59
,,	9	23.80 ±1.28	5.66 ±0.38	22.05 ±0.51	4.90 ±0.17	19.18 ±0.50	6.78 ±0.38	24.40 ±0.43	5.93 ±0.19
−10	30	23.48 ±0.60	8.46 ±0.43	25.66 ±0.21	5.86 ±0.93	20.59 ±0.31	10.87 ±0.31	24.20 ±0.24	10.30 ±0.19
,,	60	21.43 ±0.80	7.31 ±0.37	25.52 ±0.25	5.87 ±0.33	21.24 ±0.58	9.96 ±0.18	22.42 ±1.49	8.82 ±0.24
,,	90	24.36 ±0.67	5.85 ±0.31	25.48 ±0.80	5.11 ±0.13	23.27 ±0.46	9.25 ±0.43	23.29 ±1.15	6.50 ±0.22

*mg/g of wet tissues.

TABLE 2. CHANGES IN FREE FATTY ACIDS* IN BROILER MEATS DURING STORAGE

Storage temp. (°C)	Storage period (days)	Thigh		Breast	
		Raw	Cooked	Raw	Cooked
4	0	0.623 ±0.027	0.327 ±0.012	0.317 ±0.042	0.290 ±0.010
,,	3	0.830 ±0.021	0.387 ±0.012	0.547 ±0.044	0.380 ±0.017
,,	6	1.217 ±0.124	0.697 ±0.009	1.500 ±0.064	0.847 ±0.032
,,	9	1.627 ±0.050	0.787 ±0.072	1.573 ±0.019	0.973 ±0.055
−10	30	1.983 ±0.170	1.420 ±0.217	1.187 ±0.045	0.920 ±0.046
,,	60	1.990 ±0.399	1.557 ±0.133	1.533 ±0.232	1.220 ±0.089
,,	90	2.667 ±0.017	1.793 ±0.397	1.693 ±0.358	1.193 ±0.321

*mg/g of wet tissues.

phosphatidyl serine (PS), lysophosphatidyl choline (LPC), lysophosphatidyl ethanolamine (LPE), sphingomyelin (SPH), phosphatidyl choline (PC), phosphatidyl ethanolamine (PE) and phosphatidic acid (PA). The averages of different fractions in raw and cooked thigh muscles of broiler are presented in Tables 3 and 4 respectively. The averages of phospholipid fractions of raw and cooked breast muscles are presented in Tables 5 and 6 respectively. PC and PE were the major phospholipid fractions. PC was the highest followed by PE, PA, LPE, LPC, SPH and PI + PS in a decreasing order. A significant difference was observed in PC content of the thigh and breast muscles ($P<0.01$).

The extent of phospholipid fractions was similar to those reported in broiler[1,2,20]. The LPE fraction was not isolated in broiler muscle by previous workers. Presence of LPE in beef muscle was observed[14]. In the present study the presence of LPE in broiler muscle has been confirmed by comparing its R_f value with authentic LPE standard (Sigma) co-chromatographed along with experimental sample (Fig 1) and ninhydrin spraying[21].

Cooking decreased all the phospholipids except PI+ PS and LPC which showed increase. These results are in agreement with those reported by Lee and Dawson[2].

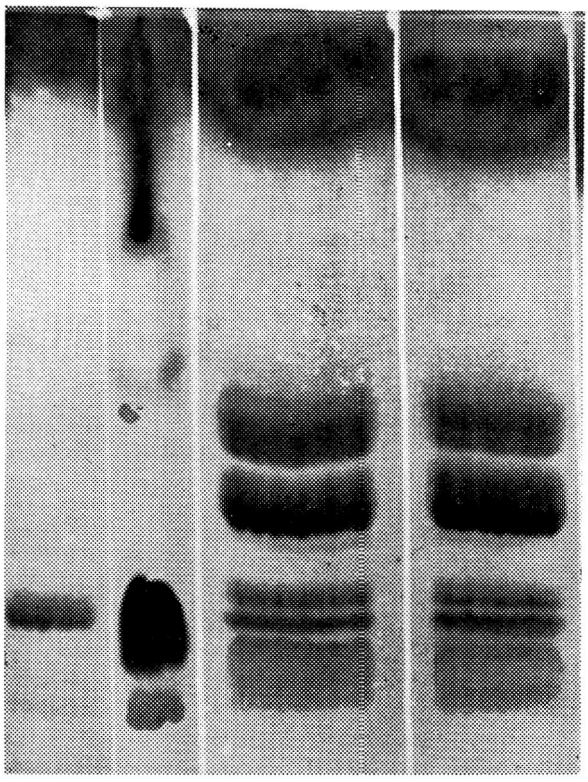

Fig. 1. TLC showing LPE Fraction of Phospholipid in Broiler

LPE = Lysophosphatidyl ethanolamine; LPC = Lysophosphatidyl choline; S 2 = Sample from breast muscle; S 3 = Sample from thigh muscle.

The regression coefficient of different fractions of phospholipid on storage time showed a significant decrease in PC,PE and PA fractions of raw and cooked meat stored at 4° and -10°C. In raw thigh and breast muscles there was increase in LPC fraction. There was a significant increase in LPE fraction of all muscles except in the cooked breast on storage at -10°C. Similar findings were also reported by Davidkova and Khan[1]. Since LPC and LPE are the hydrolytic products of PC and PE respectively[22], an increase of LPC and LPE and a decrease of PC and PE suggested that hydrolysis occurred in muscle phospholipid during cooking and storage. The rate of hydrolysis was greater at 4°C than at -10°C. Braddock and Dugan[23] also reported that the hydrolysis of lipid was affected by factors such as temperature and oxidation reaction.

TBA value: TBA values in thigh and breast muscles of broiler are presented in Table 7. Thigh muscle had significantly higher TBA value than the breast muscle ($P<0.05$). With the help of a meat model system, Igene and Pearson[24] showed that the samples containing more total lipid exhibited higher TBA value.

TBA value significantly increased after cooking both the muscles ($P < 0.01$). A progressive increase in TBA value was noticed on storage. The increase was more in the cooked meat than in the raw meat. The high TBA values obtained in the samples from cooked broiler are in agreement with the values reported by Greene and Watts[25], and Jacobson and Koehler[26]. The marked increase in TBA value and decrease of phospholipid

TABLE 3. CHANGES IN PHOSPHOLIPIDS* IN RAW THIGH MEAT OF BROILER DURING STORAGE

Storage temp. (°C)	Storage period (days)	PI+PS	LPC	LPE	SPH	PC	PE	PA
4	0	0.130 ±0.014	0.288 ±0.013	0.334 ±0.005	0.205 ±0.008	4.449 ±0.073	3.582 ±0.362	0.478 ±0.022
,,	3	0.427 ±0.039	0.597 ±0.018	0.343 ±0.049	0.158 ±0.024	4.064 ±0.145	3.004 ±0.129	0.276 ±0.003
,,	6	0.322 ±0.053	0.541 ±0.023	0.234 ±0.012	0.114 ±0.029	3.524 ±0.216	2.575 ±0.169	0.315 ±0.013
,,	9	0.143 ±0.013	0.715 ±0.062	0.429 ±0.034	0.208 ±0.015	2.291 ±0.155	1.636 ±0.111	0.257 ±0.109
-10	30	0.320 ±0.056	0.673 ±0.147	0.585 ±0.087	0.403 ±0.018	3.430 ±0.184	2.557 ±0.223	0.483 ±0.051
,,	60	0.148 ±0.012	0.515 ±0.066	0.716 ±0.051	0.306 ±0.017	3.198 ±0.200	2.130 ±0.112	0.293 ±0.044
,,	90	0.118 ±0.010	0.414 ±0.062	0.573 ±0.035	0.246 ±0.017	2.557 ±0.142	1.705 ±0.089	0.236 ±0.040

PI - Phosphatidyl inositol
PS - Phosphatidyl serine
LPC - Lysophosphatidyl choline
LPE - Lysophosphatidyl ethanolamine
SPH - Sphingomyelin
PC - Phosphatidyl choline
PE - Phosphatidyl ethanolamine
PA - Phosphatidic acid.

*mg/g of wet tissue.

TABLE 4. CHANGES IN PHOSPHOLIPIDS* IN COOKED THIGH MEAT OF BROILER DURING STORAGE

Storage temp. (°C)	Storage period (days)	PI + PS	LPC	LPE	SPH	PC	PE	PA
4	0	0.405 ± 0.067	0.469 ± 0.018	0.252 ± 0.004	0.118 ± 0.029	4.083 ± 0.032	3.464 ± 0.142	0.069 ± 0.013
,,	3	0.585 ± 0.076	0.574 ± 0.041	0.242 ± 0.013	0.269 ± 0.028	3.366 ± 0.212	2.524 ± 0.117	0.509 ± 0.019
,,	6	0.362 ± 0.023	0.404 ± 0.046	0.752 ± 0.029	0.203 ± 0.006	3.299 ± 0.099	1.600 ± 0.047	0.274 ± 0.027
,,	9	0.348 ± 0.005	0.390 ± 0.026	0.363 ± 0.062	0.233 ± 0.012	2.347 ± 0.068	0.870 ± 0.012	0.299 ± 0.049
-10	30	0.138 ± 0.017	0.200 ± 0.031	0.333 ± 0.068	0.315 ± 0.039	2.735 ± 0.483	1.899 ± 0.258	0.239 ± 0.078
,,	60	0.116 ± 0.035	0.233 ± 0.014	0.478 ± 0.080	0.228 ± 0.015	2.862 ± 0.146	1.630 ± 0.064	0.335 ± 0.018
,,	90	0.098 ± 0.028	0.202 ± 0.006	0.414 ± 0.058	0.199 ± 0.015	2.487 ± 0.048	1.419 ± 0.025	0.291 ± 0.007

*mg g of wet tissues.

TABLE 5. CHANGES IN PHOSPHOLIPIDS* IN RAW BREAST MEAT OF BROILER DURING STORAGE

Storage temp. (°C)	Storage period (days)	PI + PS	LPC	LPE	SPH	PC	PE	PA
4	0	0.259 ± 0.021	0.278 ± 0.041	0.436 ± 0.117	0.210 ± 0.049	5.094 ± 0.240	3.858 ± 0.069	0.825 ± 0.028
,,	3	0.338 ± 0.029	0.416 ± 0.084	0.434 ± 0.149	0.200 ± 0.091	5.202 ± 0.161	3.835 ± 0.033	0.708 ± 0.028
,,	6	0.438 ± 0.013	0.438 ± 0.013	0.234 ± 0.037	0.269 ± 0.126	4.155 ± 0.016	3.924 ± 0.105	0.532 ± 0.055
,,	9	0.186 ± 0.011	0.398 ± 0.076	0.356 ± 0.021	0.354 ± 0.023	2.622 ± 0.169	2.088 ± 0.156	0.436 ± 0.039
-10	30	0.366 ± 0.068	0.555 ± 0.085	0.711 ± 0.009	0.641 ± 0.019	4.633 ± 0.206	3.098 ± 0.015	0.679 ± 0.019
,,	60	0.585 ± 0.122	1.162 ± 0.109	1.100 ± 0.039	0.838 ± 0.046	4.201 ± 0.047	1.866 ± 0.024	0.206 ± 0.005
,,	90	0.545 ± 0.119	1.081 ± 0.118	1.022 ± 0.506	0.779 ± 0.048	3.900 ± 0.159	1.733 ± 0.074	0.192 ± 0.008

*mg g of wet tissues.

TABLE 6. CHANGES IN PHOSPHOLIPIDS* IN COOKED BREAST MEAT OF BROILER DURING STORAGE

Storage temp. (°C)	Storage period (days)	PI+PS	LPC	LPE	SPH	PC	PE	PA
4	0	0.332 ±0.040	0.304 ±0.026	0.413 ±0.086	0.294 ±0.043	4.792 ±0.209	3.412 ±0.530	0.750 ±0.058
,,	3	0.290 ±0.013	0.405 ±0.011	0.322 ±0.012	0.900 ±0.004	4.045 ±0.207	3.241 ±0.149	0.536 ±0.029
,,	6	0.298 ±0.006	0.272 ±0.014	0.611 ±0.154	0.443 ±0.077	3.820 ±0.326	2.124 ±0.248	0.535 ±0.028
,,	9	0.296 ±0.031	0.357 ±0.042	0.480 ±0.060	0.237 ±0.007	2.874 ±0.890	1.240 ±0.040	0.439 ±0.020
10	30	0.355 ±0.054	0.379 ±0.007	0.626 ±0.013	0.348 ±0.038	4.998 ±0.919	2.883 ±0.142	0.709 ±0.033
,,	60	0.236 ±0.028	0.293 ±0.016	0.556 ±0.037	0.282 ±0.002	4.342 ±0.068	2.550 ±0.117	0.620 ±0.030
,,	90	0.173 ±0.018	0.216 ±0.010	0.407 ±0.025	0.208 ±0.008	3.201 ±0.102	1.834 ±0.059	0.457 ±0.020

*mg/g of wet tissue

TABLE 7. CHANGES IN TBA (mg MALONALDEHYDE/1000G OF MEAT) VALUES IN RAW AND COOKED BROILER MEAT DURING STORAGE

Storage temp. (°C)	Storage period (days)	Raw		Cooked	
		Thigh	Breast	Thigh	Breast
4	0	0.320 ±0.025	0.300 ±0.006	0.823 ±0.009	0.703 ±0.047
,,	3	0.637 ±0.032	0.450 ±0.021	0.723 ±0.078	0.597 ±0.009
,,	6	1.340 ±0.058	0.997 ±0.057	1.963 ±0.054	1.067 ±0.097
,,	9	2.757 ±0.088	2.377 ±0.199	3.900 ±0.361	2.283 ±1.432
-10	30	0.353 ±0.029	0.290 ±0.031	1.493 ±0.079	1.063 ±0.094
,,	60	1.837 ±0.098	1.647 ±0.048	3.383 ±0.088	2.760 ±0.049
,,	90	2.252 ±0.092	1.397 ±0.316	3.910 ±0.080	2.723 ±0.178

in cooked meat, observed in the present investigation, indicated that cooking might have caused the oxidation of phospholipid. Phospholipid fraction of total lipid was responsible for oxidative deterioration in cooked turkey[27] and pork[28]. Total phospholipd and especially PE exerted more prooxidant effect than PC in cooked meat[24]. The rate of reduction of PE was more than PC in cooked broiler stored at both temperatures. Thus the results of this study indicated that PE was the most important phospholipid fraction responsible for oxidation of lipid in poultry meat.

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Effect of Cooking and Storage on Neutral Lipids in Broiler and Quail

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Neutral lipid composition of the thigh and breast muscles and changes in neutral lipids of raw and cooked broiler and quail meat stored at 4° and -10°C were studied. Total glyceride content in the thigh muscle was significantly greater than in the breast muscle in the two species. Cooking and storage caused an increase in the total glyceride content in the thigh and breast muscles. A significant increase of mono- and di-glycerides and a decrease of triglycerides occurred during cooking and storage. Changes in neutral lipid and its fractions were more at 4° than at -10°C of storage.

Estimation of neutral lipid and changes due to cooking and storage has received little attention in broiler[1-4]. Practically no information is available on lipid composition of quail meat which is gaining popularity as table delicacy in India. In the present paper the neutral lipid composition of broiler and quail meat and changes therein due to cooking and storage are reported.

Materials and Methods

Seventy eight broilers of 8 weeks of age and 78 adult quails (*Coturnix coturnix japonica*) were dressed. Thirty nine broilers and 39 quails were cooked in batches at 1.1 kg/cm^2 pressure for 8 min. Raw and cooked birds were packed in polyethylene bags of 150 guage and stored at 4° and −10°C. Samples from the breast and thigh muscles were taken for estimation of neutral lipid on 3, 6 and 9 days of storage from those stored at 4°C and on 30, 60 and 90 days of storage from those kept at −10°C. In broiler, samples were taken in triplicate, whereas in quails they were taken in duplicate.

Method of Folch *et al*[5]. was used for extraction of lipids. Extracted lipid was dissolved in chloroform to a known volume and applied on TLC plates coated with silica gel G of 250μ thickness. One dimensional triple development system[6] giving complete separation of neutral lipids, was used. For separation of neutral lipids, three solvent systems containing hexane: diethyl ether: glacial acetic acid were used in proportions of 60:40:1, 90:10:1, and 30:70:1 respectively. The lipid spots on the chromatoplate were located by exposing it to iodine vapour in closed chamber. Neutral lipid spots were identified by comparing their R_f values with authentic standards co-chromatographed with each run (Fig. 1). Each spot was scraped on to a glaze paper and trans-

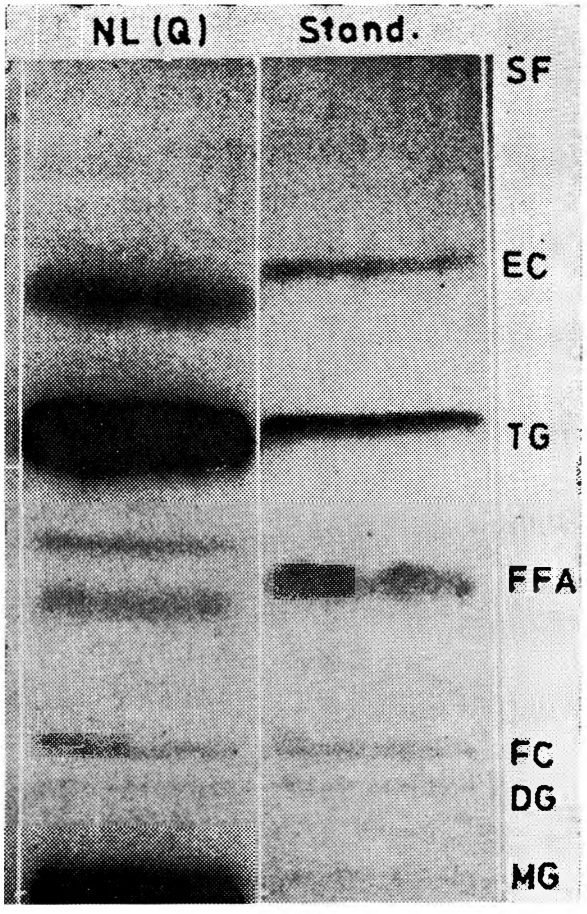

Fig. 1. Neutral Lipid Fractions of Quail

MG = Monoglyceride, DG = Diglyceride, FC = Free cholesterol, FFA = Free fatty acids, TG = Triglyceride, EC = Esterified cholesterol, SF = Solvent front.

ferred to 20 ml tubes. For eluting mono-, di- and triglycerides from the gel, 10 to 15 ml of hexane: ether (1:1, V/V) solvent was used. Corresponding gel blanks were similarly extracted. Eluted and dried neutral lipid fractions were dissolved in a known volume of chloroform and glyceride-glycerol was estimated[7].

Results and Discussion

The average total glyceride content of broiler and their fractions viz., mono-, di- and tri-glycerides in the thigh muscle are presented in Table 1 and in the breast muscle in Table 2. The average glyceride contents of thigh and breast muscles of quail are presented in Tables 3 and 4 respectively. The total glyceride content in the thigh muscle was significantly ($F<0.01$) more than in the breast muscle in both broiler and quail. The variation in total glyceride content of thigh and breast muscles was primarily due to the difference in their total lipid content, as neutral lipid is a major part of total lipid. The total lipid content in the thigh muscle was 20.71 mg/g and 40.27 mg/g in broiler and quail respectively. The breast muscles of broiler and quail contained 17.98 mg and 31.82 mg of total lipid per gram of tissue respectively.

Cooking significantly increased the total glyceride contents in both muscles of broiler, but a significant increase was not observed in quail. The total glyceride contents of thigh and breast muscles of broiler significantly increased due to storage. The increase was more in raw thigh muscle ($b=0.62\pm0.13$) as compared to that in raw breast muscle ($b=0.41\pm0.10$). A similar increase in total glyceride content due to storage was observed in cooked thigh ($b=0.37\pm0.09$) and breast muscle ($b=0.37\pm0.12$). The total glyceride contents increased in raw and cooked muscles of quail, but the rate of increase was significant in cooked breast muscle only.

The increase in total glyceride content due to cooking and storage could be due to loss of moisture and denaturation of protein. An increase in total glyceride on frozen storage of broiler was reported[4]. The reason for the increase in glyceride content in stored muscle is not known. Davidkova and Khan[4] suggested that the association of glycerides with protein as lipoprotein in fresh muscle did not permit the total extraction of glycerides. During storage, the lipoprotein deteriorated causing increased extraction of glycerides.

