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Volume 11

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CONTENTS

Research Papers

- Physico-chemical and Rheological Properties and Milling Quality of
Indian Durum Wheats** 1
A. Rahim, P. Haridas Rao and S. R. Shurpalekar
- Studies on Flavouring Compounds of Chapaties I. Carbonyls** 5
*S. B. Kannur, K. S. Premavalli, S. S. Arya, D. B. Parihar and
H. Nath*
- Proline as an Antioxidant in Fish Oil** 10
G. D. Revankar
- Effect of Homogenization and Pasteurization on Surface Tension and
Curd Tension of Buffalo Milk** 12
C. Parsad, S. M. Kulkarni, B. G. Ladkani and C. A. Mulay
- Total Chlorine Determination in Organochlorine Insecticides** 15
V. Lakshminarayana
- Some Observations on the Variation in Insecticidal Deposit on Treated
Wheat Grains** 17
A. S. Udeaan, R. P. Chawla and R. L. Kalra
- Storage Fungi Associated with Rice Weevil (*Sitophilus oryzae* L.)** 19
A. N. Ragunathan, D. Srinath and S. K. Majumder
- Quality Characteristics of Three Improved Varieties of wheat in the
Punjab** 22
K. S. Gill, K. L. Sehgal and M. S. Gill
- Research Notes**
- Identification of Beef From other Meats by Disc Electrophoresis
Method** 25
M. N. Moorjani, P. Puttarajappa and Miss M. S. Vasantha
- Toxicity of Ethylene Oxide to the Adults of *Tyrophagus Putrescentiae*
(Schränk) Acarina, Acaridae** 26
M. Muthu and B. V. Hiranniah

Continued

ห้องสมุด กรมวิทยาศาสตร์
21 ส.ค. 2517

Drainage Through Coffee Extract Foam	26
<i>A. J. Chandak and M. R. Chivate</i>	
Fractionation of Fatty Acids of Sardine Oil By Urea Adduct Method	27
<i>G. D. Revankar</i>	
A Spot Test for Detection of <i>Argemone Mexicana</i> Seeds in Mustard Seeds	28
<i>P. K. Bose</i>	
Feasibility of Using Gerber Fat Test for Rapid Estimation of Fat in <i>Khoa</i>	29
Effect of Certain Preservatives on Food Samples Preserved for Analysis	30
<i>Smt. G. Mukherjee and T. V. Mathew</i>	
Anti-Oxidant Effect of a Spice Mixture on Sardine Oil	31
<i>G. D. Revankar and D. P. Sen</i>	
Book Reviews	33
Notes and News	36
Association News	40

Physico-chemical and Rheological Properties and Milling Quality of Indian Durum Wheats

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Manuscript Received: 4 June 1973

Studies on physico-chemical characteristics of five varieties of durum wheats, viz., *Local red*, *Bijaga yellow*, *Amrit*, *Bijaga red* and commercial *Bansi* have indicated wide variations in 1000 kernel weights (36.1-47.8 g). *Bijaga red* had 12 per cent non-vitreous kernels as compared to 1.0 per cent or less for the remaining varieties. The ranges for the hectolitre weight and hardness expressed as crushing resistance were 73.5-77.0 kg and 12.5-13.8 kg per grain respectively. The chemical analysis of *maida* from durum wheats indicated variation in protein (11.2-15.5 per cent), wet gluten (35.3-52.0 per cent), falling number (431-487), pigments (1.85-2.53 ppm as beta carotene) and lipoxidase activity (8.7-25.5/ μ l $_{O_2}$ /g/min).

Farinograph studies have shown that the water absorption for all the varieties ranged from 71 to 74 per cent except 63.7 per cent in case of *Local red*. *Amrit* and *Local red* varieties had higher mixing time (7.5-8.0 min) and stability (8-12 min) as compared to the low values of 2 and 3 min respectively for *Bijaga red* which also had a significantly high mixing tolerance index of 100 B.U. Extensograph curves for *maida* indicated that ranges for extensibility and resistance to extension were 105-168 mm and 390-520 B.U. respectively. The resistance to extension was significantly high (1008 B.U.) for *Local red*.

Total semolina yields on the modified Laboratory Buhler mill was 53.7 per cent for *Bansi* and 44.1 per cent for *Bijaga red*.

With the onset of green revolution, the production of wheat in India has touched 26 million ton mark during 1971-72, more than double that of about 12 million tons during 1966-67¹. With still higher targets for the coming years, India may face the problem of economical utilisation of surplus wheat. The production of durum wheats, concentrated mainly in the States of Madhya Pradesh, Gujarat, Maharashtra and Mysore, is estimated at about 3 million tons. At present, mainly Canadian durum wheats have great demand in international market. Though this extra hard category of Indian durum wheat is priced higher than hard as well as soft wheats, little scientific information is available on their milling quality and suitability for processing into different products. As such, in years to come, the export possibility of durum wheats, highly priced in international market, is likely to prove an important avenue for the utilisation of the surplus. In this paper are presented the results of studies relating to physico-chemical and rheological characteristics and some aspects of milling quality of durum wheats, cultivated in South India.

Materials and Methods

Varieties of wheats: Samples of *Bijaga red* (from Medium Research Station, Bijapur), *Bijaga*

yellow, *Amrit* and *Local red* (from Agricultural Research Station, Annigeri) were obtained from University of Agricultural Sciences, Dharwar. For comparison, *Bansi* wheat purchased from local market was also included in the study.

Physical characteristics: Bushel weight and Hectolitre weight were determined by AACC methods². The contents of vitreous kernel and vulgare wheat were determined by visual examination. For 1000 kernel weight, a representative sample of each variety was used in triplicate. Hardness of 10 selected sound kernels of different varieties was determined using a Flat Crush and Ring Stiffness Tester (Gaydon, England) and was recorded as crushing resistance in kg per grain.

Chemical characteristics: Moisture, crude gluten, total ash, pigments and lipoxidase activity of different varieties of wheat were determined according to AACC methods². Crude protein (N \times 5.7) was estimated by Micro-Kjeldahl method. The Falling Number, an index of alpha-amylase activity, was estimated using Hagberg's apparatus².

Rheological properties: For evaluating the rheological properties, the *atta* used was obtained by grinding cleaned durum wheat in Kamas Hammer Mill (Type Slaggy 200A) using a sieve with 0.8 mm apertures.

The *maida* was obtained by milling 2 kg samples of durum wheat in a Buhler laboratory mill, model MLU-202. Farinogram and extensogram characteristics of *atta* and *maida* from different wheats were studied using Brabender farinograph and extensograph according to AACC methods².

Semolina milling quality: For milling of durum wheats for semolina, the Buhler laboratory mill, model MLU-202 was modified. The following modifications were tried to arrive at conditions of maximum yield of semolina from *Bansi* variety. The reduction rolls were kept open. Five clearances, i.e. 0.45, 0.40, 0.35, 0.30 and 0.25 mm were tried for the break rolls B₁. For break roll B₃, the clearances adjusted were 0.15, 0.20, 0.30 and 0.40 mm. A standard steel gauge was used for the adjustment of clearances. One kg samples of *Bansi* wheat were conditioned to 18 per cent moisture by adding calculated quantities of water in a 2 kg capacity tin container. The period of conditioning was 4 hr during which the containers were shaken at an interval of 15 min. Additional 0.5 per cent of water was added just before milling as described by Black and Bushuk³.

The combined overtailings from the finer sieves at break rolls B₁, B₂ and B₃ were collected as semolina fraction. The flour and the bran obtained from different break rolls were also collected separately. The bran was sieved mechanically in a Buhler laboratory plane sifter and the semolina recovered was added to the combined semolina overtailings.

The combined semolina fractions were sieved mechanically to separate fine semolina from the coarse one. The overtailings from 60 mesh and 6XX (about 74 mesh) sieves formed the fine semolina fraction, while the coarse fraction consisted of the overtailings from 32 and 45 mesh sieves.

As clearances of 0.30 and 0.20 mm between break rolls B₁ and B₃ respectively resulted in maximum yields of fine semolina, the remaining varieties of wheats were milled for semolina using these operating conditions. The yields of semolina from different wheats under modified conditions of the Buhler mill are presented in Tables 5 and 6.

Results and Discussion

Physical characteristics: The results on the physical, chemical and rheological characteristics of the wheat samples as well as those of *maida* and *atta* are presented in Tables 1, 2 and 3. The thousand kernel weight indicated that *Bijaga red* and *Local red* varieties are comparatively short grains, while *Amrit* variety consisted of larger grains. The commercial *Bansi* and the *Bijaga yellow* varieties rank in between *Amrit* and *Bijaga red* or *Local red* varieties. The hectolitre weights of *Bansi*, *Local red* and *Amrit* were somewhat higher than that of *Bijaga yellow* or *Bijaga red*. Except for the *Bijaga red* variety containing as high as 12 per cent non-vitreous soft kernels, the remaining varieties were almost free from soft wheat kernels. The hardness of the

TABLE 1. PHYSICAL CHARACTERISTICS OF DURUM WHEATS*

Variety	Bushel wt. lb	Hectolitre wt. kg	1000 kernel wt. g	Vitreous kernels %	Crushing resistance kg/grain
<i>Bansi</i>	61.5	77.0	41.5	99.6	13.8
<i>Local red</i>	61.0	76.0	36.5	99.4	12.7
<i>Bijaga yellow</i>	59.5	74.0	42.0	99.0	12.7
<i>Amrit</i>	61.0	76.0	47.8	99.8	12.8
<i>Bijaga red</i>	59.0	73.5	36.1	88.0	12.5

* Free from vulgare wheat

TABLE 2. CHEMICAL CHARACTERISTICS OF DURUM WHEATS*

Variety	Product	Moisture %	Protein %	Wet gluten %	Total ash %	Falling number	Pigments† ppm	Lipoxidase activity $\mu\text{lo}_2/\text{g}/\text{min}$
<i>Bansi</i>	<i>Atta</i>	10.0	15.9	32.8	1.27	561	2.72	23.5
	<i>Maida</i>	12.1	14.6	46.0	0.68	434	2.50	15.2
<i>Local red</i>	<i>Atta</i>	10.0	13.1	31.8	1.29	633	3.02	30.0
	<i>Maida</i>	13.1	12.2	43.3	0.61	469	2.53	12.0
<i>Bijaga yellow</i>	<i>Atta</i>	10.5	13.6	31.5	1.49	572	2.17	36.6
	<i>Maida</i>	11.7	12.5	43.7	0.67	431	1.88	13.8
<i>Amrit</i>	<i>Atta</i>	10.0	16.6	42.1	1.35	675	2.59	47.7
	<i>Maida</i>	12.1	15.5	52.0	0.77	487	1.91	25.5
<i>Bijaga red</i>	<i>Atta</i>	8.9	13.4	31.6	1.52	527	2.19	26.4
	<i>Maida</i>	9.1	11.2	35.3	0.68	448	1.85	8.7

*Figures expressed on 14% moisture basis

† as beta-carotene

TABLE 3. RHEOLOGICAL PROPERTIES OF DURUM WHEATS—FARINOGRAPH STUDIES

Variety	Product	Water absorption %	Dough develop. time min	Stability min	Mixing tolerance index B.U.
<i>Bansi</i>	<i>Atta</i>	70.6	6.0	4.5	70
	<i>Maida</i>	74.0	7.5	4.5	70
<i>Local red</i>	<i>Atta</i>	66.6	5.0	9.0	80
	<i>Maida</i>	63.7	8.0	12.0	60
<i>Bijaga yellow</i>	<i>Atta</i>	65.3	3.5	4.5	60
	<i>Maida</i>	73.0	4.5	4.5	70
<i>Amrit</i>	<i>Atta</i>	72.8	5.0	8.0	60
	<i>Maida</i>	74.0	7.5	8.0	60
<i>Bijaga red</i>	<i>Atta</i>	65.0	3.0	9.0	25
	<i>Maida</i>	71.0	2.0	3.0	100

grain was maximum for the *Bansi* wheat and lowest for the *Bijaga red* variety. *Amrit*, *Bijaga yellow* and *Local red* have values in between, in that order.