Among the glyceride fractions, the proportion of triglyceride was maximum followed by di- and mono-

Table 1. Neutral lipid composition in thigh muscle of broiler

Storage temp. (°C)	Storage period (days)	Glycerides in raw muscle: Total (mg/g tissue)	Monoglyc. (%)	Diglyc. (%)	Triglyc. (%)	Glycerides in cooked muscle: Total (mg/g tissue)	Monoglyc. (%)	Diglyc. (%)	Triglyc. (%)
4	0	9.84 ± 0.39	0.67 ± 0.13	1.85 ± 0.09	97.49 ± 0.06	12.06 ± 0.19	0.68 ± 0.12	2.28 ± 0.31	97.30 ± 0.02
,,	3	10.71 ± 0.56	0.72 ± 0.30	2.15 ± 0.22	97.17 ± 0.49	13.04 ± 0.96	0.66 ± 0.04	2.03 ± 0.15	97.37 ± 0.10
,,	6	11.97 ± 1.14	2.28 ± 0.27	3.21 ± 0.41	94.57 ± 0.59	13.70 ± 0.77	1.71 ± 0.11	4.32 ± 0.09	93.97 ± 0.20
,,	9	15.65 ± 1.02	4.20 ± 0.06	6.16 ± 0.29	89.64 ± 0.32	15.54 ± 0.70	2.23 ± 0.19	3.41 ± 0.04	94.35 ± 0.17
-10	30	12.22 ± 0.16	0.38 ± 0.04	1.81 ± 0.11	97.81 ± 0.15	17.69 ± 0.92	0.62 ± 0.04	2.04 ± 0.03	97.34 ± 0.04
,,	60	11.31 ± 0.47	1.62 ± 0.18	2.67 ± 0.18	95.71 ± 0.25	17.48 ± 0.30	2.17 ± 0.24	3.13 ± 0.30	94.70 ± 0.49
,,	90	15.01 ± 0.63	2.98 ± 0.13	4.31 ± 0.17	92.71 ± 0.34	17.81 ± 0.44	3.01 ± 0.20	3.55 ± 0.17	93.44 ± 0.35

Mono-, di-and triglycerides are expressed as percentage of total glycerides.

TABLE 2. NEUTRAL LIPID COMPOSITION IN BREAST MUSCLE OF BROILER

Storage temp. (°C)	Storage period (days)	Glycerides in raw muscle				Glycerides in cooked muscle			
		Total (mg/g tissue)	Monoglyc. (%)	Diglyc. (%)	Triglyc. (%)	Total (mg/g tissue)	Monoglyc. (%)	Diglyc. (%)	Triglyc. (%)
4	0	6.13 ±0.27	1.34 ±0.10	2.40 ±0.18	96.26 ±0.08	9.39 ±0.53	1.67 ±0.20	2.45 ±0.38	95.88 ±0.57
,,	3	6.47 ±0.30	2.39 ±0.11	3.39 ±0.15	94.31 ±0.17	10.54 ±0.74	2.61 ±0.30	4.89 ±0.76	92.50 ±0.16
,,	6	7.02 ±0.69	2.39 ±0.11	3.45 ±0.21	94.23 ±0.14	11.33 ±1.56	2.91 ±0.30	5.19 ±0.67	90.92 ±0.44
,,	9	10.08 ±0.88	3.20 ±0.09	3.90 ±0.12	92.99 ±0.26	12.91 ±0.42	3.14 ±0.07	5.95 ±0.46	90.92 ±0.44
-10	30	8.12 ±0.45	2.40 ±0.06	3.37 ±0.09	94.23 ±0.07	12.26 ±0.49	3.61 ±0.01	3.91 ±0.09	92.48 ±0.10
,,	60	9.19 ±0.63	3.61 ±0.02	4.03 ±0.07	92.36 ±0.05	11.79 ±1.34	4.34 ±0.13	4.80 ±0.36	90.86 ±0.24
,,	90	11.63 ±1.30	4.81 ±0.52	5.38 ±0.85	89.82 ±1.37	15.08 ±0.91	4.34 ±0.13	4.80 ±0.36	90.86 ±0.24

Mono-, di-and triglycerides are expressed as percentage of total glycerides.

TABLE 3. NEUTRAL LIPID COMPOSITION IN THIGH MUSCLE OF QUAIL

Storage temp. (°C)	Storage period (days)	Glycerides in raw muscle				Glycerides in cooked muscle			
		Total (mg/g tissue)	Monoglyc. (%)	Diglyc. (%)	Triglyc. (%)	Total (mg/g tissue)	Monoglyc. (%)	Diglyc. (%)	Triglyc. (%)
4	0	24.56 ±2.00	0.75 ±0.17	1.82 ±0.14	97.93 ±0.03	20.42 ±3.67	0.75 ±0.20	2.27 ±0.54	97.30 ±0.03
,,	3	19.21 ±1.13	0.89 ±0.45	2.27 ±0.33	96.90 ±0.72	24.18 ±1.74	0.69 ±0.03	1.90 ±0.13	97.41 ±0.16
,,	6	21.89 ±0.67	2.26 ±0.46	3.52 ±0.46	94.27 ±0.88	24.72 ±1.86	1.77 ±0.16	4.38 ±0.13	93.86 ±0.29
,,	9	30.60 ±3.39	4.21 ±0.11	6.34 ±0.07	93.83 ±4.47	25.28 ±0.74	2.35 ±0.27	3.42 ±0.06	94.23 ±0.21
-10	30	31.36 ±0.82	2.46 ±0.68	3.44 ±1.10	94.11 ±1.78	19.83 ±2.47	2.56 ±0.74	3.22 ±0.68	94.22 ±1.42
,,	60	21.88 ±1.46	1.55 ±0.28	2.84 ±0.13	95.62 ±0.40	23.39 ±1.14	1.83 ±0.03	2.92 ±0.52	95.25 ±0.55
,,	90	24.99 ±0.67	3.55 ±0.28	2.84 ±0.13	93.62 ±0.40	24.52 ±1.46	2.83 ±0.03	3.42 ±0.02	93.75 ±0.05

Mono-, di-and tri-glycerides are expressed as percentage of total glycerides.

TABLE 4. NEUTRAL LIPID COMPOSITION IN BREAST MUSCLE OF QUAIL

Storage temp. (°C)	Storage period (days)	Glycerides in raw muscle				Glycerides in cooked muscle			
		Total (mg/g tissue)	Monoglyc. (%)	Diglyc. (%)	Triglyc. (%)	Total (mg/g tissue)	Monoglyc. (%)	Diglyc. (%)	Triglyc. (%)
4	0	6.17 ±0.54	1.39 ±0.15	2.31 ±0.27	96.30 ±0.12	12.00 ±1.19	1.83 ±0.18	2.82 ±0.21	95.36 ±0.39
,,	3	10.56 ±3.37	2.28 ±0.02	3.43 ±0.24	94.29 ±0.22	13.52 ±1.08	1.83 ±0.49	2.93 ±1.20	95.25 ±1.69
,,	6	12.03 ±0.17	3.16 ±0.13	3.85 ±0.19	92.99 ±0.32	15.78 ±0.58	3.21 ±0.01	5.72 ±0.70	91.08 ±0.71
,,	9	11.52 ±0.78	5.84 ±0.02	6.94 ±0.14	87.23 ±0.15	17.11 ±0.11	4.86 ±0.72	4.70 ±0.60	90.95 ±0.39
-10	30	13.52 ±5.90	3.06 ±0.31	4.67 ±0.21	92.27 ±1.09	16.15 ±1.25	3.03 ±0.59	3.42 ±0.59	93.56 ±1.18
,,	60	8.53 ±.244	3.62 ±0.03	3.98 ±0.12	92.39 ±0.08	18.86 ±2.12	3.95 ±0.36	4.37 ±0.63	91.69 ±0.99
,,	90	10.09 ±2.42	3.92 ±0.03	3.99 ±0.11	92.09 ±0.08	20.65 ±1.18	3.75 ±0.14	4.34 ±0.64	91.91 ±0.50

Mono-, di-and tri-glycerides are expressed as percentage of total glycerides.

glycerides. This finding is in agreement with Gunstone[8]. Significantly higher contents of monoglycerides observed in breast muscle of broiler and quail as compared to those in thigh muscles were not in agreement with the results of Marion and Woodroof[2] who did not find significant difference in monoglyceride content of thigh and breast muscles in broiler. In white sucker fish significantly greater monoglyceride content has been reported in white than in the dark muscle.[9]

Regression coefficients of fractions of glyceride on diglycerides increased in both raw and cooked thigh and breast muscles. A significant increase of mono- and di-glycerides associated with decrease of triglyceride due to cooking and storage could be due to the hydrolysis of triglyceride to mono- and di-glycerides[10,11].

A comparison of regression coefficients of total glyceride and its fractions at 4° and -10°C revealed that the values at 4°C were greater than those at -10°C. Similar results were observed in quails. This suggested that the frozen storage of muscles caused less change in neutral lipid and its fractions than storage at refrigeration temperature.

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Persistence of Aldrin and Dieldrin Residues in Potatoes

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Aldrin EC was sprayed on the ridges and foliage of potato plant at the rate of 1.75, 2.00, 2.25 and 2.50 kg active ingredient per hectare 25 days after planting (single spray) and repeating after 25 days after the first spray (double spray) at the same doses to control cut worms (*Agrotis* spp.). In unpeeled and unwashed potatoes, the residues of aldrin and dieldrin persisted above the tolerance limit of 0.1 ppm. Peeling followed by washing or boiling and peeling of such potatoes removed the toxicants to a considerable extent, but failed to remove the toxicants completely.

Agrotis segetum (Schiff.) and *A. ipsilon* (Hfn.) are serious pests of potato crops in India. These insects have been reported to cause about 40 per cent loss in the crop yield[1,2]. Several contact insecticides like aldrin, heptachlor, toxaphene and DDT+pyrethrum in dust formulation; carbaryl as bait and chlorfenvinphos in granular formulation were found effective against potato cutworms in varying degrees[1-3]. Spraying of aldrin EC on crop foliage and drenching of ridges was also found effective against potato cutworms[4]. Earlier studies indicated that soil application of aldrin dust at the time of planting of potato crop was unsafe as residues from the treated crop persisted above the tolerance limit for a long time[7]. Since no information is available on the residue levels in the tubers harvested from the crop where aldrin EC was applied as foliar spray and drenching the soil surface, these studies were carried out and the results are reported in this paper.

Materials and Methods

Field experiments in randomised block design with nine quadruplicated treatments were conducted in fields where no chlorinated soil insecticide was applied during the last 5 years. Potato variety 'Kufri Chandramukhi' was used and the experiment was done during autumn of 1978–79 at Central Potato Research Station, Jullundur, Punjab. Each plot (3.0×4.0m) had 5 rows each having 16 plants. The space between plants was 25 cm within the rows, and 60 cm between rows. The agronomic practices recommended for the cultivation of potatoes in the region were followed.

Aldrin EC was sprayed at the rate of 1.75, 2.00, 2.25 and 2.5 kg a.i./ha in two different sets. In the first set, single spraying with the above dosages of aldrin EC (dissolved in 1250 l. of water) was given on the foliage and ridges of potato plants after 25 days of planting. In the second set two sprayings, one after 25 days of planting and the second after 25 days of the first spray were given. Both foliage and ridges were sprayed uniformly, Control plots were sprayed with water.

The average maximum and minimum temperatures were 23.4°C and 9.0°C, respectively, while average relative humidity was 78.8 per cent during the experimental period. Total rainfall during the crop season was 12 mm. The experimental plots were irrigated 10 times.

Potato tubers (6.0 kg each) corresponding to each treatment and replicates were collected at harvest i.e. after 100 days of planting for determining aldrin and dieldrin residues.

First analysis was done soon after harvest and the other two were made 30 and 60 days after harvest when they were stored in jute bags under local storage conditions (temperature ranged from 10 to 28°C). Residues were determined in (*i*) unpeeled and unwashed raw potatoes; (*ii*) peeled, cut and washed (tap water washing for 2 min) potatoes, and (*iii*) washed (unpeeled potatoes) (for 2 min) boiled, peeled ptatoes. The residues were also determined in boiled water. Extraction procedures adopted by Dewan *et al*[8], Awasthi *et al*[9]. and Misra *et al*[5]. were followed for extracting the residues.

Five hundred grams of potatoes from each set of each treatment in replicate, were cut into small pieces. After mixing, 100 g pieces were stripped with 200 ml of solvent (mixture of n–hexane and isopropyl alcohol in the ratio 4:1) for one hour. Residue level in boiled water was also determined by taking 100 ml of water followed by

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stripping with equal quantity (100 ml) of solvent mixture, described above, for one hour.

For the analysis of aldrin and dieldrin residues, known volume of extracts were cleaned up by the method of Misra *et al*[5]. The elution was done by extra pure n-hexane (washed with 5 per cent sulphuric acid and distilled over silver nitrate). The eluate was then made up to a known volume and the insecticidal residues were estimated by GLC[5] using column OV-1 at inlet temperature of 225°C and detector temperature of 250°C, in an electron capture detector for aldrin and dieldrin.

Results and Discussion

The data on residues of aldrin and dieldrin on/in (*i*) unpeeled, unwashed raw potatoes; (*ii*) peeled, washed but unboiled potatoes; (*iii*) washed, boiled, peeled potatoes and (*iv*) the water in which washed potatoes were boiled, are presented in Table 1 and 2.

Single spraying set: It is evident from the data of single spray treatment (Table 1) that the residues of aldrin persisted above the tolerance limit[10] of 0.1 ppm. in the unpeeled and unwashed potato tubers not only at harvest, but also after one month of their storage at all dosages. However, the aldrin residue levels gradually decreased with increase in storage period. After two months of storage its residue was below the tolerance limit in the lower dosages (1.75 and 2.0 kg a.i./ha), but they were above the tolerance limit in the higher dosages. Besides aldrin, high quantities of dieldrin were also detected in these tubers at harvest time.

Although dieldrin residues also decreased gradually after some period of storage, yet these were above the tolerance limit of 0.1 ppm even after two months of storage.

Peeling and washing of potatoes considerably reduced the residues of both aldrin and dieldrin on/in the tubers. As a result of these processes, aldrin residues in the tubers even soon after harvest and before storage were found below the tolerance limit in all the dosages. The quantities of aldrin residues at that time ranged from 0.05 to 0.09 ppm in these dosages. After storage for one month, residues of aldrin were not detectable in the peeled and washed potato at first two the lower dosages but their quantities ranged from 0.04-0.06 ppm at the higher dosages. Dieldrin residues in peeled and washed potatoes were found above the tolerance limit only in the higher dosages (2.25 and 2.50 kg a.i./ha) when analysed soon after harvest. The residue levels in the 2.25 kg a.i./ha treatment dissipated below the tolerance limit after one month of storage. No detectable residues of aldrin or dieldrin from peeled, washed but unboiled potato tubers were observed after two months of storage in all the dosages.

Cooking water of the freshly harvested potatoes also contained the residues. Aldrin residues were not detectable in the lowest dose; but were detected below the tolerance limit in the next two dosages (2.00 and 2.25

TABLE 1. ALDRIN AND DIELDRIN REAIDUES* IN POTATOES RESULTING FROM SINGLE SPRAY TREATMENT WITH ALDRIN EC.

Sampling period after harvest (days)	Days after spray	Aldrin EC dose (kg a.i./ha)	Residues (ppm) in tubers from single spray treatment							
			Unpeeled, unwashed		Peeled, washed		Washed, boiled and peeled		Boiled water extract	
			Aldrin	Dieldrin*	Aldrin	Dieldrin*	Aldrin	Dieldrin*	Aldrin	Dieldrin*
At harvest	75	1.75	0.18	0.28	0.05	0.06	ND	ND	ND	0.05
		2.00	0.21	0.33	0.06	0.09	0.03	0.07	0.07	0.11
		2.25	0.23	0.37	0.09	0.13	0.03	0.07	0.07	0.11
		2.50	0.23	0.41	0.09	0.13	0.03	0.07	0.11	0.17
30	105	1.75	0.12	0.19	ND	0.04	ND	ND	ND	ND
		2.0	0.14	0.21	ND	,,	,,		,,	,,
		2.25	0.14	0.23	0.04	0.06	,,	,,	,,	,,
		2.50	0.15	0.27	0.06	0.11	,,	,,	,,	0.05
60	135	1.75	0.08	0.15	ND	ND	ND	ND	ND	ND
		2.00	0.08	0.21	,,	,,	,,	,,	,,	,,
		2.25	0.11	0.21	,,	,,	,,	,,	,,	,,
		2.50	0.13	0.23	,,	,,	,,	,,	,,	,,

*Dieldrin was calculated from converting aldrin. *Average of four replications. N.D. = Not detectable

TABLE 2. ALDRIN AND DIELDRIN RESIDUES IN POTATOES RESULTING FROM DOUBLE SPRAY TREATMENT WITH ALDRIN EC

Sampling period after harvest (days)	Days after second spray	Aldrin EC dose (kg a.i./ha/ spray treatment)	Residues (ppm) from double spray treatment							
			Unpeeled, unwashed		Peeled, washed		Washed, boiled and peeled		Boiled water extract	
			Aldrin	Dieldrin*	Aldrin	Dieldrin*	Aldrin	Dieldrin*	Aldrin	Dieldrin*
At harvest	50	1.75	0.23	0.31	0.08	0.13	ND	0.03	0.09	0.11
		2.00	0.31	0.44	0.11	0.17	0.03	0.03	0.13	0.17
		2.25	0.31	0.46	0.13	0.17	0.05	0.07	0.13	0.17
		2.50	0.33	0.47	0.16	0.21	0.05	0.07	0.17	0.21
30	80	1.75	0.13	0.23	ND	0.08	ND	ND	ND	0.06
		2.00	0.18	0.28	,,	0.08	,,	,,	0.05	0.07
		2.25	0.17	0.28	0.07	0.11	,,	,,	0.05	0.07
		2.50	0.21	0.32	0.90	0.13	,,	,,	0.05	0.07
60	110	1.75	0.08	0.21	ND	ND	ND	ND	ND	ND
		2.00	0.11	0.21	,,	0.04	,,	,,	,,	,,
		2.25	0.13	0.23	,,	0.04	,,	,,	,,	,,
		2.50	0.13	0.23	0.03	0.07	,,	,,	,,	,,

*Dieldrin resulted from the conversion of aldrin to dieldrin. *Average of four replications. N.D.: Not detectable

kg a.i./ha) and above the tolerance limit (0.1 ppm) in the highest dose. Dieldrin residues were below the tolerance limit only in the lowest dose. Residues of these toxicants were not detected in the water used for cooking the potato tubers stored for one month except for the recovery of 0.05 ppm dieldrin from the highest dose (2.50 kg a.i./ha). Further, dieldrin residues were invariably more than those of aldrin residues in potatoes as well as in the water used for cooking the tubers.

Double spray: It is apparent from the data of double spray treatments (Table 2), that the quantities of dieldrin residues were invariably more than those of aldrin residues in the potato tubers collected from treated fields and the trend of dissipation of residues of both the toxicants was almost similar to that in the single spray treatment. The residues of aldrin and dieldrin were above the tolerance limit on/in unwashed and unpeeled raw potatoes even after storage for 2 months except in the lowest dose where aldrin residues were below the tolerance limit.

Although peeling and washing of harvested raw potatoes brought down aldrin and dieldrin residue levels to a considerable extent, but not below the tolerance limit except in the lowest dose (1.75 kg a.i./ha spray) where the aldrin level was 0.08 ppm. When stored for one month the residue levels were undetectable at lower levels but were 0.11 and 0.13 ppm at 2.25 and 2.50 kg a.i. aldrin/ha/spray treatment, respectively.

As in single spray treatment washing or boiling followed by peeling of potatoes resulted in eliminating aldrin and dieldrin on/in double spray treatment also. These further brought down these toxicants below the tolerance level even in fresh potatoes and below the detectability levels in one month and two month stored potatoes. Analyses of the cooking water showed, that both these toxicants passed into water from potatoes during boiling. Both the toxicants were within tolerance limits in cooking water resulting from boiling one month stored potatoes; but the boiling water obtained from 2-month stored potatoes contained practically no toxicants.

Aldrin and dieldrin residues resulting from dust formulation of aldrin had already been reported by various workers[5-7]. According to Singh and Kalra[6], aldrin and dieldrin residues were above the tolerance limit in potatoes harvested 3 months later from aldrin treated (2 kg a.i./ha) plots. Similarly Attri *et al*[7]. had observed that application of 5 per cent aldrin dust @ 25 kg/ha to the soil before planting the potatoes resulted in combined residues of aldrin and dieldrin as 0.8-1.0 ppm in the tubers at harvest. Further, they stated that total residues in peel, pulp or whole potatoes were higher than the official tolerance level of 0.1 ppm.

The present data also revealed that foliar application and drenching the ridges with aldrin EC would be risky, when freshly harvested potatoes from these fields are

to be consumed. Though (*i*) peeling followed by washing and (*ii*) washing, boiling and then peeling of such contaminated potatoes have eliminated the residues of aldrin and dieldrin to a considerable extent, even then, these failed to remove completely both the toxicants from freshly harvested potatoes. In conclusion, foliar application and drenching the ridges with aldrin EC, especially, with higher dosages (2.0, 2.25 and 2.5 kg a.i./ha) for the control of cutworms, *Agrotis* spp. on potato crop is not advisable.

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Insecticidal Residue in Vegetables Obtained from Soil Treated with Hexachlorocyclohexane

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The study deals with the extent to which the vegetables pick-up the toxic residue when grown on soil treated with hexachlorocyclohexane (HCH). The chemical has been detected in the edible portions of the crops, the concentrations being dependent on the crop and the insecticide level in the soil. Coriander absorbed highest amount of residue. The order of HCH uptake by the test plants was: coriander, amaranthus, carrot, knol-khol, chilly, cucumber and tomatoes in decreasing trend. The level of insecticide in the tissue of the same plant has not been in direct proportion to the concentration of the insecticide in the soil. Disappearance of HCH has been faster from the soil under the crop cover of coriander. Generally, the residue level did not exceed the tolerance limits established for human consumption.

Pesticides are increasingly used in agriculture to maintain high yields. Irrespective of the mode of application, these agrochemicals get accumulated in the soil due to several ecological factors. These residues are picked up by the succeeding crops and cause phytotoxicity[1]. Hence food production on contaminated soils, requires intensive study because crops grown in such areas may take up pesticides and pose health hazards.

Foods often carry organochlorine residues that are translocated from the soil. One method to overcome this is, identifying plants which do not pick up residues from soil. Though information is available on the fate of pesticides in plants, there is very scanty information

about the relative ability of food crops to translocate and transfer pesticide residues to the consumer[2]. This communication describes the results of an exploratory study conducted by growing a few vegetable crops on soil treated with the insecticide, hexachlorocyclohexane (HCH) and quantifying the residues in the edible portions.

Materials and Methods

Field studies: Field trials were conducted in the experimental plots of the laboratory measuring 3m × 1 m. in red sandy loam soil. Usually, at the recommended agricultural application rate, HCH creates soil concentration of 10 ppm or less. Therefore, for the present study concentrations were chosen both at higher and lower than this level. Technical HCH powder (obtained from MICO Farm Chemicals, Madras; Composition: *alpha*-isomer=70 per cent; *beta*=7.5 per cent; *gamma*= 12.5 per cent; and *delta*=3.5 per cent) was dusted uniformly on the soil at the rate of 0, 5, 10 and 25 kg/ha, and ploughed into the soil. Each treatment was replicated 3 times. Five 3 inch cores of soil samples were immediately taken at random from each plot and pooled for analysis. On completion of the final harvest (90 days), soil samples were again taken to determine the residue levels in the soil. Vegetable seeds of coriander, amaranthus, carrot, knol-khol, chilly, cucumber and tomato were purchased from the local horticultural department and were sown in rows and watered regularly. Harvest dates of these vegetables varied depending on the time of maturing of the specific crop. Amaranthus and coriander which are consumed as greens were harvested at the end of 6 weeks. Concentration of the test chemical was estimated in the edible parts of each vegetable.

Pesticide extraction: Soil samples collected were mixed, air-dried, passed through 2 mm sieve and extracted with a mixture of n-hexane and acetone (4:1) for 60 min on a rotary shaker. Hexane layer was separated and the soil was once again extracted. Pooled hexane layer was dried on anhydrous sodium sulphate, concentrated and stored at 5°C until taken for analysis.

Insecticidal residue from edible parts of the vegetables were extracted immediately after harvest by blending 100 g of the sample in 200 ml of acetonitrile. Macerate was filtered and washed twice with 50 ml of the above solvent. The insecticide was then transferred to hexane by partitioning. Hexane extract was further processed as described earlier. Extracts from soil as well as edible tissues were cleaned upon a florisil column as described[3]. The eluates were dried, residues were dissolved in known volume of acetone and analysed by gas chromatogrpahy.

Residue analysis: Identification of the isomers of HCH was based upon R_f values and quantities were calculated by relating peak areas to those of standards analysed on the same day. The gas chromatograph used was Varian-Aerograph series 1400 (USA) fitted with electron capture detector with tritium foil. The spiral metal column (6ft × 1/8 in i.d.) was packed with 5 per cent OV-17 coated on chromosorb-W (60-80 mesh). The operating conditions were; column temperature 160°C, detector temperature 200°C; injection port temperature 190°C; attenuation=16 and range 10^{-10}. The carrier gas was prepurified nitrogen maintained at a flow rate of 30 ml per minute. Under these conditions, R_f values obtained for alpha, gamma, beta and deltaisomers were 3.2; 4.2; 5.1 and 5.9 min respectively. Values from control samples, though negligible were deducted and the sum total of 4 isomers were shown in Fig 1 and 2. Tne method used for extraction of HCH gave recoverics above 90 per cent from soil and plant tissues.

After GLC analysis, concentrated extracts were applied on silica gel TLC plates, developed with n-hexane and dried chromatograms were sprayed with 1 per cent *ortho*-tolidine in acetone. By comparing the

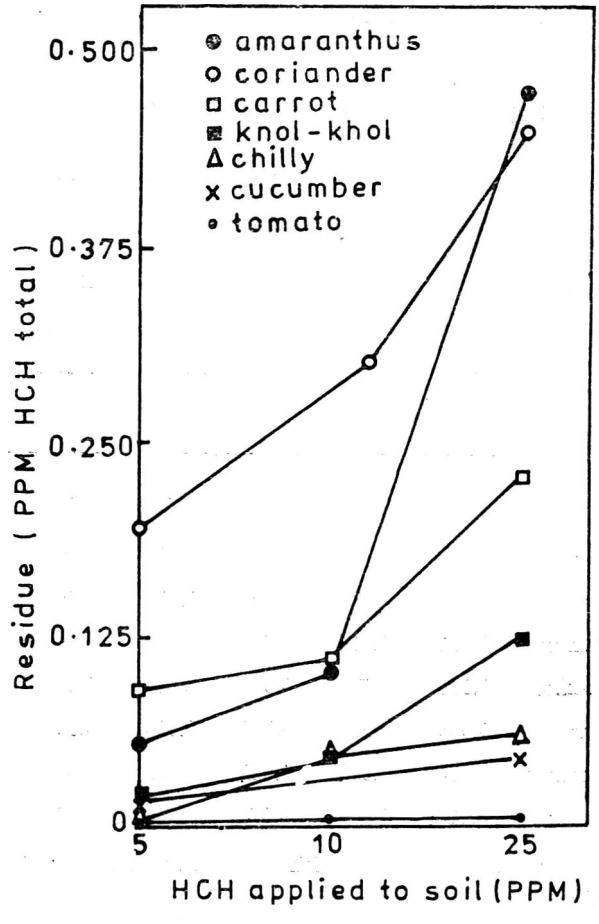

Fig. 1. Residues in edible parts of the vegetables grown on soil containing graded concentrations of HCH (ppm)

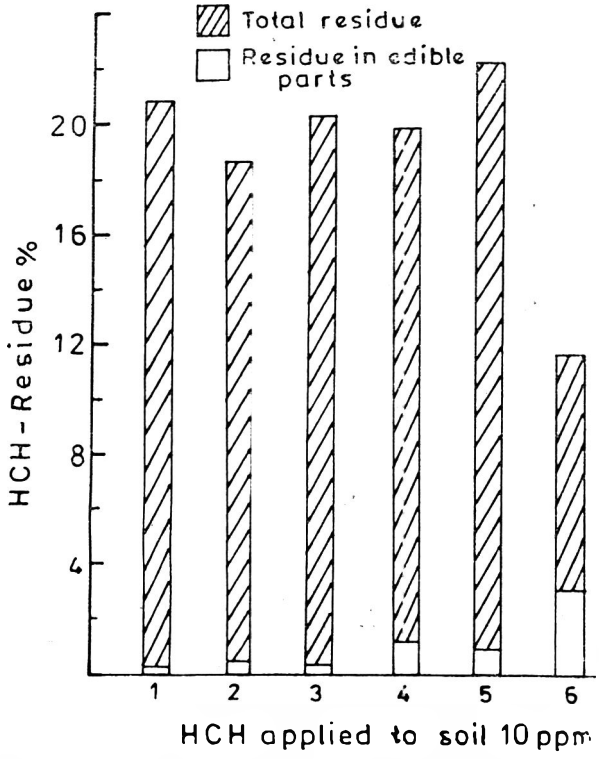

Fig. 2. Comparative distribution of HCH residues in soil and edible parts of plants.

1. Tomato, 2. Chilly, 3. Knol-khol,
4. Carrot, 5. Amaranthus, 6. Coriander

relative R_f values with the authentic samples, identification of the isomers was confirmed.

Organoleptic study: Twenty-five employees of the CFTRI drawn from different categories were explained the vegetable characteristics such as appearance, taste of the vegetable (raw and cooked) and general acceptability. All vegetables used for the study were cooked in a pressure vessel for 10 min and given for tasting with or without salt. Evaluation of these parameters was done using a four point scale of excellent-4; good-3; fair-2 and poor-1.

Results

Residue in edible parts of vegetables: Quantitative recovery of HCH from the edible parts of the plants are shown in Fig 1 (Total of 4 isomers). In general, the amount recovered was less than 1 ppm; although there appears much variation between the species. Vegetables grown in high pesticide contaminated soil always contained more residue but the levels were not proportionate to the dose applied. In the case of chilly, 0.005, 0.043, and 0.060 ppm of pesticides were recovered from the fruits harvested from plots treated with 5, 10 and 25 ppm HCH respectively. Corresponding values for coriander were 0.198, 0.303 and 0.450 ppm. Among the crops grown, tomato contained minimum residue while coriander the maximum. It is therefore, surmised that fruits contain comparatively less HCH and leafy vegetables (amaranthus and coriander) contain more residue and the tubers, in between these two.

Isomer pattern: Hexachlorocyclohexane consists of 4 major isomers of which only gamma-HCH (also known as lindane) is the insecticidal component. Other isomers are undesirable as they have either nil or negligible insecticidal activity. Environmental toxicologists are however, more concerned about isomers other than the gamma, because alpha-isomer forms bulk of the

TABLE 1. RESIDUES OF DIFFERENT ISOMERS IN EDIBLE PARTS OF VEGETABLES GROWN ON PLOTS TREATED WITH HCH*

Vegetable	5 kg/ha				10 kg/ha				25 kg/ha			
	Alpha HCH	Gamma HCH	Beta HCH	Delta HCH	Alpha HCH	Gamma HCH	Beta HCH	Delta HCH	Alpha HCH	Gamma HCH	Beta HCH	Delta HCH
Tomato	Tr	Tr	Tr	Tr	Tr	Tr	Tr	0.012	Tr	Tr	Tr	0.020
Chilly	Tr	0.001	Tr	0.003	Tr	0.015	0.015	0.013	Tr	0.025	0.020	0.015
Cucumber	0.001	0.016	Tr	Tr	—	—	—	—	0.002	0.030	0.002	0.008
Knol-khol	0.001	Tr	0.008	0.006	0.02	0.003	0.015	0.019	0.004	0.020	0.053	0.043
Carrot	0.006	0.013	0.067	0.004	0.003	0.050	0.050	0.008	0.014	0.070	0.106	0.040
Amaranthus	0.014	0.010	0.029	Tr	0.002	0.048	0.050	Tr	0.245	0.065	0.164	0.003
Coriander	0.112	0.009	0.074	0.003	0.190	0.011	0.095	0.007	0.259	0.031	0.143	0.018

*ppm calculated on fresh weight basis and is average of 3 replications.

Tr = Trace < 0.001 ppm

Control samples contained traces of isomers and the values are deducted.

TABLE 2. RESIDUAL HCH IN SOIL TREATED WITH DIFFERENT LEVELS OF HCH*

Soil taken from plot	5 kg/ha				10 kg/ha				25 kg/ha			
	Alpha HCH	Gamma HCH	Beta HCH	Delta HCH	Alpha HCH	Gamma HCH	Beta HCH	Delta HCH	Alpha HCH	Gamma HCH	Beta HCH	Delta HCH
Tomato	0.200	0.205	0.615	0.400	Tr	0.408	1.240	0.390	1.250	0.850	0.950	0.250
Chilly	Tr	0.315	0.640	Tr	0.420	0.380	0.620	0.360	0.450	1.245	1.300	1.150
Cucumber	Tr	0.105	0.405	0.040	—	—	—	—	Tr	0.310	0.620	Tr
Knol-khol	Tr	0.110	0.415	0.105	Tr	0.415	1.152	0.380	1.050	2.150	1.615	1.070
Carrot	0.115	0.315	0.590	0.106	0.105	0.410	0.615	0.620	2.425	0.620	1.115	0.425
Amaranthus	Tr	0.510	0.705	0.295	0.310	0.445	1.160	0.105	0.495	0.405	1.100	0.095
Coriander	Tr	Tr	0.398	Tr	0.002	0.105	0.385	0.059	0.096	1.050	1.425	0.405
Control	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr

Tr Trace - <0.001 ppm. *ppm calculated on dry weight basis

*ppm recovery at 0 levels of alpha, gamma, beta and delta isomers was 3.4, 0.6, 0.35 and 0.16 from 5 ppm treated plots. 7.2, 1.4, 0.73 and 0.34 from 10 ppm and 17.8, 3.1, 1.8 and 0.6 from 25 ppm plots respectively.

technical product (70 per cent), beta-isomer is known to accumulate in tissues with high lipid content, and also possesses high mammalian toxicity and delta-HCH is highly toxic to both higher plants and micro-organisms. Therefore, quality and quantity of these isomers in the edible portion of the plant is important. Table 1 gives the pattern of isomers recovered from the edible parts of the plants.

Coriander and amaranthus contained isomers in the order alpha > beta > gamma > delta. The content of alpha isomer was 0.2 ppm and delta isomer in traces. Knol-khol and carrot (tubers) contained high beta-HCH decreasing to traces of alpha-isomer. Whereas tomato, chilly and cucumber (fruits) contained more of gamma-isomer followed by beta-, delta- and traces of alpha-isomers.

Soil residue: Residue of HCH present in soil after harvest (90 days) of vegetables is presented in Table 2. It can be seen that from 5 ppm treated soil depending upon the vegetable, only 8-30 per cent of the parent molecule could be recovered (total of 4 isomers). Amaranthus grown plot gave highest level, while coriander soil contained least amount of residue. In soils treated with 10 ppm of HCH, coriander grown soil contained the least amount of HCH (5.5 per cent), while amaranthus soil had maximum of 20 per cent of the applied concentration and in others it varied between 17 and 20 per cent. Surprisingly, of the 25 ppm treated soil cucumber grown

TABLE 3. MEAN SCORES FOR SENSORY AND ORGANOLEPTIC QUALITIES OF EDIBLE PARTS OF VEGETABLES GROWN ON HCH TREATED SOIL

Vegetable*	Appearance				Taste of the raw vegetable				Acceptability after cooking			
	A	B	C	D	A	B	C	D	A	B	C	D
Tomato	4.0	3.0	3.0	2.0	4.0	4.0	3.0	3.0	4.0	3.0	3.0	3.0
Chilly	4.0	3.0	3.0	3.0	—	—	—	—	—	—	—	—
Cucumber	4.0	2.0	-	2.0	4.0	3.0	—	2.0	4.0	2.0	—	2.0
Knol-khol	4.0	3.0	3.0	1.0	4.0	3.0	2.0	1.0	3.0	4.0	3.0	3.0
Carrot	3.0	4.0	3.0	3.0	4.0	2.0	2.0	1.0	3.0	4.0	1.0	1.0

*A - Vegetables from untreated plots; B, C & D - Vegetables from plots treated with 5, 10, 25 ppm HCH respectively. Fruits of tomato, chilly and cucumber; Tubers of knol-khol and carrot.

plot contained only 3.7 per cent, while knol-khol grown plot had the maximum of 24 per cent. Large variation in the recovery may partly be due to the "rhizosphere effect" and also partly due to volatilization and non-biological decomposition of HCH.

Composition of isomers indicates that in most of the plots beta-isomer was more and alpha isomer was the least (Table 2). Degradation of isomers did not show any specific pattern.

Organoleptic evaluation: Depending on the type of vegetable, organoleptic qualities differed to considerable extent (Table 3). It is evident that the pesticide concentration in the soil is the factor affecting the taste of the vegetable. Presence of the test chemical usually altered the taste. Surprisingly carrots, obtained from 5 ppm treated soil looked bigger in size and tasted better than the control tubers after cooking. However, the raw vegetable lost the sensory score because of the unpleasant musty odour. Tomato did not differ much in appearance and taste as compared to the control. Also, concentration of the pesticide had not much influence in altering these qualities. Cucumbers harvested from treated plots were usually smaller in size. Knol-khol tubers from 25 ppm treated soil were graded low mainly because of the insect damage. Tubers had lots of wounds on them and probably due to the interaction with insects, raw vegetables did not taste good. However, after cooking there was not much taste difference between untreated and treated samples Chilly and leafy-vegetables were not tested due to inherent problems.

Discussion

Hexachlorocyclohexane forms about 48 per cent of the total pesticide used in India. This has resulted in pollution of the soil and irrigation water[4]. "Market Basket Survey" of vegetables, fruits and other food items revealed the general presence of HCH residues in them.[4-6] However, safety evaluation of foods grown on pesticide contaminated soils has not been adequately done in India. Present work done in this direction, indicates that although HCH is present in the soil, it is not likely to get accumulated by vegetables in quantities greater than 3 ppm which is the tolerance limit established for human consumption.

All the vegetables grown, had HCH residues within their edible tissues and the amounts being dependent on the type of crop and concentration in the soil. Fig 1. illustrates the close relationship between pesticide concentration in the soil and that absorbed by various vegetables. Coriander absorbed more readily than the other crops; but did not "accumulate" within the vegetable tissue as the concentration was always less than that present in the soil. Considering the lipophillic nature of HCH, relatively high recovery of the residue in coriander plant is expected because of the presence of essential oil in this plant. Relationship of oil content in seeds to residue in seed of crops grown on soil containing 1 ppm aldrin and heptachlor residue as shown by Bruce *et al.*[7] supports this observation.

Tomatoes contained minimum amount of HCH. Succulent nature of the fruit may be the reason. Similar observations were made by other workers also, while studying translocation of chlorinated hydrocarbon insecticides in plants.[8,9]

Difference between the crops in their ability to absorb HCH residues can be best revealed by the relative concentrations found in their edible portions (Fig. 2). For example, compared to tomatoes, coriander carried a load of HCH about 20 times and carrots 10 times as much under similar conditions. Such findings about differences in the content and distribution of organochlorine compounds in crops have appeared in literature[9,10]. Harris and Sans[11] while studying the absorption of DDT and cyclodiene insecticides by root crops from different types of soils have shown that, carrots absorbed the maximum amount of residues and this appears to be the case there also.

Crop rotations are frequently followed in pest management practice mainly to break the cycle of insect pests[12]. By careful selection of the rotation crop, additional benefit of pesticide decontamination can be derived. Since the recommended agricultural application rates for most pesticides create soil pesticide concentration of 10 ppm or less[13], the data presented in Fig. 2 indicate that coriander crop can be chosen under the conditions, because it aids in maximum removal of HCH from the soil, at the same time maintaining the residue level within the plant below the tolerance limit of 3 ppm.

Soil residue recovered at the time of harvesting (90 days) in the present study indicates that HCH disappears faster than reported from temperate regions[14,15]. Mac-Rae *et al*[16]. while studying the biodegradation of four isomers of HCH have reported 70 to 80 days of persistence for gamma isomer in submerged tropical soil. Similarly, Agnihotri *et al*[17]. in their work with BHC, have recovered only 2.5 per cent of the 16 ppm applied BHC (Lindane) at the end of 100 days. In our investigations, recovery of gamma-HCH from 10 ppm treated plots after 90 days (Table 2) is 0.4 ppm and is in good agreement with the above reports.