Chemical characteristics: *Atta* as well as *maida* from *Amrit* variety contained maximum protein, while the protein contents of *Local red*, *Bijaga red* and *Bijaga yellow* were in a comparable range though lower than that of *Bansi* or *Amrit* varieties. The gluten content followed a similar pattern as protein content. In view of its high gluten content, *Amrit* variety may be effectively incorporated to improve the bread making quality of *maida* obtained from indigenous wheats. *Maida* from *Bansi* or *Local red* varieties contained significantly higher amounts of pigments as compared to *Bijaga yellow*, *Amrit* and *Bijaga red*. As such, they appear to be more suitable for use in pastry goods. The falling number was maximum for *Amrit* followed by *Local red*. *Bansi* and *Bijaga yellow* varieties had significantly low falling number. It is interesting to note that *Amrit* variety which scores over other varieties with respect to important chemical characteristics has nearly three times the lipoxidase activity as that of *Bijaga red*, and nearly twice as high as *Bijaga yellow*, *Local red* or *Bansi*. Such high lipoxidase activity in *Amrit* is likely to be a disadvantage during long term storage or processing into different products. However, all these values are comparatively lesser than those reported for durum wheats grown in Western countries⁴.

Rheological properties: The data presented in Table 3 show that *maida* from all the varieties had exceptionally high water absorption ranging between 71 and 74 per cent, the only exception being *Local red* for which water absorption was 64 per cent. The dough development time for *Bansi*, *Local red* and *Amrit* was significantly higher than that of *Bijaga*

yellow or *Bijaga red*. It is interesting to note that *Local red* variety had exceptionally high dough stability of 12 min. In case of *Amrit*, the dough stability (8 min) was significantly higher than those of *Bansi*, *Bijaga yellow* or *Bijaga red*. *Bijaga red* variety had a significantly lower mixing tolerance index of 100 Brabender units as compared to 60 to 70 for the remaining varieties. The Farinograph studies indicate that *Local red* and *Amrit* varieties have excellent dough characteristics followed by *Bansi* variety. The *Bijaga red* had poor mixing time, stability as well as tolerance.

It is interesting to note that only in the case of *Bijaga red*, the stability for *maida* was significantly less than the corresponding value for *atta*. In contrast, the *atta* samples of the remaining varieties had lesser dough development time and lesser or equal dough stability as compared to their corresponding values for *maida*.

The Extensograph studies (Table 4) have indicated that for all the varieties, the extensibility was higher in the case of *maida* as compared to corresponding values for *atta*. The resistance to extension for *maida* from *Local red* variety was nearly double that of corresponding values for the rest of the varieties. The *Amrit* and *Bansi* varieties had significantly higher resistance (510 to 520 B.U.) than those (392 to 440 B.U.) of *Bijaga yellow* and *Bijaga red*. The ratio of resistance to extensibility—an index of dough consistency—practically followed the same pattern as resistance to extension. The area of the extensogram which gives a fair idea about the strength of the flour was comparable for *Bansi* and *Amrit* varieties. It was significantly higher for *Local red* variety as compared to that of *Bansi* or *Amrit*. Comparatively the *Bijaga red* had a poor strength, half as that of

TABLE 4. RHEOLOGICAL PROPERTIES OF DURUM WHEATS EXTENSOGRAPH STUDIES

Variety	Product	Extensibility(E) mm	Resistance (R) to ex- tension B.U.	R/E	Area sq cm
<i>Bansi</i>	<i>Atta</i>	115	570	5.0	82
	<i>Maida</i>	143	520	3.6	110
<i>Local red</i>	<i>Atta</i>	105	1040	10.0	136
	<i>Maida</i>	120	1008	8.4	146
<i>Bijaga yellow</i>	<i>Atta</i>	98	420	4.3	55
	<i>Maida</i>	143	443	3.1	90
<i>Amrit</i>	<i>Atta</i>	125	615	4.9	128
	<i>Maida</i>	168	510	3.0	118
<i>Bijaga red</i>	<i>Atta</i>	93	335	3.6	46
	<i>Maida</i>	105	390	3.7	56

Bansi or *Amrit* variety. Thus, the Extensograph studies also substantiated the conclusions drawn from the Farinograph studies. It may be concluded that *Bijaga red* and *Bijaga yellow* varieties have comparatively poor rheological properties, while *Local red*, *Bansi* and *Amrit* have the desirable properties in that order.

Semolina milling: As seen in Table 5, the optimum conditions for semolina milling in a Buhler laboratory mill are 0.30 and 0.20 mm clearances for break rolls B₁ and B₃ respectively. This is evident, when the flour yield and total semolina yield are taken into consideration.

The results on the yield of semolina using these clearances for different varieties of wheat are presented in Table 6. The total yields ranged between 44 and

54 per cent with *Bijaga red* having the lowest yield. The semolina yield, apart from other factors, depends mostly on the hardness of the grain. It can be seen from Table 1, the value for the hardness of the grains was maximum for the *Bansi*, which gives the highest yield of semolina, while that of the *Bijaga red* gave the lowest values for both the semolina yield and hardness. Thus hardness of the grain can be taken as one of the criteria for good semolina yield. It is however likely that, in view of the differences in their physico-chemical properties, the above mentioned modifications of the Buhler laboratory mill for semolina milling, though found optimally suitable for *Bansi* wheat, may not be optimum for other varieties milled.

This study brings out the need for processing different varieties of durum wheats for semolina milling in a mill, wherein the milling operations can be carried out under comparable conditions with respect to the clearance between the break or reduction rolls. *Local red* and *Amrit* varieties appear promising in view of their chemical characteristics and rheological properties. However, it may be concluded that as compared to *Bansi* variety which is an accepted variety for semolina milling, *Local red*, *Bijaga yellow* as well as *Amrit* varieties have shown great possibilities for improving the bread making quality of indigenous wheats or for semolina milling for use in different traditional foods and pastry goods.

TABLE 5. EFFECT OF MODIFICATION OF BREAK ROLLS ON THE YIELD OF SEMOLINA FROM BANSI WHEAT

Break rolls clearance (mm)	Break rolls clearance (mm)	Fine semolina* (%)	Coarse semolina† (%)	Total semolina (%)	Flour‡ (%)
B ₁	B ₃				
0.45	0.40	17.9	15.6	33.5	7.9
0.45	0.30	27.3	22.6	44.9	10.3
0.45	0.20	31.4	17.4	48.8	10.8
0.40	0.20	37.2	15.1	52.3	11.3
0.35	0.20	39.2	11.2	50.4	15.6
0.30	0.20	41.0	12.7	53.7	16.4
0.25	0.20	40.1	13.0	53.1	18.9
0.30	0.15	39.3	13.4	52.7	19.5

* Overtailings of 60 mesh and 6xx sieves

† Overtailings of 32 and 45 mesh sieves

‡ Throughs and overtailings of 10xx sieves

TABLE 6. YIELD OF SEMOLINA AND FLOUR FROM DURUM WHEATS*

Variety	Fine semolina** (%)	Coarse semolina† (%)	Total semolina (%)	Flour‡ (%)
<i>Bansi</i>	40.9	12.8	53.7	16.4
<i>Local red</i>	37.2	10.5	47.7	16.5
<i>Bijaga yellow</i>	39.6	10.6	50.2	16.1
<i>Amrit</i>	37.8	10.0	47.8	16.4
<i>Bijaga red</i>	35.5	8.6	44.1	20.0

* Clearances at B₁ and B₃ are 0.30 and 0.20 mm respectively

** Overtailings of 60 mesh and 6xx sieves

† Overtailings of 32 and 45 mesh sieves

‡ Overtailings and throughs of 10xx sieves

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was refluxed with 1 per cent 2, 4-dinitrophenylhydrazine solution in 5N sulphuric acid for two hours. The chloroform layer was repeatedly washed with 2N sulphuric acid for removal of unreacted dinitrophenylhydrazine followed with water and dried over anhydrous sodium sulphate. Dried extract was concentrated and made upto a known volume with methanol.

Separation of 2,4-dinitrophenylhydrazones (DNP HS) by thin layer chromatography on silica gel G: Glass plates (20×20 cm) were coated by pouring a uniform slurry of silica gel G (7 g) and water (20 ml) on them and tilting these from side to side. The plates were dried at room temperature and activated at 120°C for two hours before using. A known volume of 2,4-dinitrophenylhydrazone solution was applied in the form of a streak and the plates were developed in a solvent system consisting of carbon tetrachloride: cyclohexane: ethylacetate (10:2:1). 2, 4-Dinitrophenyl hydrazones of ethanal, propanal, butanal, hexanal, octanal, acetone, ethyl methyl ketone, cyclohexanone, furfural, crotonaldehyde, diacetyl and acetoin were also applied along with the experimental samples. Fig. 2 is a typical chromatoplate showing the resolution of 2,4-dinitrophenylhydrazones of carbonyls present in fresh and stored *chapaties*. For quantitative estimation, the different bands were scrapped and extracted with methanol. The concentration of hydrazones was determined colorimetrically¹⁷ in alkaline medium (Table 1).

Separation of 2,4-dinitrophenylhydrazones by thin layer chromatography on magnesia-celite: The plates were coated by spreading an uniform slurry of magnesia (3 g), celite (1 g), water (15 ml) and dried at room temperature for 48 hr. Experimental samples along with the known 2,4-dinitrophenylhydrazones were applied and the plates developed in a solvent system consisting of chloroform: hexane (3:1). The method was essentially that of Schwartz *et al.*¹⁸ with minor modifications. A typical chromatoplate is shown in Fig. 3. For quantitative estimation of saturated and unsaturated carbonyls the coloured bands were scrapped from the plate and extracted with methanol. The concentrations of hydrazones in methanol extracts were measured colorimetrically in alkaline medium¹⁷ (Table 2).

TABLE 2. CONCENTRATION OF SATURATED AND UNSATURATED CARBONYLS IN FRESH AND STORED CHAPATIES SEPARATED ON MAGNESIA-CELITE PLATE

Band No.	Total carbonyls %	
	Fresh	Stored
1	6.9	19.2
2	10.1	20.2
3+4 (unsaturated carbonyls)	4.1	10.3
5 (saturated carbonyls)	78.9	50.4

TABLE 1. R_f VALUES, CONCENTRATION AND TENTATIVE IDENTIFICATION OF CARBONYLS IN FRESH AND STORED (3 MONTHS) CHAPATIES.