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Characteristics of an Intermediary Strain Obtained by Crossing *Streptomyces fradiae* SCF_5 and *Streptomyces exfoliatus* MC_1

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The mixed cultivation of *S. fradiae* SCF_5 and *S. exfoliatus* MC_1 yielded a number of apparently intermediary strains. Among these, one isolate, FE-16 which was stable over a number of transfers was subjected to detailed studies. This strain resembled exclusively SCF_5 in 4 of the traits and MC_1 in 6 of others. It shared common traits of both the parents in 6 characteristics. It, (E C 5.3.1.5.) however, did not resemble either of the parents in 10 characteristics. Of particular importance was the integration of glucose isomerase hydroxylase (EC 3.2.1.8.) of SCF_5 and of MC_1 in the intermediary strain FE-16.

A strain of *Streptomyces fradiae* producing glucose isomerase, the enzyme required for the preparation of high fructose syrup (HFS) has been isolated earlier in this laboratory[1]. One of the important factors for consideration in the economic production of glucose isomerase, is the need for xylose as inducer of the enzyme. Xylose is added at a level of about 0.5 per cent in the cultivation medium[1]. Natural xylans can replace the expensive xylose in the medium provided the organism can degrade xylan to xylose. *S. fradiae* SCF_5 possesses traces of xylan hydrolases and attempts to improve it by mutation by ultra violet irradiation led to a considerable loss of glucose isomerase itself[2]. In the present work, therefore, *S. fradiae* SCF_5 was crossed

with *S. exfoliatus* MC_1 which has high titre of extracellular xylan hydrolase. Several intermediary strains having both glucose isomerase and xylana hydrolase, were derived. One of the stable intermediary strains was subjected to detailed studies and tested for its ability to produce glucose isomerase by growth on a natural xylanaceous substrate.

Materials and Methods

Microorganisms: *Streptomyces fradiae* SCF_5, *S. exfoliatus* MC_1, *S. albosporeus* 1SSK[1], *S. cyaneus* 3a have been isolated in this laboratory[3] and, were maintained on complete agar slants with fortnightly transfers.

Media: The ingredients of all the media are given in per cent W/V. (*i*) Complete medium: yeast extract 0.1, meat extract 0.1, casamino acids 0.2, glucose 1.0, pH 7.0. Agar (2.0) was used for solidification. (*ii*) Medium for xylan hydrolase production by submerged method: yeast extract 0.25, meat extract 0.25, $MgSO_4$ $7H_2O$ 0.05, wheat bran (mesh BS-6) 1.0 and corn steep liquor (50 per cent solids) 0.5, pH 7.0. (*iii*) Medium for glucose isomerase production by submerged method: peptone 1.0, yeast extract 0.25, meat extract 0.5, $MgSO_4$ $7H_2O$ 0.05, NaCl 0.5, $CoCl_2$ $6H_2O$ 0.024, glucose 0.3 and xylose 0.5, pH 7.0. The above three media were sterilized by autoclaving at 15 p.s.i. for 20 min. (*iv*) The medium for xylan hydrolase and glucose isomerase production by surface cultivation was prepared as follows: Wheat bran was wetted to 60 per cent moisture using a solution containing $MgSO_4$ $7H_2O$ 0.05, K_2HPO_4 0.25 and rice bran 0.5 per cent (W/V): pH was adjusted to 7.0. This medium was sterilized by autoclaving at 15 p.s.i. for 1 hr.

The other media used for determining the characteristics of *Streptomyces* cultures were according to Waksman[4], and Gordon and Smith[5].

Conditions for the production of xlyan hydrolase: This was produced both by submerged and surface cultivation in the respective media mentioned above.

In the submerged cultivation, 50 ml of fluid medium (Medium ii) taken in 250 ml conical flask was inoculated with two loopsful of spores of *Streptomyces* from 4 days slant cultures. The flasks were incubated at 25-30°C under shaken conditions in a gyrotary shaker (250 r.p.m., 5.0 cm stroke). After 72 hr of cultivation, the cells were separated by centrifugation and the culture fluid examined for xylan hydrolase.

In the surface method of cultivation, 50 g. of moistened wheat bran (medium iv) taken in 500 ml conical flask was inoculated with 10 ml of 72 hr old cell suspension of the *Streptomyces* cultivated by the submerged method described as above. The flasks were kept at 25-30°C for 14-15 days, after which the bran was air dried and extracted with water. The extracted fluid was assayed for xylan hydrolase and the residual wheat bran containing *Streptomyces* mycelium was assayed for glucose isomerase.

Condition for the production of glucose isomerase: The fluid medium (medium iii) (50 ml) was inoculated with 2 loopsful of *Streptomyces* spores from 4-day old slants and allowed to grow for 72 hr under shaken conditions at 25 to 30°C. The cells were harvested by centrifugation, washed twice with distilled water and resuspended in 50 ml of distilled water. Glucose isomerase was assayed in the cell suspension.

Assay of xylan hydrolase: Xylan hydrolase was assayed in 2 ml of reaction mixture containing larchwood xylan (Sigma Chem. Co., U.S.A.) 10 mg, sodium phosphate buffer (pH 6.0) 0.0125 M and enzyme about 1.5 mg protein, incubated at 55°C for 30 min. The reducing group formed was estimated using dinitro salicylic acid reagent[6]. One unit of xylan hydrolase is the amount of enzyme which caused the liberation of 1 μm of D-xylose per minute.

Assay of glucose isomerase: This was assayed in 2 ml of reaction mixture containing glucose 0.1 M, $MgSO_4$ $7H_2O$ 5 mM, $CoCl_2$ $6H_2O$ 0.5 mM, sodium phosphate (pH 8.0) 0.05 M and about 5 mg wet cells or 50 mg wheat bran *Streptomyces* culture, incubated at 60°C for 1 hr. The reaction was stopped by adding 2 ml of 0.5 M perchloric acid solution. The fructose formed was estimated by the cysteine carbazole reagent[7].

Interspecific mating of Streptomyces: The xylan hydrolase producing culture, *Streptomyces exfoliatus* MC_1 and the glucose isomerase producing *S. fradiae* SCF_5 were grown separately in the respective media for xylan hydrolase and glucose isomerase by submerged cultivation for 16 hr. They were mixed in equal volumes and diluted one and half fold with complete medium and were allowed to grow for a further 24 hr. The mixed culture was diluted and plated on complete agar containing 0.5 per cent groundnut (*Arachis hypogea*) meal as a source of hemicellulose. The plates were observed for colony morphology variants and single colony isolations were made. They were tested for their ability to produce both xylan hydrolase and glucose isomerase in the respective fluid media under submerged cultivation as well as in the common medium under surface cultivation.

Results and Discussion

The primary mating compatibility between two cultures of *Streptomyces* could be judged from the absence of antagonism between them. *S. fradiae* SCF_5 culture was streaked on the complete agar in close proximity to three xylan hydrolase producing cultures viz. *S. exfoliatus* MC_1, *S. albosporeus* $1SS_1K$, *S. cyaneus* 3a. While $1SS_1K$ and 3a were found to be

inhibitory to SCF_5, only MC_1 was without any inhibition. Hence, SCF_5 and MC_1 were considered primarily compatible for mating. These two cultures after mixed cultivation yielded a number of isolates which were apparently the intermediary strains (judged from colony morphology and colour). Table 1 lists 8 of the isolates so obtained out of about 120 isolates examined, which possessed both xylan hydrolase and glucose isomerase activities in appreciable quantities. The parental strains SCF_5 and MC_1 were also compared along with these isolates for enzyme production as indicated in Table 1.

To ascertain whether the above isolates were true recombinants they were plated several times and repeatedly single colony isolations were made. Among the 8 isolates tested, only FE-16 was found to breed true and produced simultaneously both xylan hydrolase and glucose isomerase. Hence isolate FE-16 was considered as the true recombinant strain arising out of mating between *S. fradiae* SCF_5 and *S. exfoliatus* MC_1. The other isolates which in subsequent plating segregated into parental types were possibly the heterokaryons or heteroclones. Genetic recombination among *Streptomyces* was first shown by Sermonti[8] and detailed studies have been carried out by Hopwood[9]. Although intraspecies recombinations are understandable, genetic exchanges between two species of *Streptomyces* be considered as extraordinary, due to the fact that clearcut sexual differentiation has not been demonstrated in *Streptomyces* genus. There have been some reports however, on inter specific recombination as that of Alacevic[10] who showed the mating abilities between *S. remosus* × *S. coelicolor*, *S. coelicolor* × *S. aureofaciens*, and *S. rimosus* × *S. aureofaciens*. Bradley[11] also reported the recombination between *S. aureofaciens* ATCC 10762 × *S. violaceoruber* S 99. In the present work at least one true recombinant strain could be detected from among 120 isolates. Many of the others among the 120, were apparently the heterokaryons or heteroclones. The phenomenon of heteroclones has also been observed by Sermonti *et al.*[12,13] and Hopwood *et al.*[14]. All the earlier efforts on recombination of *Streptomyces* involved artificially induced traits such as auxotrophy by induced mutations which were often associated with peculiar difficulties. For

TABLE 1. XYLANHYDROLASE AND GLUCOSE ISOMERASE PRODUCTION BY INTERMEDIARY STRAINS OBTAINED AFTER MIXED CULTIVATION OF *S. exfoliatus* MC_1 AND *S. fradiae* SCF_5

Strain	Glucose isomerase (units/ml)	Xylanhydrolase (units/ml)
FE-6	2.60	1.00
FE-9	4.70	1.37
FE-14	3.60	1.02
FE-16	3.26	2.02
FE-24	3.20	2.51
FE-25	4.50	1.08
FE-29	4.80	1.10
FE-37	5.20	1.46
SCF-5	3.60	0.04
MC-1	0.06	3.60

Medium (*iii*) for glucose isomerase and Medium (*ii*) for xylan hydrolase (vide text) were used.

TABLE 2. SEMBLANCE OF FE-16 TO SCF_5 AND MC_1

Characters	Resembling to MC_1	Resembling to SCF_5	Remarks
1) Morphology	—	—	(Aerial mycelium, wavy, branched)
2) Acid fast stain	+	+	
3) Gram's stain	—	+	
4) Growth on media			
Glucose aspargine agar	—	—	Growth white to light grey, in white background with pale yellow to pale brown soluble pigment.
Glycerol aspargine agar	—	—	
Sucrose nitrate agar	—	—	
Nutrient agar	—		
Bennett's agar	—	—	
Wheat bran agar	—	—	Colourless pasty growth
Potato plug	—	—	Colourless leathery growth
Casein agar	—	—	Scanty whitish growth
Tyrosine agar			
Growth	—	—	Leathery growth
Clearance	+	+	
Cellulose agar	+	+	
Starch hydrolysis	+	—	
5) Gelation liquefaction	+	—	
6) Litmus milk	+	—	
7) Melanin production	+	—	
8) Nitrate reduction	—	+	
9) H_2S production	+	+	
10) Carbon source utilization			
a) Good growth with acid production utilizing glucose, fructose, mannose, glycerol, arabinose	+	—	
b) Moderate growth utilising xylose	+	—	
c) Poor growth in			
Acetate and rhamnose	—	—	
Raffinose	+	+	
Lactose	—	+	
d) No growth in sucrose and adonitol	+	+	

+ Resembles — Does not resemble

example, Alikhanian and Borisora[15] working with *S. aureofaciens* found only a preponderence of arginine requiring mutants which made genetic studies extremely difficult. Polsinelli and Beretta[16] also encountered similar difficulties in obtaining suitably marked strains. Other difficulties were poor conditions of mutation induction and weak viability of mutant clones. Such difficulties were not encountered in the present case as the markers chosen for study are the natural traits with which both the parental cultures were already endowed with.

The various characteristics of isolate FE-16 as compared to its parental strains SCF_5 and MC_1 are listed in Table 2. FE-16 resembled SCF_5 in 4 of the characterics including the production of glucose isomerase. It was Gram positive, capable of reducing nitrate and utilizing lactose like SCF_5. In 6 other characteristics FE-16 was like MC_1. It produced extracellular xylanhydrolase, hydrolysed starch, liquefied gelatin, converted litmus milk, answered melanin test and had the same pattern of utilization of six of the carbon sources as MC_1. It was noteworthy that the hybrid strain was unique in not resembling either of the parents in a number of characteristics. Morphologically FE-16 produced dull white aerial mycelium in contrast to SCF_5, which possessed seashell pink colour or the MC_1 which was ash grey. FE-16 is unlikely to be a mutant of either SCF_5 or MC_1, because of the possession of various traits of both the parent cultures which were reshuffled and segregated.

FE-16 culture showed some promise of practical utility It was capable of producing glucose isomerase on wheat bran without added xylose (Table 3). It produced 47 units glucose isomerase per gram. of wet bran in the surface fermentation with a specific activity of 0.98 units/mg protein. The aqueous extract of the bran was also found to contain 7-9 units per ml of xylan hydrolase. The weights of the wheat bran after cultivation of the FE-16 culture was found to be greatly reduced (nearly half) as compared with the wheat bran on which SCF_5 was grown; it indicated the ability of the FE-16 culture to degrade native wheat bran.

TABLE 3. PRODUCTION OF GLUCOSE ISOMERASE AND XYLANHYDROLASE BY FE-16 AND SCF-5 CULTURES ON XYLAN CONTAINING MEDIA*

Culture	Glucose isomerase		Xylanhydrolase	
	Submerged fermentation (units/ml)	Surface fermentation (units/g wet bran)	Submerged fermentation (units/ml)	Surface fermentation (units/ml)
FE-16	1.62	47.00	2.02	9.00
SCF-5	0.09	11.20	0.04	0.92

*For submerged fermentation medium (*ii*) and for surface fermentation medium (*iii*) were employed under respective cultural conditions.

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Utilization of Potato for Weaning Food Manufacture

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A process was standardized for the manufacture of weaning food. Of the four combinations studied, viz, 65:35:0; 65:30:5; 65:25:10 and 65:20:15 of potato, soybean cotyledons and skim milk solids, the last had the highest protein efficiency ratio of 1.9 (as compared to 2.5 of casein). The powdered product when reconstituted as gruel with 3.5 parts of water sweetened with 10% sugar and flavoured with strawberry was acceptable.

Several attempts have been made in India and abroad to develop lowcost foods from vegetable sources of protein to help overcome malnourishment. Potato is a major crop in India and because of lack of adequate cold storage facilities, utilization of large quantity is a problem. In order to provide fair return to growers, it is necessary to find out alternative means of utilization. Hence an attempt was made to incorporate potato, a wholesome food containing some nutrients, soybean—a source of protein and fat, and skim milk—a dairy by product having a good source of nutrients other than fat, to manufacture a weaning food.

Materials and Methods

Formulation of the weaning food: The correlation coefficient between the essential amino acids content of the reference protein[1] and those contributed by various proportions of peeled potato (P), soybean cotyledons (S) and skim milk (M) was used as the basis of the formulation of PSM weaning food, as suggested by Patil and Gupta[2]. Those combinations which had the highest correlation coefficient and also provided 20 per cent protein in the finished product were selected. The four formulations of weaning food developed and tried contained potato solids, soybean solids and skim milk solids, in the ratio of 65:35:0, 65:30:5, 65:25:10 and 65:20:15.

Processing: Good quality white potatoes were boiled for 30 min. in jacketted stainless steel kettle and peeled manually, Peeled potatoes were mashed and ground using a cheese grinder. Soybeans were presoaked in 0.5 per cent sodium bicarbonate solution for 12-14 hr and blanched in water containing 0.5 per cent sodium bicarbonate, for 30 min. cooled with water, dehulled and cotyledons separated from hulls and plumules by floatation. Ground potato, soybean cotyledons and skim milk were disintegrated with water to 30 mesh. The slurry was adjusted to 12 per cent solids and pasteurised at 85°C for 5 min. and dried on Richard Simon twin roller drier (24 kg water evaporation per hour capacity) using 60 psi steam pressure. The dried product was sieved and packaged in polyethylene lined kraft paper bags (Fig. 1).

Analyses: Moisture, protein, fat, ash and crude fibre were determined in the ingredients and the finished products using standard procedures[3-5]. The total carbohydrate content was determined by difference. The protein efficiency ratio (PER) of the four weaning foods was determined using 8 male weanling albino rats per group and feeding of prepared diets (with 10 per cent protein) *ad libitum* for 28 days, and corrected with that of cow skim milk casein fed as control[6,7]. The weaning foods were evaluated for sensory characteristics by a panel of judges using a 9-point scale, as powder: fluid (1 part powder+9 parts water) and sweetened (5,6,7,8, 10 and 12 per cent sugar), flavoured (strawberry or pineapple flavour) gruel (1 part powder+3.5 parts water).

Results and Discussion

The four potato-soy-skim milk (PSM) based weaning foods contained the requisite quantity of protein, fat, moisture, ash and crude fibre as required by the Protein Advisory Group (PAG) of the United Nations[8] for cereal based supplements (Table 1). These weaning foods were similar in their proximate composition to jowar-soybean-skim milk based weaning foods developed by Gupta and Arora[9].

The protein efficiency ratio of the four formulations ranged between 1.3 and 1.9 as compared to 2.5 for casein. Thus even the highest PER of 1.9 found in 65:20:

*Data taken from the first author's Masters dissertation, Kurukshetra University, 1980.

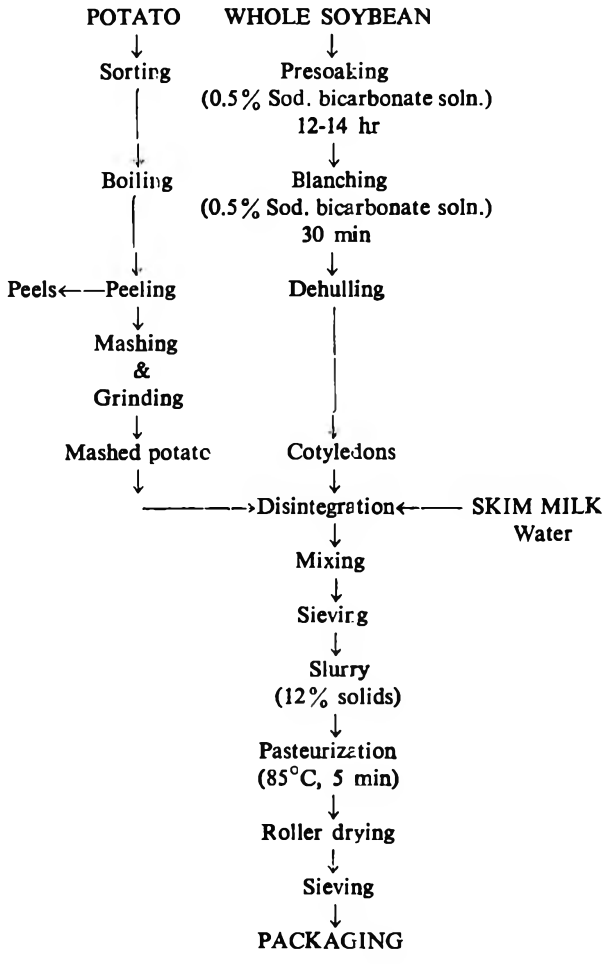

Fig. 1. Flow sheet of the manufacture of potato-soy-skim milk weaning food.

15 formulation was lower than the minimum required (2.1). The essential amino acids content of 65:20:15 combination had shown that nearly all the amino acids were present in the proportion required as per the reference protein except the sulphur amino acids (3.05 compared to 3.50 for the latter). Hence the possibility exists that, if the PSM weaning food was fortified with DL-methionine, the PER could be raised to the desired level. The PER of JSM weaning food[9] was observed to be higher than PSM. This could be due to lower initial PER of potato (0.9) compared to that of jowar (1.6). Thus on the basis of nutritional evaluation, the PSM weaning food could not be recommended as the sole source of nutrients.

If a sensory score of 6 (like slightly) is taken as the minimum acceptable level, then it was observed (Table 2), that the PSM weaning food was not acceptable in either fluid or gruel form unless it was sweetened with atleast 10 per cent sugar. Then also, combination 65:20:15 was not acceptable. Hence when it was tried with pineapple or strawberry flavour it became acceptable. In other experiements on soybean[9-11], strawberry flavour has been observed to improve the acceptability considerably. The product as gruel had an acceptable sensory quality although it had custard like body with a predominant taste of potato.

On the basis of this investigation, it is possible to utilize potato, when available in large quantitites at low prices, for the manufacture of weaning food of acceptable quality by adding flavouring agents. The protein efficiency ratio of the food, however, is lower than what is generally accepted as optimum for such foods. Further work needs to be done to improve the

TABLE 1. COMPOSITION AND PER OF POTATO-SOY-SKIM MILK WEANING FOOD

Characteristic	PSM Weaning food combinations				Reference weaning food*
	65:35:0	65:30:5	65:25:10	65:20:15	
Correlation coeff.**	0.942	0.946	0.950	0.953	—
Protein (%)	20.3	20.9	20.7	20.8	$\lessgtr 20$
Fat (%)	9.2	8.1	7.5	6.5	upto 10
Moisture (%)	5.5	6.2	7.7	9.2	5-10
Ash (%)	4.5	3.5	3.7	3.8	$\gtrless 5$
Crude fibre (%)	2.0	1.8	1.7	1.5	$\gtrless 5$
Carbohydrates (%)	58.5	59.4	58.8	58.3	—
PER***	1.3	1.5	1.7	1.9	>2.1

*PAG[8]

**Correlation coefficient between the essential amino acids content of reference protein and the PSM combinations.

***Corrected to 2.5 of casein

TABLE 2. SENSORY SCORES OF WEANING FOODS

Powder to water ratio	Sugar added (%)	Flavour added	PSM weaning food combinations			
			65:35:0	65:30.5	65:25:10	65:20:15
1:0	—	—	5.7	6.2	5.5	4.8
1:9	—	—	4.6	5.0	4.7	4.5
1:3.5	5	—	4.4	5.3	5.2	5.2
1:3.5	6	—	4.6	5.3	5.5	5.6
1:3.5	7	—	4.4	5.3	5.6	5.5
1:3.5	8	—	5.4	5.1	5.6	4.7
1:3.5	10	—	6.4	6.4	6.1	4.3
1:3.5	10	Vanilla	—	6.4	—	5.3
1:3.5	10	Pineapple	—	6.6	—	6.6
1:3.5	10	Strawberry	—	6.9	—	6.6
1:3.5	12	—	6.8	6.8	6.4	5.6

Note: 9 - point hedonic scoring consisted of: 9 = like extremely; 8 = like very much; 7 = like moderately; 6 = like slightly; 5 = neither like nor dis ike; 4 = dislike slightly; 3 = dislike moderately; 2 = dislike very much and 1 = dislike extremely.

protein efficiency ratio, body and texture of the gruel and masking of potato flavour by means of other flavours.

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Kinetics of Moisture Absorption by Soyflour

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Kinetics of moisture absorption of defatted and full fat soyflour was studied at 293, 313 and 333 K and at four relative humidity levels (75, 80, 85 and 100%). The results indicate that the absorption process could be satisfactorily described through a first order kinetics in the difference (m_e-m). The rate constant for the process was found to be a function of temperature and relative humidity. An equation to predict the moisture as a function of temperature and relative humidity of the surrounding atmosphere has been developed. Activation energies for moisture absorption for different flours were independent of the temperature and relative humidity, and ranged from 8.82 to 10.00 kcal/g-mole.

A knowledge of absorption of water vapour by flours of food materials is of practical importance in conditioning and storage of flours. The rate at which these materials absorb moisture from surrounding environment is of considerable practical and theoretical interest. This has application both in development of processes and design of equipments involving moistening with or without thermal treatment. It is desirable if the kinetics of the process could be described as a function of time, temperature and relative humidity. Soyflour is an important base material for many protein foods and textured products. Moistening of flour through absorption of water from the vapour phase is an important step in the total chain of unit operations. Generaly, the moisture contents of most food grains and their products in storage correspond to equillibrium relative humidity of 70 per cent[1]; consequently, situations of moisture absorption by such products from the surrounding environment arise only when the humidity of the atmosphere exceeds that corresponding to the storage moisture content. Therefore, the present work was restricted to atmospheric humidities above 75 per cent.

Published work on kinetics of water vapour absorption by flours pertains either to wheat flour[2,3] or its fractions[4]. Studies of Udani *et al*[2]. and Bushak *et al.*[4] have been limited to room temperature and different humidity ranges. Singh *et al*[3]. have studied the kinetics at saturation humidity and different temperatures. In all cases the rate process has been found to follow the first order kinetics. No published information is available on the process of water vapour absorption by soyflour. This paper describes the water vapour absorption kinetics of defatted and full fat soyflours.

Materials and Methods

Studies were conducted on full fat (cooked and uncooked) and defatted soyflour. Cooked soyflour was obtained by extrusion cooking of full fat dehulled soybean followed by grinding in a pin mill. Uncooked soyflour represented the material produced by grinding of dehulled raw soybean. Defatted soyflour was produced by grinding the commercially available solvent extracted food grade flakes. Physical properties and gross composition of the soyflour (on moisture free basis) were measured and are given in Table 1.

The experimental setup and the procedure described by Narain *et al*[5]. and Singh *et al*[3]. was used for the study. The product was thoroughly mixed and divided

Table 1. Physical properties and chemical composition of soyflour samples

Parametrs	Defatted	Full fat Cooked	Full fat Uncooked
Bulk density, (g/cc)	0.57	0.43	0.47
Particle density (g/cc)	1.45	1.69	1.30
Porosity (%)	50.63	74.24	63.65
*Protein (%)	55.00	38.00	40.00
*Fat (%)	0.50	23.61	33.50
*Carbohydrate (%)	37.50	31.61	33.50
*Total ash (%)	7.00	5.86	6.50
Initial moisture content, (%) (d.b.)	9.00	6.00	10.50

*On moisture free basis.

into 25 g samples. Each sample was sealed in polyethylene bag and stored at room temperature. Half of the sample (12 g) was used for studying the moisture absorption kinetics and the remaining half for determining the initial moisture content. The experiments were conducted at four relative humidity levels in the neighbour hood of 75, 80, 85, and 100 per cent, and at temperatures of 292, 313 and 333 K. Saturated salt solutions were used to maintain the required relative humidity in the test chamber. Before starting each experiment, appropriate salt solution was transferred into the test chamber, the temperature control adjusted at the desired level and the system was allowed to stabilize for 2 to 3 hr under the temperature and humidity of the test atmosphere.

To avoid moisture condensation on the sample, specially at high temperatures and relative humidities, the samples were preheated in a chamber which formed part of the system. This preheating was done to a temperature of about 2°C above the test chamber temperature. The preheating operation took about 15 min. Thereafter, the sample was quickly lowered into the test chamber. The weight of the sample recorded immediately after preheating was taken as the weight at zero time. Subsequent sample weights were measured *in situ* after every 15 min interval. The buoyancy corrections were found to be negligible and hence were not made. Each experiment was replicated thrice for a duration of 2.5 hr. Initial moisture content of the sample was determined by drying the sample at 130±1°C for one hour.

Results and Discussion

The defatted samples for the same exposure time, absorbed more moisture than the full fat flours over the entire temperature and relative humidity range. However, on comparison of moisture values corresponding to nonfat solids, it was seen that the nonfat soy solids associated with uncooked full fat soyflour had the highest sorptive capacity, followed by those of defatted soyflour and full fat cooked soyflour in that order. The extrusion cooking process used in production of full fat cooked soyflour entails thermal treatment of much higher order than that of the toasting process associated with production of defatted soyflakes. Therefore, heat denaturation of proteins in the former can be expected to be more. Denaturation is known to reduce the sorptive capacity of proteins. This must contribute towards the differences in the moisture absorption behaviour of the nonfat soy solids in the two four samples. The corresponding solids of the uncooked flour absorbed more moisture, being free from heat induced changes. The results suggest that texturization tends to compact the substance of the flour. A compact structure has lower specific surface and also would make penetration of water molecules more difficult. These effects coupled with heat induced changes must account for the differences in sorptive capacity of the different soyflour samples.

Kinetics of absorption

On the basis of first order rate kinetics

$$\frac{dm}{dt} = k\,(m_e - m) \qquad \ldots.(1)$$

$$\text{or } \frac{m_e - m}{m_e - m_o} = \exp.(-k\,t) \qquad \ldots.(2)$$

where, m represents dry basis moisture content of the flour and t, the time; k stands for rate constant. Subscripts o and e denote initial and final values. Since moisture difference and the weight difference ratios are the same for any given time, it follows that

$$\frac{m_e - m}{m_e - m_o} = \frac{W_e - W}{W_e - W_o} = \exp.\ (-kt) \qquad \ldots.(3)$$

Therefore, following Singh *et al*[3] all data were analysed in terms of sample weights to avoid carrying over of any inadvertant error involved in the measurement of initial moisture content.

It can be readily shown[3] that if data follow first order rate kinetics, then a plot of W_n versus W_{n+1}, which are consecutive sample weights, should yield a straight line provided the weight measurements have been made at equal time intervals. The present data followed this behaviour.

From eq. (3) it can also be shown that for any set of three sample weights taken at equally spaced time intervals

$$W_e = \frac{W_n(W_{n+i}) - (W_{n+2i})^2}{W_n + W_{n+i} - 2(W_{n+2i})} \qquad \ldots.(4)$$

W_n, W_{n+i} and W_{n+2i} are any three sample weights taken at time interval i. Eq. (4) is the same as recommended by Issaacs and Gaudy[6]. The parameter W_e in eq. (3) was evaluated through eq (4). Thereafter, the rate constant k was evaluated by 'least square' method of analysis using eq. (3) in its loglinearized form. The values m_o and W_o being known m_e could be directly obtained from W_e. For all the materials both k and m_e were found to vary with temperature as well as relative humidity.

The values of the final moisture content m_e could be related to temperature and relative humidity through the expression

$$m_e = A \exp.\ (B\tfrac{rh}{T}) \qquad \ldots.(5)$$

A and B represent empirical constants. The values are 0.022, 0.0252 and 0.245 for A and 725.37, 584.69

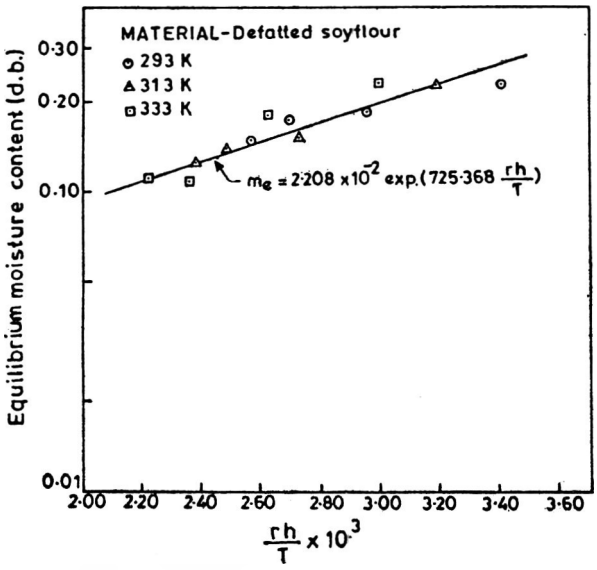

Fig. 1. The relationship between equilibrium moisture content, relative humidity and temperature.

and 640.63 for B for defatted, fullfat cooked and uncooked soyflour, respectively. A plot of eq.(5) for defatted soyflour is given in Fig. 1. The same trend has been noted by Harpal Singh[7] for extruded soyproducts. Eq.(5) could also be considered to be an extension of the relationship of Singh *et al*[3] established for wheat flour absorbing moisture from saturated atmosphere.

More precisely the final moisture content m_e should be the equilibrium moisture content of the product corresponding to the prevailing atmosphere. Comparison of the caluculated m_e values for the present data with the equilibrium moisture content values measured by Vijay Pratap[8] using static desiccator technique shows that the former are lower by 5 to 7 per cent.

Temperature dependence of k followed the Arrhenius law and the calculated average activation energy values

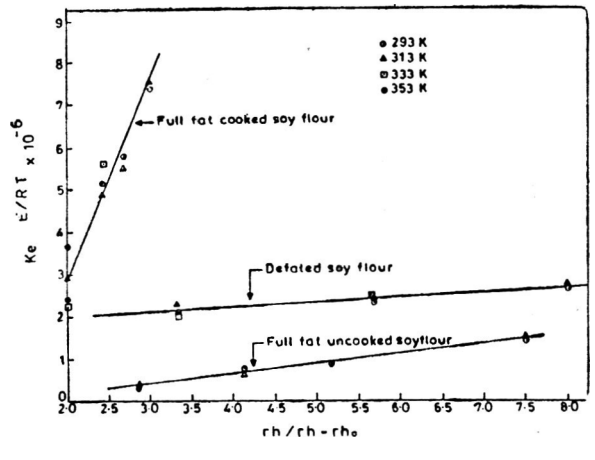

Fig. 2. Combined effect of temperature and relative humidity on rate constant.

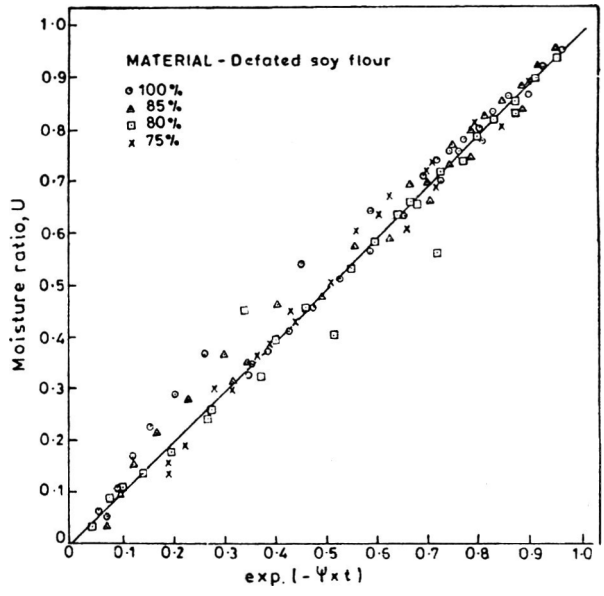

Fig. 3. Prediction graph for moisture absorption $\Psi = \Psi$ (rh, T).

for full fat (uncooked), defatted and full fat (cooked) soyflour were 8.82, 9.62 and 10.00 k-cal/g-mole respectively. These values are quite consistent with those obtained in hydration of food materials. Higher values of activation energy in defatted and full fat (cooked) soyflour clearly indicate the effect of heat induced changes as discussed earlier. Rate constant for all products decreased with increase in relative humidity.

Fig. 2 shows the combined effect of temperature and relative humidity on the rate constant for the absorption

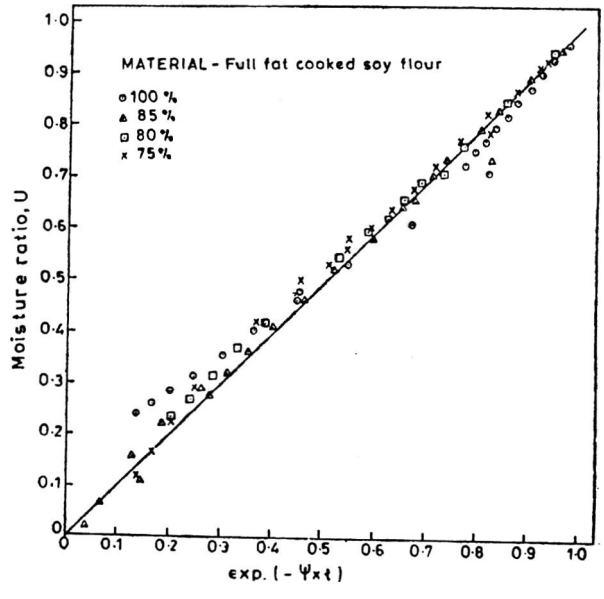

Fig. 4. Prediction graph for moisture absorption $\Psi = \Psi$ (rh, T)

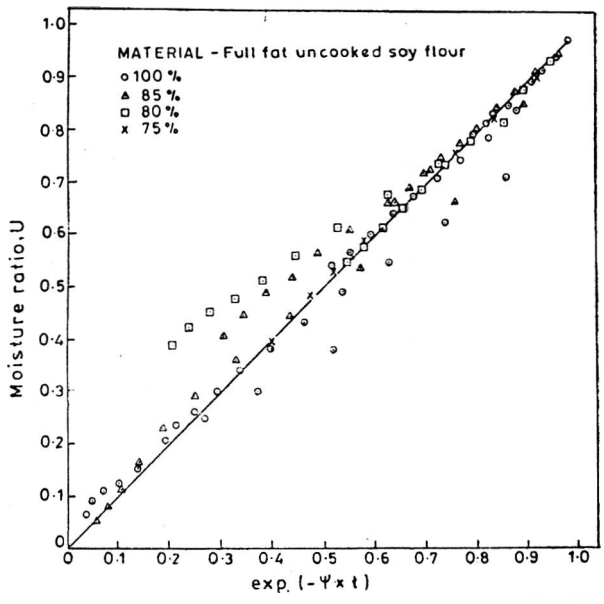

Fig. 5. Prediction graph for moisture absorption $\Psi = \Psi$ (*rh*, T)

process. The estimated values of rh_o are 0.70, 0.50 and 0.65, and the corresponding values of slopes (λ_o) and intercepts (λ_1) of the lines in Fig. 2 are 0.106×10^6, 4.58×10^6, 0.233×10^6 and 1.80×10^6, -6.264×10^6, -0.287×10^6 for defatted, full fat cooked and full fat uncooked soyflour respectively. A final equation for k could be written as

$$k=\lambda_1+\lambda_o\left(\frac{rh}{rh-rho}\right)\exp.\left(-\frac{E}{RT}\right) \qquad \ldots.(6)$$

Combining eq. (2), (5) and (6), a composite expression could be written which would fully define the kinetics of moisture absorption. Fig. 3, 4 and 5 show a comparision of the measured moisture difference ratios and the values calculated using eq. (2), (5) and (6). The line drawn through data points represent calculated values. A correlation of 0.94 to 0.98 could be obtained.

From these it could be concluded that (*i*) The absorption of water vapour by soyflours could be described through the first order rate kinetics equation. (*ii*) The rate constant was found to be a function of temperature and relative humidity. The temperature dependence could be described through Arrehenius law. The activation energies varied from 8.82 to 10 k-cal/g-mole. (*iii*) The final moisture content as evaluated through the first order kinetic model was found to be a function of both relative humidity and temperature. (*iv*) A general prediction equation for the moisture content of soyflours as a function of relative humidity, temperature and time could be established to describe the kinetics of the moisture absorption process. (*v*) Soyflours which had been exposed to heat treatment in their production process absorbed less moisture and had higher energies of activation for the absorption process.

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LIPOTROPE-LIKE ACTIVITY OF RED PEPPER

When red pepper (*Capsicum frutescens*) at 5% or an equivalent amount of its active principle capsaicin (15 mg%) was fed alongwith a choline-free diet to rats, the increase in liver neutral lipids was significantly lower than in animals not fed red pepper or capsaicin. On the other hand, paprika (*Capsicum annum*) which contains very little capsaicin did not show such activity.

In an earlier study, we had observed that red pepper fed to rats in high-fat or choline-free diets significantly lowered the accumulation of liver lipid[1]. Under the same conditions an equivalent amount of capsaicin also showed similar activity. In order to confirm that the lipid lowering activity of red pepper is due to capsaicin, a study was conducted with paprika powder which contains very little capsaicin and the results are reported in this communication.

Red pepper (*Capsicum frutescens*) was obtained from the local market and ground to 40-50 mesh size. It contained 0.3 per cent capsaicin[2]. Paprika (*Capsicum annuum*) powder was obtained from Lalocsa Paprika Co., Hungary. Its capsaicin content was found to be 0.005 per cent. Synthetic capsaicin (N-vanillyl nonanamide) was purchased from Fluka AG, Switzerland.

The basal diet composed of protein (casein) 5 per cent, sucrose 52 per cent, hydrogenated vegetable oil 40 per cent and salt mixture 2 per cent[3] and choline-free vitamin mixture 1 per cent, with adequate amounts of vitamins A, D and E[4]. Four groups of male albino rats of the Wistar strain weighing 85-90g were fed the following diets for 6 weeks. (*i*) Basal, (*ii*) basal+5 per cent red pepper, (*iii*) basal+15 mg per cent capsaicin, and (*iv*) basal+5 per cent paprika powder. Rats were fed *ad lib* and water was supplied through a polyethylene bottle.

At the end of the experimental period, rats were sacrificed under ether anaesthesia and livers were collected, washed with saline, blotted, weighed and stored in a deep freeze at - 20°C till analysis. Total lipids were extracted and purified according to Folch *et al*[5]. The purified lipid extract was used for estimating total lipids by gravimetry; phospholipids and cholesterol were estimated according to Marinetti[6] and Searcy and Bergquist[7] respectively. Neutral lipids were calculated by subtracting the sum of phospholipids and cholesterol from total lipids.