Band number	R _f values		Con. (%) of total carbonyls		Colour on silica gel G		Colour with alcoholic KOH		Major component identified
	Fresh	Stored	Fresh	Stored	Fresh	Stored	Fresh	Stored	
1	0.06	0.00	7.29	6.90	Brownish orange	Brownish orange	Blue	Blue	Diacetyl ^{1,2}
2	0.06	0.06	4.34	4.06	„	„	Violet	Violet	Acetoin ¹
3	0.18	0.18	1.45	1.67	„	„	Blue	Blue	Dicarbonyl ²
4	0.40	0.40	2.20	1.60	„	„	Violet	Violet	—
4 (a)	—	0.44	—	2.50	—	„	—	„	—
5	0.50	0.50	1.82	2.00	Yellow	Yellow	Violet	„	Ethanal ^{1,2}
6	0.60	0.60	77.57	75.80	„	„	„	„	Acetone+Propanal+Crotonaldehyde ^{1,2}
7	0.70	0.71	1.87	1.72	„	„	„	„	Ethylmethyl ketone ^{1,2}
8	0.81	0.82	2.03	1.40	„	„	„	„	Heptanal or Heptanone-2
9	0.90	0.91	1.47	0.80	„	„	„	„	Nonanal

Method of detection: 1, R_f value; 2, colour with alcoholic KOH.

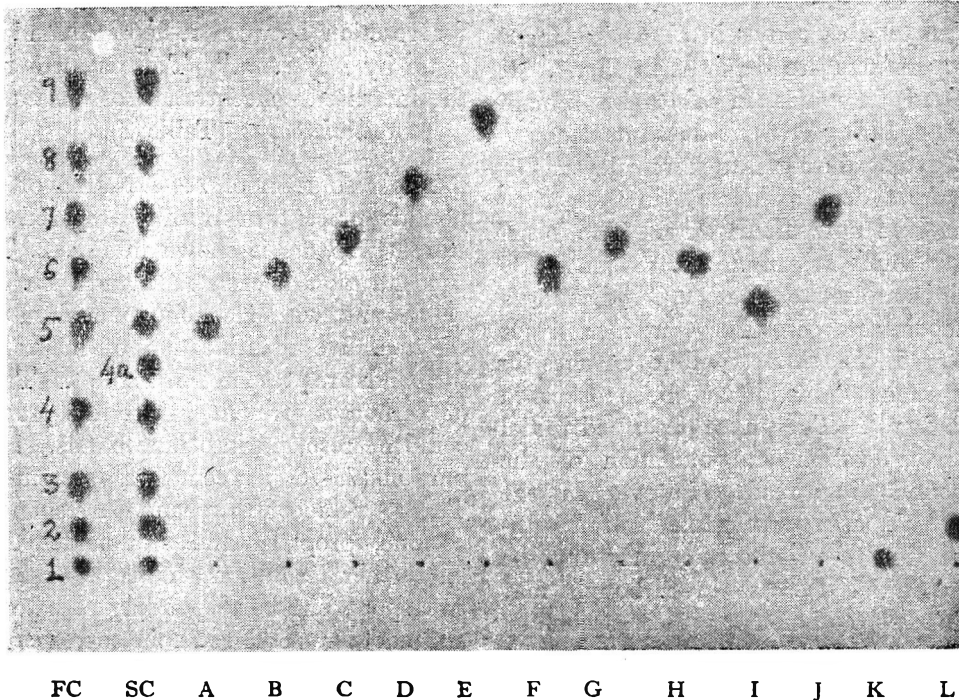


Fig. 2. Thin layer chromatogram showing the resolution of 2:4-Dinitrophenyl hydrazones of various carbonyl compounds. FC, Fresh chapati; SC, stored chapati (3 months); A, ethanal; B, propanal; C, butanal; D, hexanal; E, octanal; F, acetone; G, cyclohexa none; H, crotonal dehyde; I, furfural; J, ethyl methyl ketone; K, diacetyl; L, acetoin. Adsorbent: Silica gel G; Irrigating solvent; carbon tetrachloride cyclohexane-ethyl acetate (10:2:1); system; ascending.

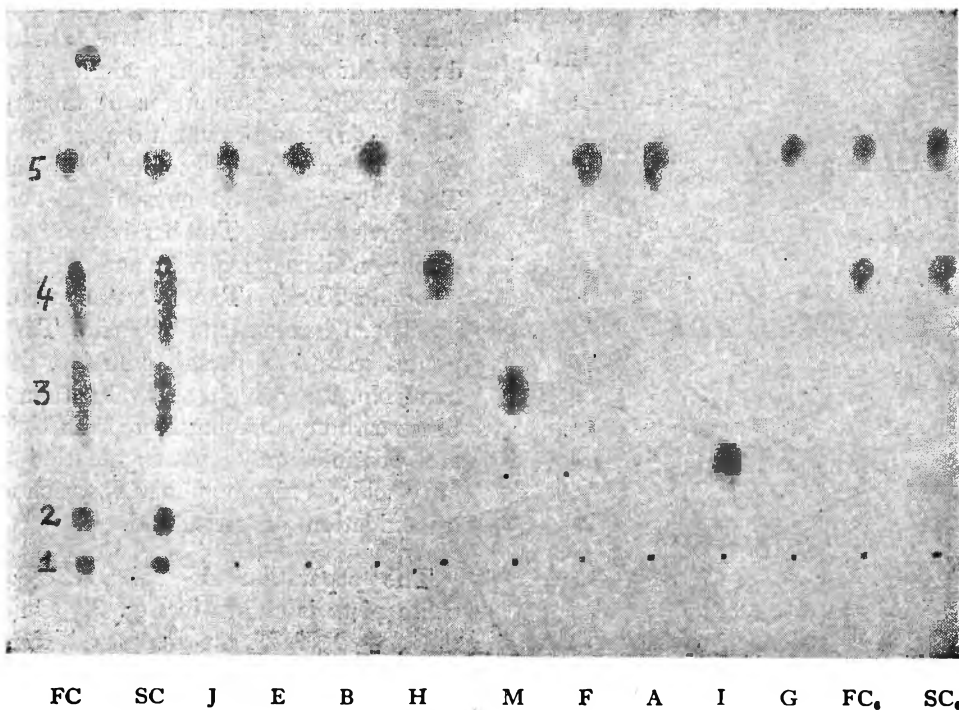


Fig. 3. Thin layer chromatogram showing the separation of 2:4-DNPHS of carbonyls on magnesia-celite (3:1) plate. Legend same as under Fig. 2; M, 2:4-hexadienal; FC₆ and SC₆ bands No. 6 of FC and SC respectively of silica gel plate (Fig. 2). Irrigating solvent; chloroform-hexane (12:42); system; ascending.

and ketones could not be separated. $\alpha\beta$ -mono- and diunsaturated aldehydes could be separated, but in case of experimental samples considerable overlapping of bands was observed and therefore both 2-enals and 2,4-dienals were estimated as total unsaturated aldehydes. The concentration of saturated and unsaturated carbonyls both in freshly prepared and stored *chapaties* are presented in Table 2. It is seen that concentration of saturated carbonyls decreased from 78.9 to 50.4 per cent, where as that of unsaturated carbonyls increased from 4.1 to 10.3 per cent during three months storage of *chapaties* at room temperature. The band No. 2 gave blue colour on magnesia-celite plate while band No. 1 had brownish yellow colour and remained at base. The R_f value and colour of band No. 2 were similar to

that of dicarbonyl compounds. The band No.1 could not be characterised authentically but concentrations of both these bands significantly increased during storage.

In preserved *chapaties* the concentration of saturated aldehydes decreased during storage due to the interaction with amino compounds forming unsaturated carbonyls which may be responsible for the lower organoleptic properties of stored *chapaties*. The unsaturated carbonyls may also be formed by oxidative degradation of sorbic acid which is used as a preservative¹⁵. Mookerjee and Chang¹⁶ have also reported the increase in concentration of 2-enals in stale potato chips. The work is in hand to establish the mechanism of changes in carbonyl compounds and their effects on organoleptic properties of *chapaties*.

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Proline as an Antioxidant in Fish Oil

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Measurement of induction period by the weighing method, and of oxygen uptake, refractive index and TBA and peroxide values at room temperature and at 50°C, all pointed to the antioxidant activity of pure L-proline in fresh sardine oil. At 0.02 per cent level in the oil, proline was as effective as, BHA and at 0.1 per cent level it was much more effective than BHA. Adverse colour and odour changes at 0.1 per cent level were less noticeable at 0.02 per cent level. Proline is non-toxic and is the first reported antioxidant with a pyrrolidine structure.

Frozen or dried fish products become rancid as a result of the oil present in them, which is highly susceptible to oxidation. Stabilisation is essential to ensure palatability. Some proteins, including defatted fish protein concentrate, were earlier reported by Sen and Padival¹ to have a stabilising effect on sardine oil. Fish protein hydrolysate was found even more effective than the concentrate in this regard (unpublished work²). Bishov, *et al.*³ reported that hydrolysed vegetable proteins stabilised the fat in soup powder.

Protein hydrolysates are mixtures of peptides and amino acids, each of which would differ in their antioxidant effectiveness. Individual amino acids have been reported^{4,5} to exhibit antioxidant activity. Initial screening in sardine oil showed that methionine, phenylalanine, alanine, leucine, isoleucine and tryptophan have slight antioxidant effect only at high concentrations of 1 and 5 per cent. Proline had significant antioxidant activity even at 0.1 per cent, and follow-up studies are here reported.

Materials and Methods

Chromatographically pure L-proline was used as the test antioxidant in these experiments. The sardine oil (FFA 0.1-0.5 per cent as oleic acid, IV 145-165) used as a medium was rendered in the pilot plant from fresh oil sardines (*Sardinella longiceps*).

Induction periods were determined by the method of room temperature storage, weighing and plotting used by Olcott and Einset⁶ and slightly modified by Sen and Padival.¹ To ensure thorough incorporation of proline, which is only slightly oil-soluble, aliquots of proline in 60 per cent alcohol were measured into cuvetts in duplicate, the solvent evaporated on a water bath, and 3 ml of oil added in small quantities with stirring and warming. In this series of experiments

sardine oil was used (a) as obtained, (b) after heating at 212°C for 5 min to destroy hydroperoxides, filtering and cooling, and (c) after heating on a waterbath at 90°C for 2.5 hr following addition of the antioxidant. The method was also used to compare BHA and proline for their antioxidant effectiveness at two concentrations (0.02 and 0.1 per cent).

Changes in the refractive index⁷ determined at 40°C were determined following storage of the oil containing 0.1 per cent proline at room temperature for 59 days, in triplicate.

Protection factors were determined by noting the time taken for the manometric absorption of 80 μ l of oxygen by 2 ml of the test sample (T_t) and the control (T_c) at 40°C in a Warburg respirometer using a vibration speed of 100-120 per min. Thereafter the protection factor was calculated at $T_t - T_c / T_c$. Sardine oil was heated at 212°C for 5 min to destroy hydroperoxides, and 20 ml each added to several petridishes of 10 cm diameter containing proline, which was thereafter incorporated with slight warming. The dishes carrying this layer of oil were kept exposed at room temperature, and the oils tested manometrically for oxygen absorption after 1, 8, 16 and 22 days of storage.

Both peroxide values (as O.D./mg oil) and TBA values ($1000 \times$ O.D./mg oil) were determined by earlier methods¹ on controls and proline-containing (0.1 per cent) sardine oils which were held for upto 15 days at 50°C.

Results and Discussion

Proline at 0.1 per cent level prolonged the induction period, as determined by weight increase, under all the three conditions studied. In the oil as such, the increase in induction period was from 228 to 546 hr, in the oil heated at 212°C for 5 min to destroy hydroperoxides, it was from 288 to 1230 hr, and in the oil

heated at 90°C for 2.5 hr after antioxidant incorporation, it ranged from 150 to 1100 hr. Proline shows up better in a peroxide-free oil, as is to be expected. The superior effect following fairly prolonged heating after antioxidant addition could be due to increased incorporation of proline, which at room temperature is only slightly oil-soluble, either by physical solubilisation or by solubilisation following chemical reaction with the components of the oil.

When storage of all three types of oils was continued for 62 days, the controls turned very viscous, while the treated samples had not changed very greatly. In triplicate experiments, sardine oil itself, on keeping at room temperature rapidly increased in refractive index from 1.4727 to 1.4800, as calculated from graphs, on the 22nd day, while with 0.1 per cent proline, it took 59 days to reach this figure.

Protection factors were determined manometrically. Immediately after addition of 0.1 per cent proline, the negative protection factors obtained (-0.33 and -0.52 in duplicate experiments) revealed a pro-oxidant effect. With storage of the oil containing the added proline for 8 days, the values turned positive (2.91, 1.60) and after 16 days a strong antioxidant effect was seen (protection factors 40.0, 31.1). After 22 days the protection factors fell again (7.1, 7.1), perhaps as a result of antioxidant destruction.

Fig. 1 shows the changes in peroxide value and TBA value of sardine oil held at 50°C without and with 0.1 per cent proline for 15 days. The suppressive action of proline is evident. The characteristic drop in the curves after a maximum indicate more rapid transformation than formation of the compounds being measured.

Proline was next evaluated in comparison with the well-known antioxidant BHA at concentrations of 0.02 and 0.1 per cent of each by the storage at 50°C and weighing procedure. The induction periods obtained were:

- Control for all experiments—135 hr
- With 0.02% BHA—215 hr
- With 0.1% BHA—285 hr
- With 0.02% proline—285 hr
- With 0.1% proline—1275 hr

At the lower concentration, both BHA and proline have a slight and similar antioxidant effect. At the higher (0.1 per cent) concentration BHA shows little protection improvement, but proline is many times as effective. However, at the higher concentration, adverse colour and odour changes were seen to be induced in the oil by proline. These changes were much less evident at the lower concentration, but this aspect calls for further study.

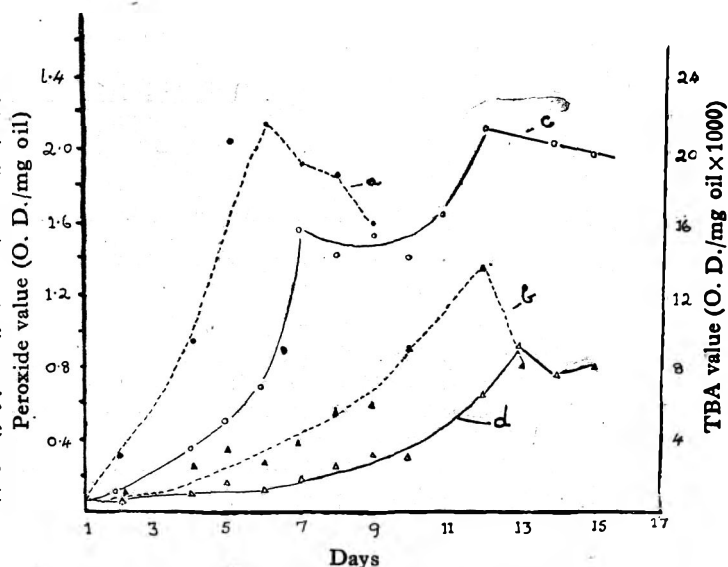


Fig. 1. Changes in TBA values and peroxide values of sardine oil with and without proline.

Changes in TBA values: (a) Sardine oil; (b) sardine oil with 0.1 per cent proline.

Changes in peroxide values, (c) sardine oil; (d) sardine oil with 0.1 per cent proline.

Unlike BHA and BHT, proline is non-toxic at any concentration. It is present in high proportions in such proteins as gliadin, zein and gelatin, while its synthesis is also known. If found suitable, it may be possible to obtain it at a low price for use as an antioxidant.

This may be the first report of antioxidant potency for a substance such as proline carrying a pyrrolidine structure, and further synthetic approaches on this basis seem possible.

Acknowledgement

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Effect of Homogenization and Pasteurization on Surface Tension and Curd Tension of Buffalo Milk

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Study was carried out to find out the effect of homogenization and Pasteurization on surface tension and curd tension of buffalo milk. The sequences practised for homogenization, was prior (HPP) and after (HAP) pasteurization. Pasteurization was done by holder method and heating to high temperature under laboratory conditions.

Average surface tension of raw buffalo milk was observed to be 45.95 dynes/cm at 30°C which increased by 0.89 and 0.81% in case of pasteurization by holder method and heating to high temperature respectively. Over-all increase in surface tension was 8.08% for double stage against 5.01% for single stage homogenization.

Curd tension of raw buffalo milk was observed to be 44.54 g. Pasteurization by holding method and heating to high temperature resulted in reduction in curd tension values by 32.5 and 28.1% respectively. Overall average reduction in curd tension were 73.1 and 72.7% for single and double stage homogenization respectively.

Surface tension is one of the important physical properties of milk. Surface tension measurements have been carried out for determination of adulteration, foaming ability, emulsion stability, rancidity development and bacteriological growth in milk.¹ Surface tension of cow milk ranges between 40-60 dynes/cm with an average of 46-47.5 dynes/cm at 20°C as reported by Jenness and Patton.² The surface tension values obtained by Traube's Stalagmometric method were 49.6 dynes/cm at 27°C for buffalo milk as reported by Chandan and Dastur³ whereas Sharma⁴ and Sat Parkash⁵ reported 46.2 and 44.58 dynes/cm at 27°C respectively by using DuNouy ring method.⁶ Hetrick and Tracy⁷ reported decrease in surface tension from 44 to 35 dynes/cm in case of raw homogenized milk which was attributed to increased lipolysis. Trout *et al.*⁸ and other workers reported increase in surface tension on pasteurization and homogenization.

Cow or buffalo milk form comparatively much tougher curd than human milk in the stomach. Homogenization of milk is commonly practised to reduce the curd tension making it suitable for infant feeding. Hill regards curd tension of 30 g or under as soft curd milk. Average curd tension values of raw cow and buffalo milk are reported in between 50-70 g and 40-45 g respectively. Spur⁹ observed average curd tension of 11.6 and 11.2 g for homogenized grade A and grade B milk respectively. A number of research workers have reported reduction in curd tension due to homogenisation of milk.

In the present investigation, the work on surface tension and curd tension has been carried out to find out the effect of homogenization and pasteurization on buffalo milk.

Materials and Methods

Raw material: Bulk buffalo milk from the experimental dairy of the National Dairy Research Institute, Karnal was used in the experiment.

Standardisation: Raw buffalo milk samples were standardised to 6 per cent fat with buffalo skim milk.

1. **Holder pasteurization:** Standardized milk was held in a waterbath at 80°C so as to raise the temperature of milk to 63°C in about 5 min. Later, the milk was held in another waterbath maintained at 63°C for a period of 30 min.

2. **High temperature heating:** Standardized milk was held in a waterbath maintained at 80°C such that milk was allowed to come to temperature of 73°C within 15 min and maintained at this temperature for 15 sec.

The sequence of homogenization was as follows:

- (i) Homogenization after pasteurization (HAP)
- (ii) Homogenization prior to pasteurization (HPP)

Milk was homogenized with Rannie Piston type homogenizer, the pressure for the first and second stage being 180 kg/cm² and 40 kg/cm² respectively.

Standardized raw milk samples were preheated to 60-63°C before homogenization and then pasteurized

(high temperature heating or Holder). The second lot of milk samples were pasteurized (high temperature heating or Holder) and later homogenized at 60-63°C. All samples were cooled and maintained at 4-6°C for a minimum period of 3 hr prior to determine the surface tension.

Method of determining surface tension: du Nouy⁹ tensiometer was used for the determination of surface tension values of milk samples tempered to 30°C.

After standardizing of the du Nouy tensiometer the surface tension of milk samples was determined as follows:

A small quantity of milk sample was taken on a watch glass already rinsed with the milk sample. The watch glass was kept on the circular table and height was adjusted such that the liquid level was brought about one centimeter below the ring. The pointer and the main scale were adjusted to zero. The milk level was raised by means of the screw till the ring dipped in the milk sample. The torsion head was turned until the index-line was again opposite the reference line. The milk level was slowly lowered and at the same time, torsion head was turned so that index line and reference line remained opposite to each other. The stage at which the ring suddenly left the liquid level indicated the surface tension in dynes/cm of milk sample. The ring was cleaned before each determination by

rinsing with distilled water and then with petroleum ether and finally with alcohol.

Method of determining curd tension: The method adopted by Chandrasekhara¹⁰ *et al.* was used in the experiments. Fifty ml of milk samples were taken in beakers and the temperature was brought to 37°C, curd tension knife was placed in each of the beakers and 2 ml of 0.2 per cent Hansen rennet solution were added rapidly to the milk. The milk in each of the beaker was stirred and the beakers placed in thermostatically controlled water bath at 37°C. After three hours holding at 37°C, the curd tension was determined by loading the pan with lead shots till the curd tension knife cut its way through the curd. The lead shots were weighed to determine the curd tension.

Results and Discussion

From the statistical analysis presented in the Table 1 it is observed that pasteurization, homogenization and the sequence of homogenization had significant effect in increasing the surface tension of milk. Overall holder pasteurized unhomogenized and holder pasteurised homogenised milk increased the surface tension to a greater extent as compared to corresponding treatment of pasteurisation by high temperature method. Overall double stage homogenization increased surface tension more effectively than did

TABLE 1. SURFACE TENSION OF (30°C, DYNES PER CM) BUFFALO MILK UNDER DIFFERENT METHODS OF PASTEURISATION, STAGES AND SEQUENCES OF HOMOGENIZATION (13 REPLICATES)

Homogenization	Unhomogenized			Homogenized			
	Raw milk	Pasteurization holder	H.T.H.	Holder pasteurization		H.T.H.	
				Single stage	Double stage	Single stage	Double stage
H.A.P.	44.85	45.29	45.22	46.59	48.14	46.77	48.27
H.P.P.	47.05	47.42	47.42	50.03	51.26	49.77	50.98
Average	45.95	46.36	46.32	48.31	49.70	48.27	49.63
Increase %	Overall	0.89	0.81	5.14	8.16	5.05	8.01
	HAP	0.89	0.82	3.88	7.33	4.28	7.65
	HPP	0.79	0.79	6.33	8.95	5.78	8.33
C.D.	—	—	0.37				
Analysis of variance				F			
Between treatments				145.6*			
Between sequence of homogenization				728.7*			
Interaction				271.1*			

* Significant at 1% level; CD: Critical difference; HTH: High temperature heating

TABLE 2. CURD TENSION (G) OF BUFFALO MILK UNDER DIFFERENT METHODS OF PASTEURIZATION, STAGES AND SEQUENCES OF HOMOGENIZATION (13 REPLICATES)

Sequence of homogenization	Unhomogenized			Homogenized			
	Raw milk	Pasteurization		Holder pasteurization		H.T.H.	
		Holder	H.T.H.	Single stage	Double stage	Single stage	Double stage
HAP	44.62	31.92	33.23	13.15	12.31	13.15	12.31
HPP	44.46	28.15	30.83	11.77	10.77	11.92	11.15
Average	44.54	30.04	32.04	12.46	11.54	12.54	11.73
Percentage	Overall	32.5	28.1	72.0	74.1	71.8	73.6
Reduction	HAP	28.4	25.5	70.5	72.4	70.5	72.4
	HPP	36.6	30.6	73.5	75.7	73.2	74.9
Analysis of variance		C. D=1.38		'F'			
Between treatments				715.74*			
Between sequence of homogenisation				19.36*			
Interaction				1.3*			

* Significant at 1 % level. HTH: High temperature heating

single stage homogenisation so also overall HPP treatment of milk resulted in increased surface tension values as compared to HAP treatment. Trout *et al.*⁸ and a number of workers have reported increase in surface tension on pasteurisation and homogenisation of cow milk. The increase in surface tension of milk on homogenisation might be due to the fat globule membrane which becomes dispersed in the plasma (perhaps denatured) as a result of homogenization and the new membrane formed due to the adsorption of plasma protein on the increased fat surface, composed of the same or different enzymes, proteins, lipids, etc., proportional to their abundance, mobility and ability to affect the surface tension. Webb and Johnson¹¹ attribute the increase in surface tension on homogenization of milk to reduction in the amount of free fat of homogenized milk.