The influence of feeding red pepper, capsaicin or paprika powder on liver lipids is shown in Table 1. Both red pepper (5 per cent) and capsaicin (15 mg per cent) significantly reduced the accumulation of liver lipids particularly neutral lipids, whereas paprika powder at 5 per cent level did not exhibit any such activity. Phospholipids and cholesterol were not affected in any of the experimental animals. Such a low protein diet as used in the present study has been used earlier[8] for producing fatty livers in rats. Moreover the lipotrope-like activity of red pepper was also demonstrated by us[1] on a 15 per cent groundnut protein diet, which is adequate with respect to protein content but is deficient in methionine.

TABLE 1. INFLUENCE OF RED PEPPER, CAPSAICIN OR PAPRIKA POWDER ON LIVER LIPIDS IN RATS FED CHOLINE-FREE DIET

Ingredients added		Total lipids (mg/g)	Phospholipids (mg/g)	Cholesterol (mg/g)	Neutral lipids (mg/g)
Name	Quantity %				
Nil	—	84.8±2.14	28.1±1.08	10.7±0.54	46.0±2.53
Red pepper	5	71.9±4.19*	28.8±1.18	9.50±0.37	31.6±3.39**
Capsaicin	15 mg	72.5±2.45**	27.7±1.65	10.4±0.75	34.4±2.73**
Paprika	5	85.3±4.36	27.5±1.05	10.5±0.32	47.3±3.31

Values are mean±SEM for 6 rats

*P<0.05; **P<0.01

These results are in conformity with the earlier observations with red pepper and capsaicin[1]. The absence of lipotrope Like effectin the group fed paprika powder which is similar to red pepper in all respects, except for the negligible capsaicin content lends support to the conclusion that the effect of red pepper is due to capsaicin. The lower lipid levels would be due to any one or a combination of the following factors: decreased synthesis, increased oxidation or increased transport from the liver. The influence of red pepper and capsaicin on these processes is under investigation.

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THE ROLE OF DISSOLVED OXYGEN IN THE IMPROVEMENT OF COLD SOAKING PROCESS OF PADDY

Spoilage of paddy as indicated by foul smell, cloudiness, slimy growth in the soak water and loss of dry matter during cold soaking is related to changes in the dissolved oxygen (DO) content of the soak water. The DO of the soak water was completely used up within 24 hr of soaking, when the above spoilage changes were noticed. Addition of sodium chromate at 0.05% (w/w) to soak water, chemical sterilization of paddy before soaking and refrigerated soaking at 5-10°C, kept the DO of soak water between 1.5 and 10.4 ppm at the end of soaking and thus helped in eliminating the spoilage and minimising the dry matter loss. Addition of hydrogen peroxide, sodium peroxide and potassium permanganate to soak water increased the DO initially, but completely depleted within 48 hr resulting in the spoilage and dry matter loss.

Soaking of paddy for 36 to 72 hr in water with or without prior steaming is adopted in most of the conventional rice mills. During such soaking, an undesirable off smell develops due to the multiplication of yeasts and bacteria on paddy.[1,2] The spoilage changes also result in dry matter loss of about 1-2 per cent in dry season paddy and 3-4 per cent in wet season paddy.[3] The microbial growth and consequent development of foul smell can be checked if the conditions of soaking are kept more or less sterile during the period of soaking.[1,2] Blowing of air and repeated change of water during soaking were reported to eliminate the foul smell.[1] Application of sodium chromate to the soak water was found to eliminate the foul smell and reduce the leachate loss in rice.[3,4] The correlation between the dissolved oxygen (DO) content of soak water with the spoilage changes and the dry matter loss in soaking of paddy were studied and results are reported here.

'Co 25' variety of paddy, cleaned in the dockage tester was taken in 1.0 kg lots in polyethylene buckets, soaked in 1.5 l. cold water for 72 hr and shade dried. The difference in the weight of paddy on moisture free basis before and after soaking was taken as the dry matter loss. Observations like smell and condition of paddy, cloudiness and slimy growth in soak water were made by sensory evaluation. Water alone and water with paddy were taken as control experiments. In the continuous water flow experiment, constant quantity of water was allowed to pour in from the top and drain out simultaneously from the bottom of the vessel through a special tap arrangement. After trying various rates of flow of inlet and outlet water, it was found that a minimum flow rate of 3.5 ml per minute per kg of paddy has to be maintained for getting smell free paddy and

TABLE 1. DIURNAL AND NOCTURNAL VARIATION IN DISSOLVED OXYGEN CONTENT OF SOAK WATER FROM 'CO 25' PADDY

Soaking period	DO (ppm) of soak water after indicated periods (hr)			
	0	6	12	24
6.00 a.m. to 6.00 p.m.	3.4	4.9	2.0	0
6.00 p.m. to 6.00 a.m.	3.4	4.8	0.0	0

hence this flow rate was maintained. Care was taken to maintain the soak water level above the paddy to avoid exposure of paddy to the atmosphere. Chemical sterilisation was done by soaking 1.0 kg of paddy in 1.5 l. of solution containing 1.0 per cent copper sulphate and 0.1 per cent mercuric chloride for 10 min and then washing it with sterilised water to remove the adhering chemical on the paddy.

Chemicals like sodium chromate, hydrogen peroxide, sodium peroxide and potassium permanganate (all of LR grade) were added at 0.05, 0.2, 0.2 and 0.01 per cent (W/W) respectively to increase the DO in the soak water. Refrigerated soaking was carried out by keeping the samples in the refrigerator at 5 to 10°C. The moisture content of paddy was determined by air oven method.[5] The DO was determined by the Winkler method using $MnSO_4$ and $Na_2S_2O_3$ reagents.[6] The day and night experiments (Table 1) were started at 6.00 a.m. and 6.00 p.m. respectively. Samples from both the experiments were taken at 6, 12 and 24 hr intervals. Soak water samples from experiments given in Table 2 were taken at 9.00 a.m. every day.

TABLE 2. DIFFERENT TREATMENTS AND THEIR EFFECT ON THE UTILISATION OF DISSOLVED OXYGEN DURING SOAKING OF 'CO 25' PADDY

Treatment	Dissolved oxygen content at (ppm)			
	0 hr	24 hr	48 hr	72 hr
Control (water alone)	3.5	3.3	3.3	3.0
Control (water+paddy)	3.5	0	0	0
Raw paddy	3.5	3.0	2.2	0.3
Steamed paddy	3.5	3.0	3.0	3.0
Chemically sterilised paddy+ sterile water	3.5	1.8	1.6	1.5
Hydrogen peroxide 0.2%	33.6	0	0	0
Sodium peroxide 0.2%	36.8	0	0	0
Potassium permanganate 0.1%	4.0	2.9	0	0
Sodium chromate 0.05%	28.2	27.7	19.7	10.4
Refrigerated soaking	3.5	5.4	3.7	3.0

Raw paddy and steamed paddy had continuous water flow

TABLE 3. EFFECT OF DIFFERENT TREATMENTS ON THE SOAKING CONDITIONS AND DRY MATTER LOSS IN 'CO 25' PADDY

Treatment	Sampling time (hr)									Dry matter loss (%)
	24			48			72			
	Cloudiness	Off smell	Slimy growth	Cloudiness	Off smell	Slimy growth	Cloudiness	Off smell	Slimy growth	
Control (water+paddy)	+	+	+	++	++	++	+++	+++	+++	1.81
Raw paddy	—	—	—	—	—	—*	—	—	—*	2.47
Steamed paddy	—	—	—	—	—	—	—	—	—	1.15
Sterilised paddy & sterile water	—	—	—	—	—	—	—	—	—	1.09
H_2O_2 0.2%	+	+	—	++	++	+	+++	+++	+++	1.65
Sod. peroxide 0.2%	+	+	—	++	++	+	+++	+++	++	1.70
Pot. permanganate 0.1%	—	—	—	+	+	—	++	++	—	1.50
Sod. chromate 0.05%	—	—	—	—	—	—	—	—	—	1.00
Refrigerated soaking	—	—	—	—	—	—	—	—	—	0.58

—: No spoilage; +, ++, +++: Denotes increasing order:; *: Germinated

Raw paddy and steamed paddy only had continuous water flow.

The depletion of DO was found to be faster during night than during the day (Table 1). The effect of light in minimising the growth and activity of microbes might be the reason for this difference. However, the DO was completely used up within 24 hr of soaking. The growth of microbes was found to be retarded by light.[7] DO was also found to increase during the first 6 hr of soaking followed by a decrease. The release of entrapped air in the space between the husk and kernel might contribute to this increase.

The spoilage and the dry matter loss noticed were found to be interrelated with the change in the DO (Table 2 and 3). Complete depletion of DO was significantly noticed by the beginning of the spoilage changes within 24 to 48 hr of soaking as seen in the control (water+paddy), hydrogen peroxide, sodium peroxide. and potassium permanganate treatments. In the chemically sterilised paddy, sodium chromate treated, refrigerated soaked and in continuous water flow with steamed paddy, where the DO was kept at the end of 72 hr, at 1.5 to 10.4 ppm no spoilage was observed. In all these treatments, the proliferation of the microbes was controlled and this might be the factor for the maintenance of DO in soak water. The proliferation of microbes in the cold water soaking of paddy has been reported.[2] However, a slight protrusion of the rice germ was noticed in about 40 per cent of the grains during the 48 hr of soaking in the continuous water flow treatment with the unsteamed paddy. At the same time the DO of the outlet water was also reduced to 2.2 ppm which finally came down to 0.3 ppm. at the end of soaking. The rapid depletion of DO might be due to the germination of the grains. Increased oxygen uptake by the germinating seeds under submerged condition was reported by Pradet *et al.*[8] and Woodstock *et al.*[9] who have also reported a positive and significant correlation between oxygen uptake by germinating seeds and their germinability and seedling growth. The germination and the depletion of DO were absent in the same treatment where presteamed paddy was used. Consequent on the destruction of the seed viability due to presteaming there was no germination and hence the absence of any significant change in the DO of the outlet water. The partial sterilisation of paddy in the steaming process in arresting the rapid proliferation of microbes was also a factor in controlling the drop in DO. Moreover, since there is continuous movement of water in the system, the nutrients diffusing from the grains are removed by the running water and hence the chances for proliferation of the microbes are greatly reduced. Since the microbes and the seed enzymes are known to remain inactive at 5-10°C, in the refrigerated soaking there was only a very negligible drop in the DO after 72 hr and consequently no spoilage changes were noticed. Eventhough there was significant reduction in the DO when chemically sterilised paddy was soaked in sterile water, the DO was 1.5 ppm after 72 hr and hence there were no spoilage changes. It is significant to note that the DO of continuous water flow with steamed paddy and the refrigerated soaking treatments at the end of soaking were not much reduced and were the same as that of the control (water alone) indicating a highly reduced microbial activity in these two treatments.

Like the spoilage, changes in the dry matter loss was also directly correlated with the change in the DO. The dry matter loss was very significant in the control (with paddy), hydrogen peroxide, sodium peroxide and potasium permanganate treatments where the DO was, completely depleted within 24 to 48 hr of soaking. However, it was relatively higher than the control (water+paddy) in the continuous water flow treatment with unsteamed paddy. Since there was about 40 per cent germination in the paddy during soaking, there was a higher dry matter loss. Vasan[10] reported that the loss of dry matter in the germinated grains was more since the kernel in the germinated grains was exposed to the soak water. In the case of steamed paddy, there was no germination and consequently there was lower dry matter loss. The existing double steaming process perhaps owes its development from this observation. Wherever the DO was kept at 1.5 ppm and above till the end of the soaking the dry matter loss was less, as seen in the continuous water flow with steamed paddy, chemically sterilised paddy, sodium chromate and refrigerated soaked treatments. The loss was almost similar in all the above treatments from 1.00 to 1.15 per cent, except in the refrigerated soaking where it was only 0.58 per cent. However, presence of a higher level of DO (more than 10.4 ppm.) throughout the soaking as noticed in the sodium chromate soaking does not correspondingly reduce the dry matter loss.

The present study points out the correlation of dissolved oxygen with the spoilage changes and the dry matter loss during cold soaking of paddy. Complete depletion of DO invariably results in the spoilage changes and keeping the DO at and above 1.5 ppm at the end of soaking not only avoids the spoilage changes but, also reduces the dry matter loss.

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TOXICITY OF ETHYLENE DIBROMIDE AND METHYL BROMIDE MIXTURE TO *SITOPHILUS ORYZAE* (L.) AND *TRIBOLIUM CASTANEUM* (HERBST)

Toxicity of 1:1 (w/w) ethylene dibromide and methyl bromide mixture against the adults of *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst) is reported. The mixture reduced the multiplication potential of *T. castaneum*.

Instances of insect resistance towards individual fumigants including methyl bromide and phosphine have been reported[1]. The use of fumigant mixtures may solve the problem of insect resistance towards a single fumigant. Kazmaier and Fuller[2] studied the toxicity of ethylene dibromide and methyl bromide mixture to *Tribolium confusum* Duval. The mixture has been found particularly suitable for the disinfestation of stored products in India[3]. The purpose of this study was to collect basic toxicity data of the mixture against *Sitophilus oryzae* (L.) and *T. castaneum* Herbst. The post fumigation potential of the latter for multiplication was also investigated

Two to three week old adults of *S oryzae* and *T. castaneum* collected from established cultures were fumigated with a 1:1 (w/w) mixture of ethylene dibromide and methyl bromide in batches of 30, in 0.85 L desiccators at 25-30°C and 40-70 per cent R.H. for 24 hr. The fumigation procedure has been described elsewhere[4]. Seven (*S. oryzae*) or five (*T. castaneum*) dosages with 12 replicates per dose were studied. Equal number of controls were also included. At the end of the exposure period mortality was recorded. *S. oryzae* were, then placed in plastic vials containing sorghum and held at the rearing temperature and R.H. On the 23rd day, final mortality was noted and it was corrected for control mortality[5]. *T. castaneum* adults from each of the four replicates of a dose and control were placed in 150g whole wheat flour containing 5 per cent dried yeast. The adults were discarded on the 23rd day after recording the mortality. F_1 progeny (larvae) were counted and discarded once in a week.

From the final mortality data, LD_{50} and LD_{95} values were estimated after probit analysis[6].

The lethal dosages (mg/l) with the fiducial limits were as follows:

	LD_{50}	LD_{95}
S. oryzae	1.36(1.44, 1.29)	2.40(2.66, 2.16)
T. castaneum	1.16(1.19, 1.13)	1.78(1.85, 1.71)

Rajendran and Muthu[4] estimated LD_{50} values of methyl bromide and ethylene dibromide against *S. oryzae* as 1.14 and 1.49 mg/l respectively; against *T. castaneum* the values were 2.65 and 0.72 mg/l respectively. The mixture was more toxic to *S. oryzae* when compared to ethylene dibromide alone. If methyl bromide was taken as the criterion the mixture was less toxic to *S. oryzae*. The mixture was better than methyl bromide against *T. castaneum*. Based on mortality data arrived on '1' post-fumigation day, Kazmaier and Fuller[2], however, claimed that the mixture was more effective than the individual fumigants to *T. confusum* exposed for 5 hr.

The mixture like ethylene dibromide[3] affected the multiplicative potential of *T. castaneum*. The possible reasons for reduced potency for multiplication could be, that the treated insects laid more of infertile eggs, and that the mixture has selectively killed a particular sex of the population or that their reproductive physiology was affected. Kazmaier and Fuller[2] noted that whenever ethylene dibromide exceeded 1 mg/l in ethylene dibromide-methyl bromide mixtures of different ratios, *T. confusum* failed to produce viable progeny. In the present investigation surviving insects after fumigation with 1:1 ethylene dibromide-methyl bromide mixture consisting of 1.5 mg/l or ethylene dibromide laid viable eggs.

TABLE 1. MULTIPLICATIVE POTENTIAL OF *T. CASTANEUM* EXPOSED TO 1:1 W/W ETHYLENE DIBROMIDE AND METHYL BROMIDE MIXTURE

Fumigation dose (mg/l)	Surviving insects (nos.) After treatment	On 23rd day	Progeny produced during the holding period (nos.)
0	360	360	17115
1.00	360	265	6986
1.50	359	42	523
2.00	343	13	338
2.50	131	1	214
3.00	74	7	6

In India, it has been the experience that fumigation with aluminium phosphide tablets usually warrants repeated application in a short period due to the low toxicity of phosphine to eggs and pupae, whereas treatment with ethylene dibromide ensured more effective disinfestation, as further treatment was generally not required for a longer period. The effect of ethylene dibromide, either alone or in a mixture, in reducing the multiplication potential of insects may be one of the reasons.

In conclusion, the toxicity of 1:1 (w/w) ethylene dibromide and methyl bromide mixture was in between either of the fumigants applied alone. The mixture reduced the productivity of *T. castaneum*.

In practical fumigation, there would be various permutations and combinations of a mixture of fumigants. The effective concentrations would be revealed by bioassays and gas chromatographic studies.

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STUDY OF THE OCCURRENCE OF AFLATOXIN B_1 IN FOODGRAINS

Of the 125 samples of foodgrains and oilseeds analysed, aflatoxin B_1 was found in 23.2% of the samples. Among the 15 samples of each foodgrain analysed, aflatoxin B_1 was found in 2 samples of rice, 6 samples of jowar, 10 samples of raw groundnut, 5 samples of roasted groundnut and in 6 samples of maize.

Aflatoxins are a group of structurally similar difuranocoumarin compounds classified as aflatoxin B_1, B_2, G_1, G_2, M_1, M_2, B_{2a} and G_{2a} produced as secondary metabolites by certain strains of *Aspergillus flavus* Link Ex Fries and *Aspergillus parasiticus* Speare including other species of *Aspergillus*, *Penicillium* and *Rhizopus*[1,2]. Aflatoxin B_1 is occasionally found in rice, ragi, sorghum, wheat, cotton, soybean, spices[5], oil seeds, pulses, green coffee, tobacco, meat, cheese and feedstuff[3,4]. Aflatoxin contamination of foodgrains is now recognised to be a potential health hazard. It has been reported that aflatoxicosis caused the death of several persons in Banaswara and Panchamahal districts of Rajasthan and Gujarat respectively in India[6,7]. The primary objective of the present study is to find out the aflatoxin B_1 contamination of food commodities collected from various parts of the country as it has been reported to be the most potent of all carcinogens known[8].

A total of 125 samples of different foodgrains and oilseeds, such as 15 each of paddy, rice, parboiled rice, wheat, jowar, roasted groundnut, maize and 20 samples of raw groundnut were collected from adjoining villages and markets of Hapur, Muzaffarnagar, Dasna, Gwalior, Calcutta, Udaipur and Patna (India) during 1970–80. The samples from adjoining villages and Hapur were collected from traditional storage structures and the samples from Muzaffarnagar, Dasna, Gwalior, Calcutta, Udaipur and Patna were collected from conventional godowns. One kg. of sample was collected from different parts of the structure randomly. After proper mixing, 50g of the material was taken for analysis of aflatoxin B_1 using the method of Pons *et al*[9].

The cleaned up extract was dried by the passage through a bed of anhydrous sodium sulphate and after evaporation, the residue was dissolved in chloroform and made upto 5 ml. 5μl, 10μl and 20μl aliquots of the sample extraction were spotted on the TLC plate. On the same plate, was spotted 5μl and 10μl of aflatoxin standard containing 0.0025μg and 0.005μg of aflatoxin B_1 respectively. The plates were developed in chloroform: acetone (88 : 12 v/v). Chromatographic plates were examined under long wave U.V. light (363 nm) for flourescent compounds. Further confirmation was made by spraying the plates with 10 per cent HCl in 95 per cent

TABLE 1. AFLATOXIN B_1 IN DIFFERENT COMMODITIES

Commodity	No. of samples analysed	Samples contaminated	No. of samples with Aflatoxin B_1 concn (ppb) in the range of				
			0	1-50	51-150	151-300	301-700
Paddy	15	Nil	—	—	—	—	—
Rice	15	2	13	2	—	—	—
Parboiled rice	15	Nil	—	—	—	—	—
Wheat	15	Nil	—	—	—	—	—
Jowar	15	6	9	1	2	1	2
Raw groundnut	20	10	10	3	3	2	2
Roasted groundnut	15	5	10	3	2	—	—
Maize	15	6	9	3	2	—	1

ethyl alcohol[10]. The samples, which were having more than 50 ppb toxin, were determined with Beckman spectrophotometer by the method of Nebney and Nesbit[11]. The samples having less than 50 ppb of aflatoxin B_1 were analysed as per "comparison of standard"[12] procedure to determine the concentration of aflatoxin B_1.