From the statistical analysis presented in Table 2, it is observed that pasteurisation, homogenization and the sequence of homogenization had significant effect on reducing the curd tension of milk. Overall holder pasteurised unhomogenised and holder pasteurised homogenised milk resulted in lower curd tension—than did the corresponding lots treated by high temperature treatment. Overall double stage homogenisation HPP treatments were more effective in reducing curd tension than single stage and HAP treatments.

Rao *et al.*¹² noticed only 10 per cent reduction in

curd tension of holder and high temperature treatment as against 3.25 and 28.1 per cent in the holder and high temperature method respectively noted in the present study. Kelly¹³ noted almost equally effective reduction of curd tension both by single and double stage homogenization whereas the present result indicated that double stage homogenization reduced the curd tension to a greater extent (73.8 per cent) as compared to single stage (71.9 per cent). Henderson¹⁴ reported 50 per cent reduction in curd tension. Whereas Kraus *et al.*¹⁵ observed 61.5 per cent reduction in curd tension on homogenizing milk at 2500 lb/sq in (175 kg/cm²) pressure. Babcock¹⁶ observed that pressure in excess of 2000 lb/sq in appeared to be of little practical value in further reducing the curd tension. Sommer¹⁷ attributed the reduction in curd tension of homogenized milk to the increase in number of fat globules serving as points of weakness in coagulum. According to Wolman¹⁸ the adsorption of protein on the increased fat surface on homogenisation was the major factor in lowering the curd tension.

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Total Chlorine Determination in Organochlorine Insecticides

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A simple procedure for the determination of total chlorine in aldrin, endrin, dieldrin and lindane formulations using xylene and metallic sodium and the estimation of the resulting chloride electrometrically is described.

One of the most commonly used methods of liberating organically bound chlorine from compounds is the well known Stepanov's method using metallic sodium and isopropyl alcohol. This method is applicable to organochlorine insecticides and their formulations. However, this method has some disadvantages. One is the need to use a large amount of sodium (usually 2-3 g) and another is the occurrence of large blanks.

Beckman¹ has noted that many of the organochlorine insecticides may be dechlorinated with sodium without isopropyl alcohol merely by using a hydrocarbon solvent with a boiling point above 110°C. At this temperature which is above the melting point of the sodium sufficient activity is achieved so that most of the insecticides yield sodium chloride. The advantages of a method based on this observation is that a small amount of sodium (about 0.2 g) has to be used and it also results in low blanks.

Details of the method for the determination of total chlorine in aldrin, endrin, dieldrin and lindane based on the above observation have been worked out and are described below. The chloride is estimated by an electrometric method.

Materials and Methods

Sodium: Prepare fine grain by heating about 5g of sodium with 50ml xylene in a flask under a reflux condenser until the sodium melts. Remove the heat, disconnect the flask, loosely stopper with a cork, cover with a cloth and shake vigorously when sodium is divided into small grains.

Solution 1 (for reference cell): Dissolve 10 g of potassium nitrate and 14 g of sodium oxalate in one litre of distilled water and add 25 ml of 0.1N silver nitrate. The precipitate formed should be shaken up into the solution when pouring into the reference cell. An excess of silver oxalate appears desirable. The solution should be stored in a dark bottle.

Solution 2 (plating solution): Dissolve 25 g of potassium nitrate in a litre of 0.1N sulphuric acid and add 1 drop of 0.1N silver nitrate. Store in a brown bottle.

Galvanometer: Spot reflecting galvanometer. Resistance 500 ohms giving a full scale deflection at 1 microampere. A suitable instrument is manufactured by M/s. Umatson, Angappanaicker street, Madras.

Electrodes: Pure silver wire 1 mm diameter.

Tapping key

Reference electrode: It consists of a glass tube constricted at one end as in Fig. 1. The constricted end is plugged with a small filter paper wad and the cell filled about two thirds of its volume with the solution 1. The tube is fitted with a cork carrying the silver wire.

Procedure: Weigh into a 250 ml R. B. flask with a standard ground glass neck a quantity of the formulation containing 25-40 mg active ingredient accurately. Emulsion concentrate formulations can be directly weighed out into the flask and in the case of low concentration dusts the active ingredient can be extracted with xylene and an aliquot taken for the determination. Add 10 ml xylene and 0.2 g of sodium and reflux under a condenser for 30 min. Remove the heat and add cautiously through the condenser 15 ml of isopropyl alcohol and heat till all the sodium dissolves (about 10 min). Stop heating. Wash down the condenser with 25 ml of water. Disconnect the flask and add cautiously 5 ml (20v/v) hydrogen peroxide and warm the flask to destroy the excess. Add a drop of phenolphthalein and neutralise with 2N nitric acid. Add about 10 ml in excess.

Transfer the contents of the flask to a 250 ml beaker and titrate with standard silver nitrate (0.05 N) electrometrically as described below.

Carry out a blank for the reagents by omitting the sample.

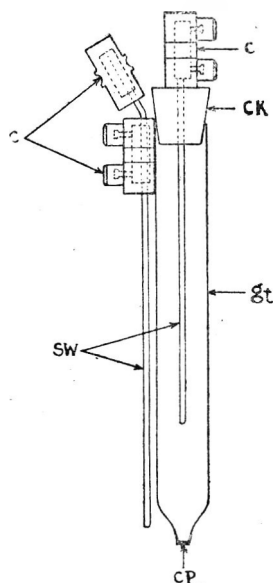


Fig. 1. Reference electrode

C: Plastic connectors; SW: 1mm dia. Silver wire; CP: Cotton plug; gt: Glass Tube; CK: Cork

Electrometric chloride determination: The procedure is based on that described by Northrup². It depends on the use of a reference cell in which a silver electrode is suspended in a medium containing a small but constant concentration of silver ions. The reference cell is connected through a suitable bridge medium to the titration cell. When there is no excess of silver ions in the latter, a galvanometer joining the two electrodes (both silver) shows a deflection. As soon as a slight excess of silver ions equal to that of the reference cell is established in the titration cell, the potentials of the two half-cells balance one another and the galvanometer returns to zero. With a suitable galvanometer this is a very sensitive arrangement.

The electrodes are immersed in the solution containing the chloride and connected to the galvanometer through a tapping key as in Fig. 2. The solution is suitably stirred and titrated with standard (0.05N) silver nitrate solution until no deflection in the galvanometer is observed on momentarily depressing the key. The key must be depressed momentarily to avoid polarisation of the cell. The silver nitrate must be added slowly from the burette with all the resistance in circuit until the deflection of the galvanometer is about 1 small division from zero, when the resistance is cut out and the titration is completed.

Some precautions: The reference cell solution must be changed daily, the bottle being shaken before adding the solution to the cell so that the latter contains some of the silver oxalate precipitate as well as the solution.

The titration electrode must be left in distilled water during short periods of rest and the reference electrode always in the reference cell solution. At night 'plating solution' should be put into a 250 ml beaker and the two electrodes connected without any resistance in circuit.

The electrodes may require occasional cleaning with fine emery paper after which they should be left in the plating solution overnight. Calculation is done as follows:

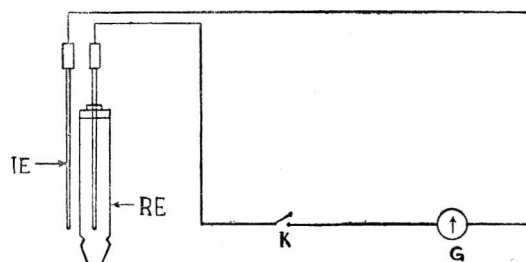


Fig. 2. Circuit diagram

IE: Indicator electrode; RE: Reference electrode; K: Key; G: Galvanometer

$$\text{Active ingredient\%} = \frac{3.545 (V - V_1) \times n \times f}{W}$$

Where, V = ml of standard silver nitrate consumed by the reaction.

V_1 = ml of standard silver nitrate consumed by the blank

n = normality of silver nitrate

f = 1.79 for endrin or dieldrin (HEOD)

= 1.72 for aldrin (HHDN)

= 1.367 for lindane

W = weight of sample taken

Acknowledgement

The author is thankful to Dr S. N. Banerjee, Plant Protection Adviser to the Govt. of India and Dr K. K.

Nirula, Deputy Director, Central Plant Protection Training Institute, Hyderabad for their interest and encouragement.

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Some Observations on the Variation in Insecticidal Deposit on Treated Wheat Grains

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Immersion of wheat grains in acetone for 24 hr and analysis of the extract as such by gas-liquid chromatography gave almost quantitative recovery of pirimiphosmethyl, a potential grain protectant. This method was found suitable for the estimation of insecticide on a single grain. Grain to grain variation in the deposit of the insecticide on wheat treated with the PAU grain treating machine was studied. The variation in deposit ranged from 0.45 to 1.90 μ g per grain. An optimum sample size for residue estimation of the insecticide was considered to be 10 g.

The distribution of a grain-protectant in a grain mass is of great importance from the point of view of its effectiveness as well as its residue implication¹⁻³. Recently, Bindra *et al.*⁴ developed a simple machine (PAU grain treating machine) for the application of grain-protectants. The present studies were carried out to ascertain the extent of variation of insecticide in grain mass treated with this machine. This included the estimation of the insecticide on single grain basis and the determination of an optimum sample size for the insecticide residue studies. To avoid variations in estimations due to quick degradation, the relatively stable insecticide pirimiphosmethyl (2-diethyl amino-6-methyl pyrimidin-4-yl-dimethyl phosphorothionate) was purposely chosen⁵. It is a new organophosphorus insecticide having low mammalian toxicity (2050 mg/kg body weight) and has also been found to compare favourably with malathion as a grain-protectant⁶.

Materials and Methods

Technique for the estimation of the insecticide on single-grain basis: Wheat grains (variety *Kalyan Sona*) were treated singly by topical application using Hamilton microsyringe with 1 μ l of the acetone solution (1 mg/ml) of technical grade pirimiphosmethyl*. Each treated grain was kept separately in a test tube for 24 or 48 hr. The grain was then crushed with the help of a glass rod and was rinsed with another 2 ml of acetone. The volume of the final extract was reduced to 2 ml from which an aliquot of 2 μ l was injected into Packard gas chromatograph (Model 7624) using pyrex glass column packed with 10 per cent DC 200 on 80 to 100 mesh gas chrom Q. The flame-ionization detector modified by coating with KCl was employed. The operating conditions of the GLC were essentially the same as described by Chawla and Kalra⁷. The temperature of the column, injection block and detector were

* ICI Plant Protection Ltd., Berks, U.K.

200, 210 and 220°C respectively. The flow rates of gases were maintained at 100 ml/min for nitrogen (carrier gas), 40 ml/min for hydrogen and 200 ml/min for air. The chromatograms obtained were compared on the basis of peak height with standards of known concentration injected under identical operating conditions. The consistency of the response was checked by repeated injections of standards.

Treatment of wheat bulk and sampling for residue analysis: About 50 kg of wheat was treated at 10 ppm with pirimiphosmethyl (Actellic^R, 50 per cent e.c.) employing the PAU grain treating machine as per procedure described by Bindra *et al.*⁴. Immediately after treatment, about 100 grains were randomly picked with the help of forceps and the insecticidal deposit was estimated as described above.

Samples of 1, 5, 10 and 20 g were taken and crushed in a Wiley mill through 20-mesh sieve. The crushed grains were immersed in acetone (1:5) and kept overnight. The slurry was filtered using Whatman No. 1 filter paper having a layer of anhydrous sodium sulphate followed by washings with 5-10 ml of acetone. The combined extract was made to a suitable volume and an aliquot ranging from 1 to 2 μ l was injected into the GLC for analysis.

Results

The solution of pure pirimiphosmethyl gave a single peak with retention time of 6.5 min. A linear response in peak height was observed in concentration range of 0.1 to 20 μ g used. The blank extract of the single wheat grain did not give any interfering peak whereas the extract from the insecticide treated single grain gave measurable response (Fig. 1). The recovery of insecticide ranged from 85 to 95 per cent. It was found that the deposits of pirimiphosmethyl on a single grain ranged from 0.45 to 1.90 μ g, the mean being 0.93 μ g. Green *et al.*¹ and Tyler and Green², however, found a much wider range of deposits (0.1 to 424 ppm of bromophos) in the case of equipment used by them for the treatment of the grains. The results, therefore, showed that the treatment with the PAU grain treating machine gave much more uniform insecticidal deposit on wheat grain.

The results further revealed that the estimation of pirimiphosmethyl in a sample of 10 and 20 g gave almost the same coefficient of variation (Table 1). The residues obtained with the sample size of 10 and 20 g ranged from 0.2 to 10.3 ppm (with mean value of 9.2 ppm) and from 0.3 to 10.7 ppm (with mean value of 9.0 ppm) respectively. However, the smaller samples of 1 and 5 g resulted in greater variation. The results, therefore, suggest that the sample size of 10g is

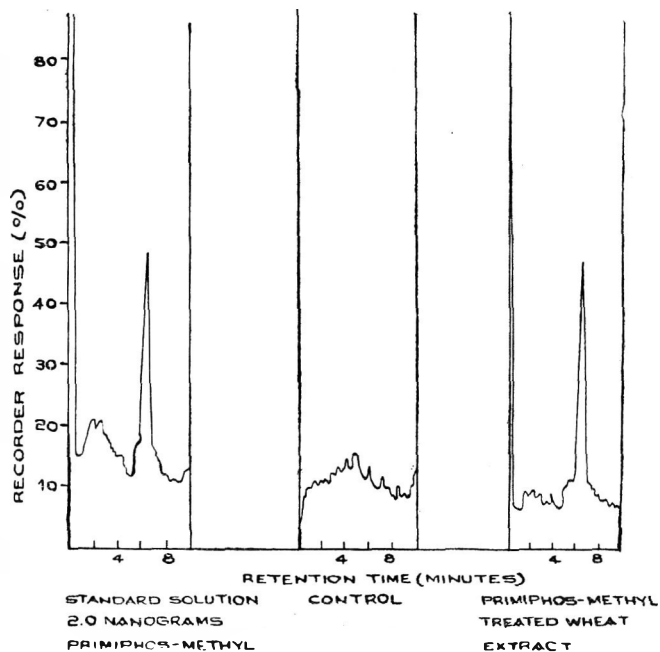


Fig. 1. Chromatograms of standard solution and extracts of control and treated wheat samples

optimum for the estimation of insecticidal deposits/residues on grains. It is most likely that the observations made in the case of distribution of pirimiphosmethyl may hold good for other grain protectants as well.

The immersion of crushed grains in acetone for 24 hr gave almost quantitative recovery of the residues of pirimiphosmethyl even after a month of the treatment. The extension of the immersion period from 24 hr (9.4 ppm) to 48 hr (9.71 ppm) did not substantially improve the recovery of the residues in the samples examined. Comparative extraction of the residues by Soxhlet apparatus also gave similar recovery (9.7 to 10.2 ppm; based upon 3 samples). The reproducibility of this technique was checked by the fortification of the filtered extract with the insecticide prior to the estimation by GLC. The amount of the insecticide ranged from 7.5 to 8.0 ppm, with a mean of 7.7 ppm.

TABLE 1. ESTIMATES OF PIRIMIPHOSMETHYL RESIDUES IN DIFFERENT SAMPLE SIZES OF TREATED WHEAT

Replicates	Sample size g	Residue range (ppm)	Mean \pm S.D.	Coefficient of variation
63	Single grain	0.45-1.90	0.93 \pm 0.38	40.9
10	1	6.19-9.10	7.31 \pm 0.84	11.5
8	5	7.20-10.05	9.26 \pm 0.90	9.7
8	10	8.20-10.25	9.18 \pm 0.73	8.5
8	20	8.3-10.70	9.02 \pm 0.74	8.2

The coefficient of variation was only 2.68 per cent. Thus, it can be inferred that the immersion technique is equally suitable for the extraction of the insecticide residues from grains.

Acknowledgement

The authors are thankful to Dr O. S. Bindra, Head of the Department of Entomology for his keen interest in the work and his valuable suggestions.

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Storage Fungi Associated with Rice Weevil (*Sitophilus oryzae* L.)*

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In rice weevils, (*Sitophilus oryzae* L) storage fungi were present internally in grubs, pupae and adults but not in eggs. Among the insect stages screened, grubs were found to carry more species of fungi. The number of adult weevils associated with the fungi does not vary according to the commodity in which the insects were breeding (wheat 67; Sorghum 66; and rice 55 per cent).

However the most frequently observed fungal species was differing according to food material. *A. flavus* was dominant in weevils from rice and wheat, whereas *A. restrictus* was dominant in weevils from sorghum. The species of internal fungi isolated from rice weevil also include *A. candidus*, *A. ruber*, *A. niger*, *A. fumigatus*, *A. ochraceus*, *A. chevalieri*, *Penicillium rugulosum* and *Amblyosporium* species.

The rice weevil, *Sitophilus oryzae* L. is the most common pest in sorghum, wheat and rice under tropical conditions. It is stated that the stored product pests can act as vectors of storage fungi¹. However, there is no information available on the type of fungi associated with *S. oryzae*. In this investigation the association of storage fungi with different stages of *S. oryzae* and the pattern of mycoflora present with the adult weevils living in different commodities like sorghum, wheat and rice were undertaken.

Materials and Methods

The infested grains were soaked in warm water (40-45°C) for 5 min, drained off and covered with acid fuchsin solution (acid fuchsin, 0.5 g; glacial acetic acid, 50 ml; water, 950ml) for 2 to 5 min. Then the stain was poured off and the grains were washed

in tap water to remove excess dye. The egg plugs take the stain which is deep cherry red². The grains having egg plugs were further soaked for 20 min in water to facilitate easy recovery of eggs, grubs and pupae.

Ten samples each of sorghum, wheat and rice having natural infestation of *S. oryzae* were obtained from consumers and market. From each sample adult insects and their different stages of development were collected as mentioned earlier. The insects were first washed with soap in running water for two minutes, surface sterilized for one minute in 1 per cent sodium hypochlorite, rinsed in sterile 7.5 per cent sodium chloride solution and cultured on malt salt agar (malt extract, 2 per cent; sodium chloride, 7.5 per cent; agar, 2 per cent) Czapek's agar and potato dextrose agar were also used in one experiment, as differential media to isolate fungi from

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different stages of rice weevil. The petridishes were incubated at room temperature (25 to 28°C) and the fungal counts were made after five days.

Results and Discussion

(i) *Fungi present in different stages of development of rice weevil*: Weevils breeding in wheat were used for this experiment. The different stages of insect such as egg, grub, pupa and adult when plated on three differential media showed that fungi were present internally in all except the egg. With the three differential media used *viz.* malt salt agar, Czapek's agar and potato dextrose agar, the internal fungi recovered are as follows. When malt salt agar is used as the medium, 83 per cent of adult insects, 63 per cent of pupae, and 42 per cent of grubs recorded the presence of fungi. In the same order 34.5 and 19 per cent of insects had fungi with Czapek's agar medium, and 18, 60 and 38 per cent of insects had fungi with potato dextrose agar medium. Nine species of *Aspergillus*, one each of *Penicillium* and *Amblyosporium* were isolated from grub. The pupa and adult were each having six species of *Aspergillus* (Table 1). Among the media used malt salt agar was superior, permitting the growth of osmophilic fungi such as *Aspergillus restrictus* and *A. chevalieri*, besides the other saprophytic fungi.

(ii) *Internal fungi from weevils breeding in sorghum, wheat and rice*: The storage fungi were isolated from 25-100 per cent of adult weevils breeding in sorghum. *Aspergillus restrictus* was found to occur predominantly followed by *A. chevalieri*, *A. candidus*, *A. flavus*, *A. ruber* and *Penicillium rugulosum*. The

TABLE 1. SPECIES OF INTERNAL FUNGI ISOLATED FROM DIFFERENT STAGES OF *S. oryzae* ON DIFFERENTIAL MEDIA

Egg grub	Pupa	Adult
<i>Aspergillus flavus</i> ^{1, 2, 3*}	<i>A. flavus</i> ^{1, 2}	<i>A. flavus</i> ^{1, 2, 3}
<i>A. ochraceus</i> ^{1, 2, 3}	<i>A. restrictus</i> ¹	<i>A. candidus</i> ^{1, 2}
<i>A. sydowii</i> ^{1, 2}	<i>A. terreus</i> ¹	<i>A. ochraceus</i> ^{1, 2}
<i>A. candidus</i> ^{1, 3}	<i>A. chevalieri</i> ¹	<i>A. chevalieri</i> ¹
<i>A. restrictus</i> ¹	<i>A. ochraceus</i> ^{2, 3}	<i>A. versicolor</i> ³
<i>A. versicolor</i> ²	<i>A. candidus</i> ^{2, 3}	<i>A. niger</i> ³
<i>A. niger</i> ³		
<i>A. terreus</i> ³		
<i>A. tamarii</i> ¹		
<i>Penicillium rugulosum</i> ¹		
<i>Amblyosporium</i> sp. ¹		

* 1. Malt—salt agar 2. Czapek's agar
3. Potato-dextrose agar

TABLE 2. INTERNAL FUNGI OF *S. oryzae* ADULTS BREEDING IN WHEAT, SORGHUM AND RICE

Sample	Wheat	Sorghum	Rice
1	64	74	28
2	74	25	92
3	86	60	58
4	72	36	25
5	100	34	58
6	91	94	74
7	44	98	20
8	32	100	22
9	46	74	94
10	60	68	78
Range	32-100	25-100	20-94
Average	67	66	55

TABLE 3. FUNGAL SPECIES ISOLATED FROM *S. oryzae* ADULTS BREEDING IN WHEAT, SORGHUM AND RICE

Fungi isolated	Wheat	Sorghum	Rice
<i>A. flavus</i>	+	+	+
<i>A. chevalieri</i>	+	+	+
<i>A. candidus</i>	+	+	+
<i>A. restrictus</i>	+	+	+
<i>A. ruber</i>	+	+	+
<i>A. niger</i>	+	—	—
<i>A. fumigatus</i>	—	—	+
<i>A. ochraceus</i>	—	+	+
<i>P. rugulosum</i>	+	+	+
<i>Amblyosporium</i> sp.	+	—	—

+ Fungal species isolated; — Fungal species not isolated

weevils breeding in wheat had internal fungi to an extent of 32-100 per cent. *A. flavus* was the common species isolated, besides *A. restrictus*, *A. ruber*, *A. chevalieri*, *A. niger*, *A. candidus*, *P. rugulosum* and *Amblyosporium* sp. The incidence of fungi in weevils obtained from rice varied from 20-94 per cent. As in the case of wheat *A. flavus* was the predominant species followed by *A. restrictus*, *A. niger*, *A. candidus*, *A. fumigatus*, *A. ochraceus* and *P. rugulosum* (Table 2 and 3).