Of the 125 samples of foodgrains and oilseeds analysed, only 29 samples were contaminated with aflatoxin B_1 (Table 1). Aflatoxin B_1 was detected and confirmed in two out of the 15 samples of rice (20 and 50 ppb), six out of the 15 samples of jowar (22.8–550 ppb), ten out of the 20 samples of raw groundnut (33-440 ppb), five out of the 15 samples of roasted groundnut (10–85 ppb) and six out of the 15 samples of maize (15–680 ppb). None of the samples of paddy, parboiled rice and wheat were contaminated with aflatoxin B_1.

The present investigation reveals that the foodgrains and oilseed samples collected from the traditional storage structure were found contaminated with variable amounts of aflatoxin B_1 ranging from 10–680 ppb. The amount of aflatoxin B_1 found in this study is quite low as compared with the amount (10,000 ppb) reported in a study of contaminated cereal grain in North America[13].

All the samples of paddy, parboiled rice and wheat analysed were free from aflatoxin B_1. It might be due to the fact that these samples were collected from conventional godowns wherein optimum environmental conditions for mould development are not easily available. In addition to this, as already pointed by Majumder[14], the regular use of fumigants and insecticides in the godowns might have killed the fungal spores.

Out of all the contaminated foodgrain samples, maize grains, jowar and groundnuts (raw) were contaminated with highest amounts of aflatoxin B_1. The lowest amount, however, was found in rice. It is interesting to note that the samples of roasted groudnut also were contaminated with aflatoxin B_1 more than the suggested commercial tolerance limit of 30 ppb.

These observations suggest the need for a systematic survey to collect food commodities from different climatic zones of the country to analyse the occurrence of aflatoxin B_1 which will enable us to forecast and prevent health hazards to human beings, livestock and poultry.

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COMPARISON OF POUR PLATE WITH OVERLAY AND SPREAD PLATING FOR ENTEROBACTERIACEAE COUNT IN MINCED MEAT

Enterobacteriaceae counts were enumerated in retail samples of ground mutton and sausage meat using Violet Red Bile Glucose agar medium. Two methods of plating the selective medium viz. pour plate with overlay and spread plating were compared. Spread plating showed higher recovery of Enterobacteriaceae counts.

Enterobacteriaceae, coliforms, fecal coliforms, *Escherichia coli* and enterococci are used as indicators of fecal contamination of foods[1]. Unlike coliforms and fecal coliforms, Enterobacteriaceae group of indicator organisms taxonomically well defined[2], an usually present in foods in more numbers[3] and includes slow and non lactose fermenters[2]. Organisms of this group associated with animal products have been reported from this country[4-6]. Enumeration of all organisms belonging to Enterobacteriaceae as an indicator system has been reported in foods and feeds[7-10].

Enumeration of Enterobacteriaceae is done either by most probable Number (MPN) technique or by colony count method. Violet Red Bile Glucose (VRBG) agar has been recommended for colony counts of Enterobacteriaceae using thickly layered pour plates with overlay of the same agar[11]. Hechelmann *et al*[12]. obtained from poultry meat the highest yield of Enterobacteria on this medium with spread plating and aerobic incubation. The present communication compares pour plate having an overlay with spread plating of VRBG agar for the numbers and kinds of Enterobacteria recovered from ground mutton and sausage meat.

Fourteen samples of ground mutton and sausage meat were used in this study. Twenty five gram samples of meat were blended in 225 ml of 0.1 per cent peptone water for 1 min in a presterilized blender. Serial decimal dilutions were prepared for both the methods of plating in 9 ml volumes of 0.1 per cent peptone water. Pour plates with overlay[11] and spread plates[12,13] were prepared in duplicate for each dilution using VRBG medium and incubated at 37°C for 18–24 hr. The number of purple colonies surrounded by purple haloes of precipitated bile salts were counted.

Typical colonies isolated from some of the samples were subjected to confirmatory tests of Enterobacteriaceae by fermentation of glucose in glucose–salt medium and by oxidase test[14]. The confirmed isolates obtained from both the methods of plating the selective medium were identified[15].

Counts of presumptive colony forming units of Enterobacteriaceae by spread plating were consistently higher in all the samples tested. The difference in the log counts of Enterobacteriaceae between the two methods range from 0.18 to 0.92 with a mean difference of 0.47 (Table 1). A paired '*t*' test demonstrated a significant difference

TABLE 1. ENTEROBACTERIACEAE COUNTS OF GROUND MUTTON AND SAUSAGE MEAT ON VRBG MEDIUM BY POUR PLATE WITH OVERLAY AND SPREAD PLATE METHODS

Meat type	Sample no.	Colony count as $\log_{10}$/g by	
		Pour plate with overlay	Spread plate
Ground mutton	1	3.16	3.34
	2	3.21	3.83
	3	3.22	3.65
	4	3.42	3.95
	5	3.75	3.94
	6	3.79	4.26
	7	4.41	4.96
	8	4.78	5.70
	9	5.23	5.90
Sausage meat	10	4.30	4.84
	11	4.48	4.93
	12	4.67	5.00
	13	4.70	5.06
	14	5.09	5.43

($P<0.01$) in the recoveries of colony forming units between the two methods of plating The coefficient of variation was 12 and 13 per cent for pour plate with overlay and spread plating respectively. The difference in the Enterobacteriaceae counts between the two methods may be due to oxygen requirements of the bacteria predominating in the food. According to Mossel *et al*[11], pour plate with overlay secures sufficiently anaerobic conditions for suppressing the growth of strictly aerobic Gram negative rods and allows optimal utilization of glucose by an anaerobic pathway to favour the formation of clearly visible colonies. Hechelmann *et al*[12]. reported low Enterobacteriaceae counts under anaerobic incubation. The results reported in this study are in general agreement with that of Hechelmann *et al*[12].

In both the methods, 85 per cent of typical colonies were true Enterobacteriaceae suggesting that spread plating is not prejudiced by false positives (Table 2). The typical colonies on spread plates are easily distinguishable from other colony types of obligate aerobic non-Enterobacteriaceae. Further studies are needed to inhibit the growth of these organisms. The spectrum of Entrobacteriaceae was more or less similar in both the methods of plating (Table 2). Quantitative differences in the profiles of Enterobacteriaceae between the two methods of plating could not be ascertained from the small number of isolates identified. However, using pour plate with overlay method, Mercuri and Cox[16] showed more non coliform species resulting in lower proportions of faecal coliforms and *E. coli* in Enterobacteriaceae counts on VRBG medium. By spread plating, isolation of enteropathogenic *E. coli* has been reported in broilers[17].

TABLE 2. DISTRIBUTION OF ENTEROBACTERIACEAE BY POUR PLATE WITH OVERLAY AND SPREAD PLATE METHODS

Organism	Pour plate with overlay	Percentage	Spread plate	Percentage
E. coli	5	25	11*	39
Enterobacter	4	20	1	4
Citrobacter	3	15	9	32
Erwinia	2	10	1	4
Serratia	2	10	0	0
Klebsiella	0	0	1	4
Non-typable	1	5	1	4
Non-*Enterobacteriaceae* (False positives)	3	15	4	14

*Includes one slow lactose fermenter.

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CANNING OF FRUITS IN NATURAL FRUIT JUICES. I. CANNING OF PEACHES IN APPLE JUICE

Free stone peaches were canned in apple juice sweetened with sugar or in sugar syrup. On comparison, peaches canned in apple juice prepared from concentrate were found to be quite acceptable. The use of apple juice in canning of peaches, besides increasing the nutritive value and total fruit content also reduces the sugar required.

A substantial quantity of peach fruit grown in, Himachal Pradesh is canned in cane sugar syrup. Apple juice and concentrate are also commercially manufactured in the state. Apple juice which contains about 10 per cent sugar and 0.30 per cent acid, can partially replace sugar and acid in the covering syrups used in the canning of fruits. This product would provide more nutrition and fruit content to the consumer. It would also help to utilise more of apple juice. In the past, some work has been done to replace sugar syrup with corn syrup in the canning of fruits, which gave quite encouraging results[1]. However, very little work appears to have been done on the canning of different fruits in fruit juices[2]. A study was undertaken to find out the suitability of using apple juice in the syrups for canning of peaches and the results are reported here.

Seven fold apple juice concentrate manufactured by the horticultural processing and marketing corporation, Simla, and apple juice manufactured by the department of horticulture, Simla and stone free peach fruit of canning variety grown around Simla town, were used. Apple juice concentrate had a total soluble solids (TSS) content of 73° Brix and 2.0 per cent acidity as citric acid. It was diluted with water in the ratio of 1:6 to yield single strength apple juice of TSS 11° Brix and 0.30 per cent acidity as citric acid. Straight apple juice used had a TSS of 10° Brix and 0.31 per cent acidity as citric acid. Reconstituted as well as straight apple juices were adjusted to 42° Brix with cane sugar, resulting in a final acidity of 0.2 per cent as citric acid and used as covering media. A covering media of the same composition was also prepared using water, cane sugar and citric acid. The composition and the quantities of cane sugar and citric acid required for preparing the three covering media are given in Table 1. Sound and fully mature hard fruits were lye peeled, dipped in 0.1 per cent citric acid solution and washed in water. They were cut into halves and filled into A 2½ cans. Syrups of the above three different compositions were used as covering media and 12 cans from each of them were processed following the standard method described.[3]

The chemical analysis of the products was carried out after two months of storage at room temperature according to the procedure described by Ranganna.[4] Organoleptic evaluation of the product was done after 60 days of storage by 5 semitrained panelists in our laboratory. Coded samples were presented in random to the pane lists and asked to evaluate the product on a 9–point

TABLE 1. COMPOSITION OF THE COVERING MEDIA AND THE INPUT REQUIREMENTS

Covering media	Final TSS (°Brix)	Cane sugar required (g/100 g)	Sugar contributed by juice (%)	Final acidity (% citric acid)	Citric acid required (g/100 g)	Citric acid contributed by juice (%)
Sugar syrup	42.0 (0)	42.0	—	0.20 (0)	0.20	—
Apple juice (reconstituted) sugar syrup	42.0 (11)	31.0	26	0.20 (0.30)	0.0	100.0
Apple juice (straight) sugar syrup	42.0 (10)	32.0	24	0.21 (0.31)	0.0	100.0

Figures in parenthesis represent the original TSS and acidity of the juices used.

TABLE 2. CHEMICAL COMPOSITION AND ORGANOLEPTIC SCORE OF THE PEACH HALVES CANNED IN DIFFERENT COVERING MEDIA

Parameters	Plain sugar syrup	Apple juice (Recons.) sugar syrup	Apple juice (straight) sugar syrup
TSS (°Brix)	21	22	22
Acidity (% citric acid)	0.38	0.42	0.42
pH	3.7	3.7	3.7
Drained wt (%)	55	57	56
Vacuum (lb/sq. in.)	10	10	10
Syrup	Organoleptic score		
Clarity	7.5	7.0	6.5
Taste & flavour	7	7.2	6.8
Peach halves			
Colour	7.5	7.0	7.0
Taste & flavour	7.0	7.2	7.0
Texture	7.5	7.5	7.5
Syrup penetration	7.0	7.0	7.0
Overall acceptability	7.0	7.5	7.3

hedonic scale on the basis of attributes like colour, texture and taste of the fruit halves, and clarity and flavour of the covering syrups.

The results of the chemical and organoleptic analysis of the product presented in Table 2 show that there was no appreciable difference in the drained weights and cut out TSS of the three products, which were found to be in the range of 55 to 57 per cent and 21 and 22° Brix respectively. The flavour and taste of the peaches canned in apple juice from concentrate scored well over the other two combinations. Since the apple juice from concentrate has mild flavour, the characteristic peach flavour is not masked by the apple flavour. As regards the texture of peach halves and penetration of the syrup, there was no appreciable difference in all the three cases, which indicates that apple juice in the syrups does not interfere in the penetration of the syrups into the fruit. Although a slight brownish tinge was observed on the peach halves canned in apple juice syrups, which may be due to the original colour of the juices used, yet the peach halves remained sufficiently attractive to be acceptable. The product prepared using apple juice from concentrate appears to be preferred over the other two products. Data given in Table 1 show that the use of apple juice in the covering syrups for the canning of peaches saves about 25 per cent sugar and adidtion of citric acid is not necessary.

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PREPARATION AND STORAGE STUDIES ON SOME INTERMEDIATE MOISTURE VEGETABLES

Intermediate moisture (IM) vegetables (mushrooms, cauliflower and carrots) of moisture content about 20 and 40 per cent and salt content about 8 to 10 per cent, have been successfully prepared by steeping overnight in 10 per cent brine containing 0.1 per cent potassium metabisulphite and 0.2 per cent citric acid, followed by draining and drying. Products with 20 per cent moisture content packed in 300 gauge polyethylene pouches, with an outer card board cover have a shelf life of about 3 months at an accelerated temperature of 37°C and relative humidity of 42 to 74 per cent.

Conventional hot air drying of vegetables in cabinet drier to moisture levels of about 7 per cent, results in adverse changes in texture and cooking quality. Intermediate moisture (IM) foods with moisture content, ranging from 20 to 50 per cent though difficult to preserve, are expected to have superior texture and cooking quality and are therefore, receiving considerable attention.

In this investigation, attempt has been made to prepare mushrooms, cauliflower and carrots of IM content by incorporating common salt in high concentration, so that the final, free water content of dried vegetables, is not able to provide, favourable environment for microbial growth. Since the end use of the products is as vegetable curry, their high salt conent gets diluted in their preparation and is, therefore not a

problem in the final acceptability. Similarly, a high concentration of sulphur dioxide (SO_2) decreases during cooking.

Mushrooms (*Agaricus bisporus*), cauliflower (var. 'Giant Snow Ball' and carrots (var.'C.29'), were procured from the local market and washed throughly in water. The mushroom quarters were blanched for 5 min and other vegetables for 3 min in boiling water. After cooling to room temperature, they were dipped overnight in 10 per cent brine solution, containing either 0.1 per cent potassium metabisulphite (KMS) alone or along with 0.2 per cent citric acid. The vegetables were dried in a cross flow cabinet drier at an initial temperature of 80° C for one hour and subsequently at 60° to a final moisture conent of about 20 and 40 per cent. They were packed in low density polyetheylene (LDPE) pouches of 300 gauge thickness with an outer card board container and stored at room temperature (19–26°C) as well as at 37°C during February to May. The relative humidity varied from 42 to 74 per cent during storage period. Each polyethylene pouch contained 100 g of dried material.

The samples were tested for moisture, salt content, residual SO_2 and browning at 45–day intervals. They were evaluated for rehydration ratio and organoleptic qualities after three months. The moisture was determined by AOAC method[4], sodium chloride by chromate indicator method[3], and residual SO_2 by distillation method[5]. The brown colour was extracted with 60 per cent aqueous methanol and its colour intensity was measured at 440 nm using Photochem-Colorimeter. The samples were rehydrated in boiling water for 15 min

TABLE 1. EFFECT OF PRETREATMENTS AND STORAGE PERIOD ON THE MOISTURE AND SALT CONTENT OF THE INTERMEDIATE MOISTURE VEGETABLES

Vegetable Treatment	Moisture level	Initial moisture (%)	Moisture/salt at 19-26°C 45 days (%)	90 days (%)	Decrease in moisture* (%)	Increase in salt* (%)	Moisture/salt at 37°C 45 days (%)	90 days (%)	Decrease in moisture* (%)	Increase in salt (%)
Mushrooms										
KMS	High	39.0 (8.7)	37.8 (8.9)	37.0 (9.1)	5.1	4.5	38.4 (9.0)	37.3 (9.2)	6.7	5.7
,,	Low	22.5 (9.8)	21.6 (10,0)	21.4 (10.1)	4.5	3.0	22.0 (10.2)	21.5 (10.2)	3.1	4.0
KMS+CA	High	40.0 (8.8)	37.9 (9.1)	36.4 (9.2)	6.6	4.5	38.0 (9.1)	36.6 (9.3)	8.5	5.6
,,	Low	22.2 10.1)	21.8 (10.2)	21.5 (10.3)	4.4	2.0	21.0 10.2)	20.9 (10.3)	5.8	2.9
Cauliflower										
KMS	High	40.0 (8.7)	39.2 8.8)	38.2 (8.9)	4.5	2.3	39.0 (8.9)	38.1 (9.0)	4.7	3.4
,,	Low	17.8 (9.7)	17.5 (9.8)	17.2 (9.9)	3.2	2.0	17.2 (9.8)	17.0 (9.9)	3.3	2.0
	High	39.5 (8.5)	38.2 (8.6)	37.6 (8,8)	4.8	3.5	39.0 (8.7)	37.8 (8,9)	4.3	4.7
KMS+CA	High	39.5 (8.5)	38.2 (8.6)	37.6 (8.8)	4.8	3.5	39.0 (8.7)	37.8 (8.9)	4.3	4.7
,,	Low	18.5 (9.7)	18.1 (9.8)	17.8 (9.9)	3.8	2.0	18.2 (9.9)	17.8 (10.0)	3.8	3.1
Carrots										
KMS	High	40.0 (8.6)	38.5 (8.8)	37.1 (9.1)	7.2	5.7	38.0 (8.8)	36.9 (9.2)	7.7	6.9
,,	High	43.3 (8.5)	42.0 (8.8)	39.6 (9.0)	8.5	5.8	41.5 (8.9)	39.5 (9.1)	8.7	7.0

*Recalculated, taking intial as 100 for comparison of rate of increase or decrease of mositure or salt.

KMS: Potassium metabisulphite; CA: Citric acid.

and from the increase in weight, rehydration ratio was calculated.

The moisture content was found to be 90.1,92.0 and 95.0 per cent for fresh mushrooms, cauliflower and carrots respectively. Among the IM products carrot with 20 per cent moisture was most unstable with respect to its colour; fading of colour occurred within a week and therefore, these were prepared, at about 40 per cent moisture for storage studies.

The sodium chloride content after soaking overnight in all the vegetables ranged from 8.5 to 10.1 per cent which increased by 2.0 to 5.8 per cent during storage, but there was decrease in moisture content which was lowest (3.1 per cent) in mushrooms at low moisture level and highest (8.7 per cent) in carrots at high moisture level (Table 1). The increase in salt and decrease in moisture was more at 37°C as well as at high moisture level.

The residual SO_2 was minimum (960 ppm.) at low moisture level in mushrooms and cauliflower and maximum (1088 ppm.) at high moisture level in mushrooms and carrots which decreased during storage (Table 2). The decrease was minimum (9.2 per cent) in mushrooms at low moisture level and maximum (18.7 per cent) in mushrooms and cauliflower at high moisture level. The browning was negligible at initial stage but it increased during storage. The decrease in SO_2 as well as increase in browning was more at 37°C. The samples with high moisture content were more brown, than those having low moisture content. There was no significant difference in retention of SO_2 by the treatments, but the samples treated with KMS and citric acid were

TABLE 2. EFFECT OF PRETREATMENTS AND STORAGE PERIOD ON THE RESIDUAL SULPHUR DIOXIDE (PPM) BROWNING IN INTERMEDIATE MOISTURE VEGETABLES

Vegetable/treatment	Moisture level	Initial moisture (%)	Storage at 19-26°C (days)		Loss of SO_2 (%)	Storage at 37°C (days)		Loss of SO_2 (%)
			45	90		45	90	
Mushrooms								
KMS	High	10.24 (0.08)	920 (0.18)	864 (0.30)	15.6	896 (0.25)	832 (0.55)	18.7
,,	Low	9.60 (0.09)	896 (0.15)	864 (0.25)	10.0	928 (0.12)	864 (0.34)	10.0
KMS+CA	High	10.88 (0.07)	960 (0.09)	896 (0.12)	16.6	1024 (0.12)	928 (0.18)	15.2
,,	Low	10.24 (0.08)	960 (0.09)	896 (0.11)	12.4	928 (0.12)	928 (0.16)	9.2
Cauliflower								
KMS	High	10.24 (0.08)	960 (0.10)	864 (0.15)	15.6	864 (0.12)	832 (0.18)	18.7
,,	Low	9.60 (0.08)	928 (0.08)	896 (0.12)	6.6	928 (0.10)	864 (0.15)	10.0
KMS+CA	High	10.24 (0.06)	928 (0.07)	896 (0.12)	12.4	960 (0.09)	864 (0.12)	15.6
,,	Low	10.24 (0.07)	960 (0.09)	928 (0.10)	9.3	960 (0.08)	928 (0.10)	9.3
Carrots								
KMS	High	10.24 (0.03)	992 (0.05)	928 (0.07)	9.3	992 (0.06)	896 (0.07)	12.4
KMS+CA	High	10.88 (0.03)	1024 (0.04)	960 (0.05)	11.0	992 (0.05)	928 (0.06)	14.7

Figures in parentheses represent O.D. to indicate browning
KMS: Potassium metabisulphite; CA: Citric acid

TABLE 3. EFFECT OF PRETREATMENTS AND STORAGE FOR 90 DAYS ON THE REHYDRATION RATIOS AND ORGANOLEPTIC QUALITY OF INTERMEDIATE MOISTURE VEGETABLES

Treatment	Moisture level	Mushrooms		Cauliflower		Carrots	
		19-26°C	37°C	19-26°C	37°C	19-26°C	37°C
KMS	High	1.6	1.6	2.3	2.2	2.2	2.3
		(77)	(69)	(63)	(54)	(68)	(61)
,,	Low	1.9	1.8	2.6	2.5	—	—
		(81)	(75)	(77)	(71)		
KMS+CA	High	1.6	1.8	2.2	2.2	2.2	2.3
		(78)	(71)	(74)	(70)	(69)	(65)
,,	Low	1.8	1.9	2.7	2.6	—	—
		(89)	(79)	(88)	(81)		

The Total score of 100 given in parentheses is divided into: texture 40; colour, 25; flavour, 20; and overall acceptability, 15
KMS – Potassium metabisulphite; CA – Citric acid

less brown, than those treated with KMS alone. The browning was less in carrots as compared to mushrooms and cauliflower.