The internal fungi of adult weevils grew out most commonly through thorax and anal region and less commonly through snout (Fig. 1). At a later stage the fungi also grew from inter segmental regions of the abdomen in the adult weevils. In the case of grubs and pupae fungal growth is not restricted to any specific region.

The importance of internal fungi of the stored product pests can be stressed well by the existence of selective association of fungi and insects. Sikorowski³ has stated that *Tribolium confusum* can breed well with mycelium and spores of *Aspergillus versicolor* as sole food. The association of

A. glaucus group fungi with *Sitotroga cerealella* was studied by Misra *et al.*⁴ Agarwal *et al.*⁵ have concluded that *Sitophilus granarius* on wheat, was the main cause in transferring *A. restrictus* to healthy grains. They have isolated *A. restrictus* and *A. repens* as internal fungi from adult weevils. The fungal species like *A. flavus* and *A. ochraceus*, which are known to produce mycotoxins were also found in this study to be carried internally by the rice weevil. Thus the occurrence of internal fungi of insects will reveal definite possibilities of transmitting such fungi to healthy grains.

The observation of *Aspergillus* sp. as the predominantly associated fungi with the insects than the other saprophytes like *Penicillium* invites further studies on this subject. The presence of fungi even in the pre-emergence stage of pupa is interesting to note, as the histolytic changes in the pupa do not seem to have any effect on the survival of fungi. The type of fungi associated with weevils breeding in rice, sorghum and wheat were similar, except that *A. ruber* was not observed in weevils breeding in rice. However, the most frequently observed fungal species in adult *S. oryzae* differed according to the commodity. *A. flavus* was predominantly observed in weevils from rice and wheat, whereas *A. restrictus* was predominant in weevils from sorghum.

Besides fungi, bacteria and yeasts were also observed in the various stages of the rice weevil either alone or in combinations with fungi. Pant and Fraenkel, have stated about the symbiotic relationship of insects like *Stegobium paniceum* and *Lasioderma serricornis* with yeast like organisms. Unlike yeasts and bacteria⁶ which could multiply in insects, the saprophytic fungi are apparently carried in the alimentary canal of the insect, mechanically, along with the food. It is likely that some of the spores are digested and utilised by insects. This has been proved at least in *T. confusum* which can complete its life cycle on fungus food.³ However under natural condition fungal spores are observed in the pellets of many stored product pests, revealing that all the fungal material was not consumed or utilised by the insects.

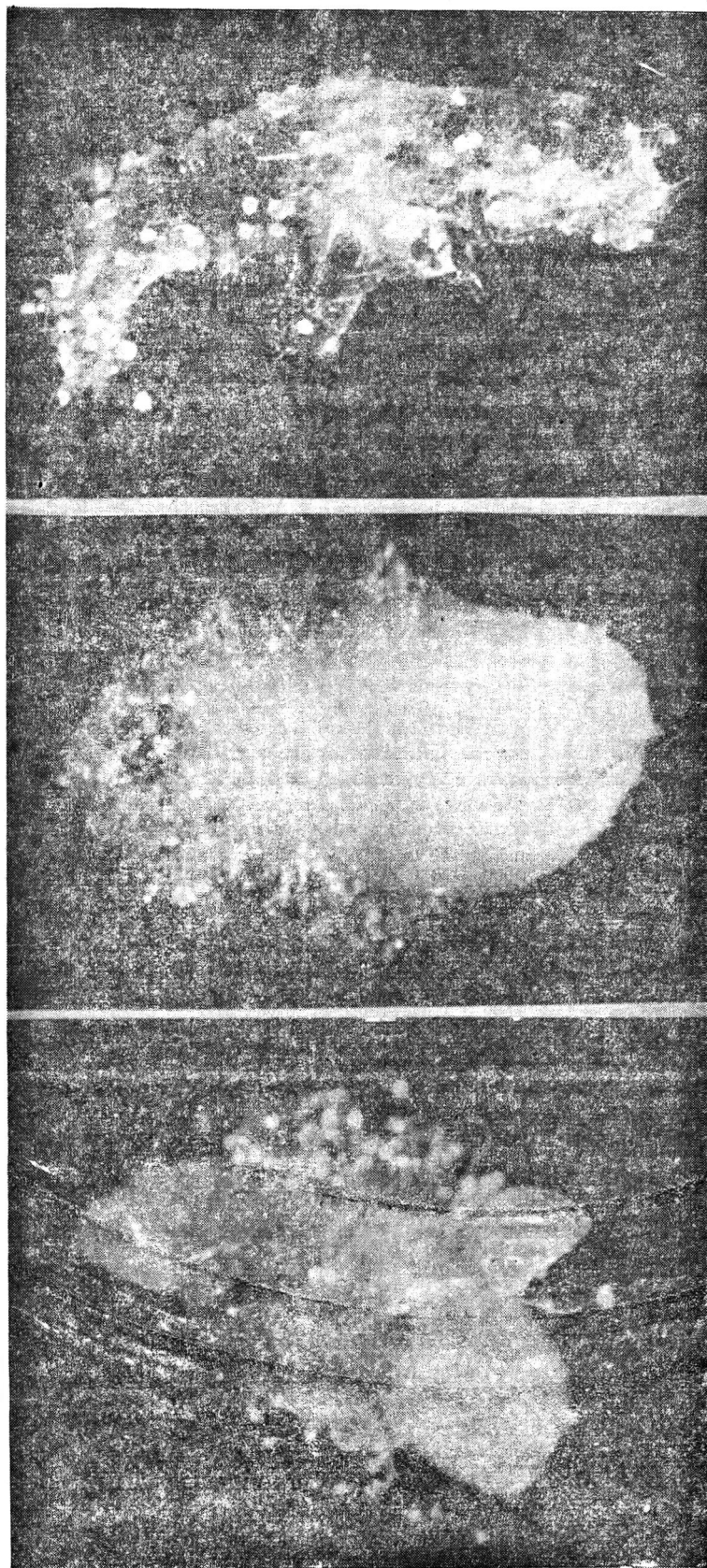


Fig. 1. Internal fungi present in different stages of rice weevil
 1. Adult weevils showing conidial and cleistothecial stages of *Aspergillus ruber*.
 2. Grub 3. Pupa of rice weevil showing growth of *A. flavus* and *A. candidus*

Further work on the preference of rice weevil to storage fungi and their breeding habits with various fungi are under progress.

Acknowledgement

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Quality Characteristics of Three Improved Varieties of Wheat in the Punjab

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Three improved wheat varieties namely WG 357, Kalyansona and WG 377 tested in replicated trials at five different locations in the Punjab were studied for their various quality characteristics. The difference due to varieties were significant for grain moisture, protein, ash, pearling index, Pelschenke value and developing time indicated by mixographs but were non-significant for rest of the characteristics. The differences due to locations were significant for all the characteristics except pearling index, flour moisture, developing angle and width of the curve in mixograph, stability in alveograph and loaf weight. The variety WG 357 had higher protein content, pearling index, Pelschenke value and loaf volume than Kalyansona and WG 377. The quality characteristics of Kalyansona and WG 377 were also satisfactory.

The Punjab is an important wheat growing state in the country. The wheat production has increased from 1.9 million tonnes in 1965-66 to 5-6 million tonnes in 1971-72. The variety *Kalyansona* has occupied about 80 per cent of the total area under wheat in the State. Recently, two new varieties, *WG 357* and *WG 377* have been developed and released for general cultivation in the State. The quality characteristics of these two wheats as compared with those of *Kalyansona* are reported in this paper.

Material and Methods

Three varieties namely *Kalyansona*, *WG 357*, and *WG 377* were grown in comparative trials at five locations (Ludhiana, Gurdaspur, Samrala, Jullundur City and Jullundur Cantt.) in 1970-71. These varieties were sown in the first fortnight of November and the crop was applied the recommended doses of fertilizers (N50, P 25 and K 12 per acre). The normal cultural and irrigation practices were followed¹. For quality analysis, a random sample from the produce of each variety at each location was taken, dried in the sun and kept free of any insect infestation. The samples were analysed for the following quality characteristics.

Moisture, ash and baking characteristics were determined according to the method of AACCS². Nitrogen was estimated by Kjeldahl method³ and the protein was calculated by multiplying it with 5.71.

Pelschenke value (whole wheat-meal fermentation time test) were estimated by the method of Welsh and Normann⁴ and the pearling index was determined according to Taylor *et al.*⁵. Sedimentation test was carried out by the method of Pinkney *et al.*⁶ and Zeleny⁷.

Milling quality (flour recovery percentage): The cleaned sample of wheat grain was tempered to 15 per cent moisture content and kept overnight at room temperature before being milled in a Brabender Quaderumat junior flour mill.

Mixographic characteristics: Mixograms were prepared on a National-Swanson-Working Mixograph using 35 g flour (14 per cent moisture basis) at 65 per cent absorption spring setting tension at 10. Calculations for various mixographic characteristics were made following the methods of Swanson and Johnson⁸ and Mehdi *et al.*⁹

Alveographic characteristics: This test was performed according to the method of Kent Jones and Amos¹⁰.

Results and Discussion

The data on different quality characteristics are given in the Table 1. The grains of all the three improved wheat varieties were of amber colour, those of *WG 357* being bold, attractive and lustrous. The initial moisture content of 11.02 per cent in *WG 377* was significantly higher than the other two varieties. The differences in flour moisture contents were not significant, because the grains were tempered to 15 per cent moisture before milling.

The protein content varied both for whole wheat grains and wheat flour. The mean protein content of whole wheat was higher in *WG 357* (13.44 per cent) as compared to those of *Kalyansona* and *WG 377* (12.28 and 12.94 per cent respectively). The mean

values for wheat flour proteins followed similar pattern.

The difference in the ash content of whole wheat grain as well as wheat flour were rather small. *WG 377* had significantly lower ash content in the wheat grain but significantly higher in the wheat flour than the other two varieties.

The pearling index which is a measure of grain hardness was significantly greater at 5 per cent level in case of *WG 357* as compared to *Kalyansona* and *WG 377*.