The maximum rehydration ratios of 1.9, 2.6 and 2.3 were found in mushrooms, cauliflower and carrots, respectively (Table 3). It was more in samples having low moisture level. The samples stored at 19-26°C when evaluated organoleptically, for texture, colour and flavour after 90 days, were acceptable but the samples stored at 37°C, though slightly of poorer quality, were still acceptable (Table 3). The samples at high moisture level were organoleptically poor as compared to those at low moisture level. The samples treated with KMS in combination with citric acid were superior to those treated with KMS alone. Slight fading of orange colour in carrots was noticed even at high moisture level.

It is concluded from the above study, that the IM vegetables like mushrooms, cauliflower and carrots of reasonable shelf life, can be successfully prepared, by dipping them in 10 per cent brine solution having 0.1 per cent KMS and 0.2 per cent citric acid.

Grateful thanks are due to Dr. K. Kirpal Singh, Director, Food Technology, Processing & Marketing, Punjab Agricultural University, for his keen interest in this investigation.

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BOOK REVIEWS

Nutrition and Food Science, Vol. 1, *Food and Nutrition Policies and Programmes*, Edt. W. Santos, N. Lopes, J. J. Barbosa, D. Chaves and J. C. Valente, Plenum Press, New York; 1980; Pp: 808; Price: $ 69.50.

One can hardly come across a more concise caption and a book based on reviews and research papers presented at an International Congress of Nutrition than the one provided by the editors, who are members of committee assigned with the task of organising the papers presented in the XI International Congress of Nutrition in 1978 in Rio de Janeiro, Brazil, in a book form.

The topics covered in the book provide ample proof of multidisciplinary nature of the subject. In all, the book has seven sections dealing with programme planning at national, regional and local levels at the one end and to consumer protection programme at the other.

Section I of the book discusses at length, nutrition as part of overall national and regional development plans and policies. Relevance of nutritional consideration in health and agricultural sector planning—two closest correlates of nutrition, is well illustrated in intercountry experiences narated in this section. Results emerging out of different study models are ; health model (the Colombian experience), income model (the Brazilian experience) other integrated models (the Indian, Chilean etc.), strongly suggest that the equation between food (nutrient) consumption and nutritional status of population, particularly of the poorer segments, is not simple, needs the support of other disciplines for comprehension.

The effect of an increase in nutrient (protein and calorie) consumption on nutritional status cannot be predicted without the knowledge of health status, which in turn is a function of availability of health and sanitary services. This concept has been clearly broughtout in the Colombian experience. Similarly, there are studies to show that the increased food production did not always result in the improvement of nutritional status of the malnourished groups namely, the marginal farmers and labourers. The reason being that the enhanced food production does not contribute to the increase in real incomes of the poor and thus does not enhance their purchasing power. The resultant effect is failure to create effective market demand commensurate with their nutritional needs. Despite strong links between the food production and the problem of malnutrition, one cannot be certain of eliminating the latter, by solving the aggregate food problem through agricultural research and modern farm technology, unless the agricultural sector planning provides for specific measures to increase purchasing power that would equate need. These measures are: generation of gainful employment, direct nutritional interventions such as transfer payments, food subsidies, fairprice shops (ration shops) and supplementary feeding.

A number of target oriented intervention programmes undertaken by different countries, designed to contain the problem of malnutrition, both of general (protein-energy malnutrition—PEM) and specific (vitamin A deficiency etc.) nature are presented in sectionV. Cultural constriants, operational and evaluation difficulties socio-economic, eduational and political implements of such intervention strategies are discussed. The advocates of supplementary feeding programmes, though concede that such programmes besides being expensive even when the foods are donated are fraught with operational and organisational difficulties, they hasten to point out that these programmes have the potential of reaching large number of mothers and children, who otherwise are not drawn towards existing health and educational services in developing countries. The results of CARE-India study (reported here) and the study of India Population Project (IPP-Karnataka) carried out under the auspices of World Bank, SIDA and Govt. of India (not reported here) indicated that the supplementary food distribution (take-home) programme, when delivered as 'part of health package' did show the spin-off benefits in terms of improved image of health workers and greater acceptance of health services. These results indicate that the nutritionists while evaluating the impact of nutritional programme need not or should not always look for only nutritional improvement. Instead, he should be prepared to measure the impact in terms of non-nutritional parameters also and assess its relevance in the totality of health and development programme before pronouncing its success or failure.

Elaborate descriptions of the role of international and voluntary agencies, and the private sectors in obtaining solutions for food and nutritional problems are provided in Section II & III of the book respectively. Concerned with the sharp increase in the number of severely undernourished people in the developing countries despite increase in GNP and food production, the widening gap between the rual and urban-poor and rich, and the dwindling buffer food stocks at global level, these agencies are reorienting their policy goals and trying to adopt innovative strategies

with humanistic orientation focussing more on native technology, small farmer and landless labourer and participatory endeavours.

In the remaining sections of the book, are discussed the problems and prospects of organizing programmes for: (*i*) feeding the organised groups such as school children and factory workers, (*ii*) mass feeding under stress situations such as floods and famine, (*iii*) a system of evaluation and surveillance to forewarn the impending food crisis and enable prompt action, and (*iv*) programmes for consumer protection.

Thus, the book provides full details with relevant tables, graphs, charts etc., and extend over numerous inter-related aspects of food and nutrition. An over all summary for the topics covered in the book, though is impossible, one wished to see a resume at the end of each section devoted to a specific aspect of the subject. The book besides presenting opinions contains much data and many observations, gained first hand from inter-country experiences regarding which it may be said that they, topicwise, are broad enough to interest generalists and narrow enough to hold the attention of subject-matter specialists in the field of food and nutrition and thus appeal to a broad readership. The volume should be at hand to all concerned with food and nutrition science in general, and its application for eliminating the problems of malnutrition in particular.

N. PRAHLAD RAO
NATIONAL INSTITUTE OF NUTRITION, HYDERABAD

Advances in Biochemical Engineering. Vol. 16. *Plant Cell Cultures I:* by A. Fiechter; Springer-Verlag GmbH & Co. KG Heidelberger Platz 3 Postfach D-1000 Berlin 33; 1980; 148: Pp, Price: $ 42.50.

The aim of this series is to "Uncover the characteristic features of biochemical engineering and to report on current developments observed in the application of basic knowledge to this discipline". It is a welcome feature of this series that a broad view is taken of biochemical engineering and in addition to microbiology, enzymes, and plant and animal cell culture are also covered. Volumes 16-18 deal with plant cell and tissue culture. The five chapters in this volume cover a wide range of topics. The first chapter on continuous culture of plant cells using the chemostat principle deals with equipment, media and other parameters which will be of interest to bioengineers. The second chapter on embryogenesis will be of primary interest to those working on plant tissue culture. It deals with factors which influence embryogenesis, the isolation of protoplasts and regeneration of plantlets from them and the use of tissue culture for mutation breeding. The remaining three chapters on biotransformations, steriod metabolism and lipids of plant cell and tissue cultures review recent advances and indicate the potential uses of these cultures for basic studies and for the production of useful chemicals.

The volume, which is dedicated to the memory of Professor H.E. Street, will be invaluable not only to those working on plant tissue culture but also to biochemists, organic chemists and bioengineers interested in this new and rapidly developing field of research.

V. JAGANNATHAN
NATIONAL CHEMICAL LABORATORY, PUNE

Proceedings of the National Workshop on Alga Systems: Edited by C. V. Seshadri, Sebastian Thomas and N. Jeeji Bai, Published by the Indian Society of Biotechnology, New Delhi; 1980; Pp. 275; Price: 100.

The book is the outcome of a National Workshop on Algal Systems held at Madras on Oct. 1980. Forty research papers have been included under the following four major areas: (*i*) algae as biofertilizers (14 Papers): (*ii*) algae as feed stock for drug, chemicals and energy (1); (*iii*) algae as food/feed (12); and (*iv*) as environmental aids (13).

Each section is preceded by a key note paper covering the work done in India in that area.

Algal biofertilizer using a mixed culture of blue greens has already gone into the practical application for paddy crops in several parts of India. The papers report valuable technical data on the simplification of techniques, biofertilizer production, cost economics, comparison with utilization of azolla complex, etc. for routine application.

Marine algae as feed stock for drugs and chemicals has received much less attention as evidenced by only two papers. There is also potential for bioconversion of seaweeds as fuels to meet the energy requirements.

Algae as feed and food, primarily deals with both clean water and sewage studies with an emphasis on rural application and use of algal biomass for poultry and fish feeds; considerable technological data have been generated which emphasise the need for urgent practical application.

Algae as environmental aids has received only recent attention particularly to monitor pollution problems. Algae can be used as indicator for different types of industrial pollutants. It is possible to convert waste water from that of a problem to one of biomass productivity.

There are many printing mistakes and rather poor

reproduction of figures which could have been avoided. The editors need to be complimented for bringing out the full proceedings with comprehensive account of research work carried out in India in a record time.

L. V. VENKATARAMAN
C.F.T.R.I., MYSORE

Palatability and flavour use in animal feed: First International Symposium on Palatability and Flavour Use in Animal Feed, Zurich, Switzerland, 10-11 October, 1978, Edited by Prof. Dr. Hans Bickel, Published by Scientific Publishers Lindenstr, 44-47, Berlin 61; 1980; Pp. 147; Price: $ 34.10

This publication contains contributions from 17 speakers from North America and Europe. It reviews comprehensively the chemistry of taste and odour imparting substances in human foods and feeds; the formation and occurrence of odour and taste perception in animals; their anatomical and physiological fundamentals; techniques to evaluate taste preferences of animals, their behavioural responses to gustatory effects of sugar and sweetness.

A few pages deal on factors influencing the palatability of toxic components of feed stuffs, the significance of the physical forms in compound feeds; the influences of flavour components on primary feed intake and feed conversion; storage, handling and admixing of feeds for commercial utilization are effectively dealt.

At present there is a need to achieve better growth and breeding of livestock. The new fields of interest are the role of flavours in animal nutrition and the technology of their use in compound feeds. Some of the above aspects are discussed briefly.

Perhaps, a few aspects on food legislation, feed standards and feed composition would have been helpful, if covered in the symposium. The problems of ration formulation with ingredient procurement and mixing and specialised/flavoured ration supplements could also have helped the researchers and the technologists in animal feeds.

There are some obvious printing errors which could have been avoided. The book will be valuable to those interested in animal nutrition and feed technology.

M. K. KRISHNAKUMARI
C.F.T.R.I., MYSORE

ADDENDUM

In the paper entitled 'Identification and estimation of some volatile carbonyls in three types of Khoa by GLC' by Gyanendra Kumar and M. R. Srinivasan in Vol. 18 July/August Issue of this journal, 1981 on page 158, the following foot note is to be included in the table—

"The number of C atoms for the compounds mentioned in the table could be computed from the nomenclature".

ERRATUM

In the paper entitled 'Malathion poisoning in non-target species....' by P. S. Rajini and M. K. Krishnakumari in Vol. 18 November/December Issue of this journal, 1981, on page 239 in line 1 of the abstract the words "Technical Malathion and its 50 per cent EC formulation exclusively...." should read as "Technical Malathion and its 50 per cent EC formulation extensively...."

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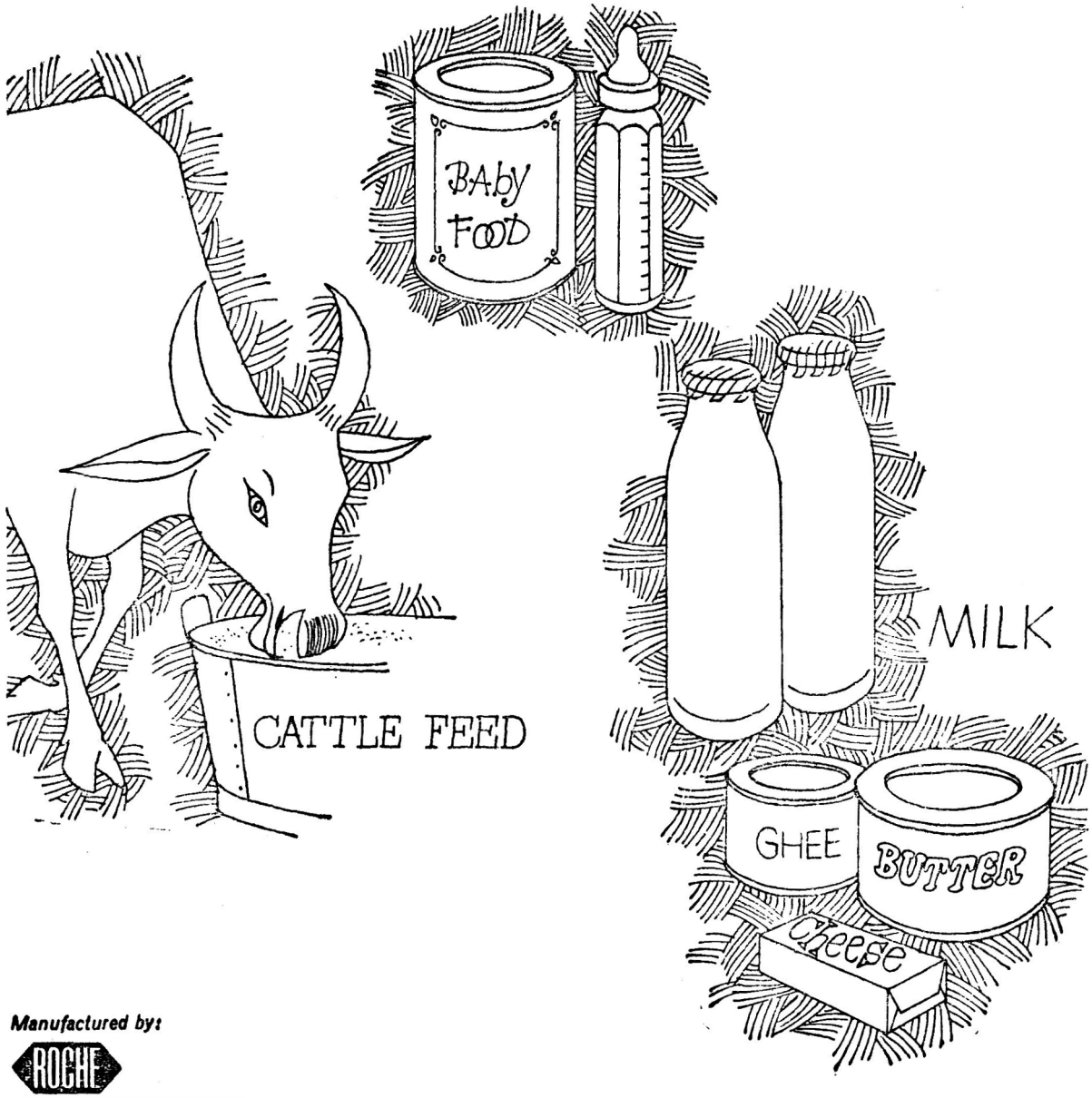

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SUMMER SCHOOL IN MICROBIOLOGY

Association of Microbiologists of India, Mysore Unit, in collaboration with Central Food Technological Research Institute, Mysore, is organising a Summer School in Microbiology on "Microbiological Techniques in Food Industries". This will be of five days duration during May/June 1982 for the persons working in the Food Industries. The course will provide training in Food Microbiology and Sanitation. The broad areas to be covered are indicated below: (*a*) Role & Significance of microorganisms in food material, (*b*) Enumeration of microorganisms in food material, (*c*) Isolation and identification of coliforms and pathogenic organisms (*d*) Occurrence of microbial toxins in food,

For further details, please contact Sri J. D. Patel, Secretary-cum-Treasurer, Association of Microbiologists of India, Microbiology & Fermentation Technology Discipline, C.F.T.R.I., Mysore-570 013.

PUBLICATIONS
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(Central Food Technological Research Institute Campus, Mysore)

*

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   Price: India **Rs. 8/–**
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Research Papers

Research Notes

Review Paper

Printed and Published by Dr. P. Narasimham, Secretary AFST (India), CFTRI, Mysore-570 013, at Sharada Press, Mangalore-575 001.

INSTRUCTIONS TO AUTHORS

1. Manuscripts of papers (*in triplicate*) should be typewritten in double space on one side of bond paper. The manuscripts should be complete and in final form, since only minor corrections are allowed at the proof stage. The paper submitted should not have been published or data communicated for publication anywhere else. Review papers are also accepted.

2. Short communications in the nature of Research Notes should clearly indicate the scope of the investigation and the salient features of the results.

3. Names of chemical compounds and not their formulae should be used in the text. Superscripts and subscripts should be legibly and carefully placed. Foot notes especially for text should be avoided as far as possible.

4. **Abstract:** The abstract should indicate the principal findings of the paper. It should be about 200 words. It should be in such a form that abstracting periodicals can readily use it.

5. **Tables:** Tables as well as graphs, both representing the same set of data, should be avoided. Tables and figures should be numbered consecutively in Arabic numerals and should have brief titles. They should be typed on separate paper. Nil results should be indicated and distinguished clearly from absence of data, which is indicated by '—' sign. Tables should not have more than nine columns.

6. **Illustrations:** Graphs and other line drawings should be drawn in *Indian ink* on tracing paper or white drawing paper preferably art paper. The lettering should be in double the size of printed letters. For satisfactory reproduction, graphs and line drawings should be at least twice the printed size 16cms (ox axis) × 20cms (oy axis); photographs must be on glossy paper and must have good contrast; *three copies* should be sent.

7. Abbreviations of the titles of all scientific periodicals should strictly conform to those cited in the World List of Scientific Periodicals, Butterworths Scientific Publication, London, 1962.

8. **References:** Names of all the authors along with title of the paper should be cited completely in each reference. Abbreviations such as *et al.*, *ibid. idem*, should be avoided.

   The list of references should be included at the end of the article in serial order and the respective serial number should be indicated in the text as superscript.

   Citation of references in the list should be in the following manner:

   (*a*) *Research Paper:* Jadhav, S. S. and Kulkarni, P. R., Presser amines in foods, *J. Fd Sci. Technol.*, 1981, **18,** 156.

   (*b*) *Book:* Venkataraman, K., The Chemistry of Synthetic Dyes, Academic Press, Inc., New York, 1952, Vol. II, 966.

   (*c*) *References to article in a book:* Joshi, S. V., in the Chemistry of Synthetic Dyes, by Venkataraman, K., Academic Press, Inc., New York, 1952, Vol. II, 966.

   (*d*) *Proceedings, Conferences and Symposia Papers:* Nambudiri, E. S. and Lewis, Y. S., Cocoa in confectionery, *Proceedings of the Symposium on the Status and Prospects of the Confectionery Industry in India*, Mysore, May 1979, 27.

   (*e*) *Thesis:* Sathyanarayan, Y., Phytosociological Studies on the Calcicolous Plants of Bombay, 1953, Ph.D. Thesis, Bombay University.

   (*f*) *Unpublished Work:* Rao, G., unpublished, Central Food Technological Research Institute Mysore, India.

9. Consult the latest copy of the *Journal* for guidance.

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