The mean Pelschenke value of 101.6 for *WG 357* was significantly higher than the other two varieties (*Kalyansona*, 84.4 min and *WG 377*, 81.6 min). But the sedimentation values did not vary significantly in the three varieties.

Flour recovery percentage was high (72.7 to 74.5) in all the three varieties. The differences were, however, not significant. The mixographic characteristics did not vary significantly except for the mixing

TABLE 1: QUALITY CHARACTERISTICS OF THREE IMPROVED VARIETIES OF WHEAT AND THEIR MEAN VALUES WITH RESPECT TO LOCATION

Characteristic	WG 357	Kalyan Sona	WG 377	C.D. at 5%*	Location				C.D. at 5%	
					Samrala	Jullundur		Gurdaspur		Ludhiana
						City	Cantt			
A. Grain										
Moisture (%)	10.8	10.8	11.0	0.21	10.8	11.3	10.7	10.9	10.60	0.30
Protein (%)	13.4	12.3	12.9	0.42	13.8	12.4	12.7	12.5	13.1	0.59
Ash (%)	1.54	1.58	1.50	0.05	1.52	1.55	1.53	1.49	1.63	0.07
Pearling index	42.5	33.4	33.1	2.27	34.3	36.3	39.0	34.7	37.3	N.S.
Pelschenke value (min)	101.6	84.4	81.6	9.4	88.3	79.0	79.71	95.3	103.7	13.3
B. Flour										
Moisture (%)	13.6	13.2	13.4	N.S.	13.5	12.8	13.7	13.5	13.3	N.S.
Protein (%)	11.4	10.5	10.9	0.54	11.6	10.7	11.2	11.3	10.0	2.01
Ash (%)	0.64	0.64	0.70	0.14	0.60	0.72	0.65	0.63	0.71	0.18
Flour recovery (%)	74.0	72.5	74.5	N.S.	75.2	74.6	77.4	73.0	68.2	4.54
Sedimentation value (ml)	23.1	23.3	19.7	„	22.4	23.8	22.5	24.0	17.5	N.S.
C. Mixographic characteristics										
Developing time (min)	2.34	2.74	2.70	0.42	2.60	3.00	2.23	2.40	2.73	0.76
Height of the peak (cm)	6.74	6.68	7.24	N.S.	7.50	6.23	7.47	6.97	6.27	0.72
Developing angle	33.6	27.0	32.2	„	28.3	25.0	36.3	31.3	35.3	N.S.
Weakening angle	22.8	20.4	25.4	„	25.7	15.0	32.3	21.0	20.0	6.12
Mixing tolerance angle	123.6	130.8	121.6	„	126.0	140.0	109.3	127.7	123.7	14.92
Developing area (cm ²)	19.6	24.0	24.4	„	27.0	22.3	22.3	24.7	17.0	3.83
Width of the curve at peak RM value	0.90	0.80	0.88	„	0.93	0.83	0.80	1.03	0.83	N.S.
D. Alveographic characteristics										
Baking strength (area in cm ²)	24.8	24.6	29.3	N.S.	29.4	22.6	27.2	28.3	26.9	2.86
Stability (height P in mm)	80.6	83.7	102.3	„	100.1	83.2	74.8	97.3	92.3	N.S.
Extensibility (length L in mm)	57.5	55.5	52.5	„	51.3	35.7	79.3	54.3	55.2	12.63
L/P ratio	0.77	0.67	0.54	„	0.53	0.38	1.09	0.62	0.65	1.00
E. Baking characteristics										
Loaf weight (g)	137.0	140.0	141.0	N.S.	139.3	142.0	137.3	138.7	139.3	N.S.
Loaf volume (ml)	487.5	457.5	456.2	„	471.7	440.0	503.3	453.3	467.3	34.75

* N.S.: Not significant

time which was significantly lower in case of *WG 357* (2.34 min) than the other two varieties (*Kalyansona*, 2.74 and *WG 377*, 2.70 min).

No significant differences were observed in alveographic characteristics and the loaf volumes.

The differences due to locations were significant for all the characters studied except for pearling index, dough stability (P value) and baking quality.

The percentage of grain and flour protein of wheat grown was at Samrala significantly higher than at other locations. The percentage of ash both in the grain and the flour was significantly less at Gurdaspur than at other locations. The Pelshenke value was significantly higher at Ludhiana than other locations except Gurdaspur whereas the flour recovery was the lowest at this place. Probably on account of higher protein content at Samrala the values of height of the peak, mixing tolerance and developing area in the mixographs and baking strength as determined in the alveograph were also higher at this location than others. The values for developing time and mixing tolerance were maximum at Jullundur city but the value of weakening angle was the lowest at this place. The value of extensibility of the dough and L/P

ratio were the highest at Jullundur Cantt than other locations and correspondingly the loaf volume was also the maximum at this location.

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RESEARCH NOTES

IDENTIFICATION OF BEEF FROM OTHER MEATS BY DISC ELECTROPHORESIS METHOD

The identification of beef from sheep and goat meat employing disc electrophoresis on acrylamide gels is described. Beef can be identified in fresh condition using the myoglobin pattern of bands, which was found to give totally three myoglobin bands—one major and two minor bands, whereas goat and sheep meat gave only two bands, one major and the other minor band.

Cook and Sturgeon¹ identified horse meat, pork and beef by the use of gas chromatography on the unsaponifiable matter, first fractionated by column chromatography. Hill *et al.*² applied electrophoresis using agar gel as the supporting medium in the identification of fish species. Thompson³ employed starch gel zone electrophoresis for identification of animal species. Mackie⁴ and Chu⁵ used disc electrophoresis for the identification of fish species, while Cowie⁶ employed thin-slab polyacrylamide gel electrophoresis for the same purpose. Hoyem and Thorson⁷ identified species of meat based upon the migration of the myoglobin bands.

The present investigation was undertaken to detect presence of beef because religious restrictions prohibit its use by a large section of the population in India and, at the same time, other meats are adulterated with beef because beef is much cheaper as compared to other meats.

Representative samples of fresh meat from cattle, goat and sheep were collected at slaughter house. Five gram of meat freed from fatty tissue was homogenized with 5 ml of distilled water and the homogenate was centrifuged at about 3000 rpm for 15 min. Aliquots of the supernatant were used for disc electrophoresis, according to the method described by Davis⁸ with slight modifications. The disc electrophoresis was conducted at room temperature (26-28°C) by passing a current of 5 milliamperes and 230 volts per tube for a period of 50 min. The size of polyacrylamide column was 6.5 cm long and 0.55 cm diameter. The gels (7.5 per cent polyacrylamide) were prepared by the same method as given by Davis; the pH of the buffer solution (tris-glycine) was 8.3.

Six trials were conducted for each kind of meat. Brownish myoglobin bands can be easily recognised

TABLE 1. R_b VALUES OF MYOGLOBIN BANDS IN DIFFERENT MEATS

Species of meat	Concentration (μ l)	R_b values*	
		Major band	Minor bands
Goat	100	0.72	— 0.81
"	150	0.75	— 0.80
Beef	100	0.72	0.16 0.80
"	150	0.72	— 0.81
Sheep	100	0.73	— 0.80
"	150	0.70	— 0.80

*Average of six trials for each concentration

during and after electrophoresis without staining with Amido Black. The migration rates (R_b values) of myoglobin bands compared with the migration rate of bromophenol blue (tracking dye) are given in the Table 1. Very little difference in R_b values of major and minor bands was observed in different meats. The protein concentration of the extract was standardized before electrophoresis and even by increasing the amount of protein loaded into the gel, it was found to have no effect on the R_b values of the myoglobin bands.

Two myoglobin bands were common to all the three species of meats and the R_b values of each type of band was almost same in the different meats. The R_b values of the major myoglobin band ranged between 0.70 to 0.75 and that of minor band between 0.79 to 0.82. In the case of beef, another extra minor band appeared in addition to the two bands, which are common to all the meats. This new band was found on the top of the major band with an R_b value ranging between 0.15 to 0.18, which was found specific to beef only.

These results indicate that beef can be identified in fresh condition using the myoglobin pattern of bands, which was found to give totally 3 myoglobin bands—one major and two minor whereas goat and sheep meat gave only two bands—one major and another minor.

We are thankful to Mr D. Narayana Rao for his valuable advice and assistance in this work.

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TOXICITY OF ETHYLENE OXIDE TO THE ADULTS OF *TYROPHAGUS* *PUTRESCENTIAE* (SCHRANK) ACARINA, ACARIDAE

Ethylene oxide is an effective fumigant for control of *T. putrescentiae*.

Toxicity of ethylene dibromide, methyl bromide and phosphine to the storage mite *Tyrophagus putrescentiae* (Schrank) has been reported^{1,4}. No work however has been reported on the toxicity of ethylene oxide (EO), another important fumigant to *T. putrescentiae*. Unlike methyl bromide, EO does not impart any taint to the treated foodstuff.

The mites were reared as reported earlier⁴. There were four dosages with 5 replicates for each dosage. Thirty to forty two mites were exposed in special glass cells inside 2.5 litre glass desiccators. Ethylene oxide was pressure filled into an all glass 20 ml gas-tight syringe from a 500 g capacity stainless steel lecture-bottle with a fine-control needle valve and the required dose injected through a septum cap on the desiccator lid under a slightly reduced pressure. Atmospheric pressure was restored immediately by opening the stop-cock of the desiccator. After an exposure period of 2 hr the mites were held in plastic humidity boxes for 48 hr before recording the mortalities, which was done under a binocular microscope.

The mortality responses against the dosages were statistically evaluated by the method of Litchfield and Wilcoxon². The data are shown in Table 1.

The results as demonstrated by the LD₅₀ (3.8 mg/l) and LD₉₅ (7.6 mg/l) values show that EO is more toxic than either methyl bromide or phosphine to *T. putrescentiae* adults, but less toxic than ethylene dibromide. Corresponding LD₅₀ of methyl bromide, phosphine and ethylene dibromide were 13.8, 5.1 and 0.3 mg/l respectively⁴.

TABLE 1. TOXICITY OF ETHYLENE OXIDE TO *Tyrophagus putrescentiae* ADULTS

		At 95% Confidence limits	
		Upper	Lower
LD ₅₀	3.8 mg/l	3.9	3.3
LD ₉₅	7.6 mg/l	9.5	6.1
Slope function	1.5	1.7	1.3

Temp: 26±1°C; R. H. 80%

Ethylene oxide is well known for its microbicidal effect apart from its toxicity to insects and hence is used for fumigation of spices, fruits, etc.³ It has a higher vapour pressure (1095 mm Hg at 25°C) than ethylene dibromide (15 mm Hg at 25°C) and hence penetrates better into commodities. This study has shown that EO holds out promise as an effective fumigant for the control of *T. putrescentiae* infesting food commodities in preference to either methyl bromide or phosphine.

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DRAINAGE THROUGH COFFEE EXTRACT FOAM

Some investigations by photography of drainage through foams obtained from coffee extract are reported. Various theoretical models have been put forth by different workers to describe the interstitial flow for specific type of foams. The theoretical model put forth by Brady and Ross, for foam drainage through thin vertical channels, bounded by non-rigid and parallel planes has been found to be valid in this study.

Essentially all the current know-how on foam preparation is empirical and learned by trial and error¹⁻³. No definite conclusions can be arrived at regarding the relationship between the geometric structure of foams and other physical parameters, on the basis of the existing state of knowledge in this field.⁴

Several half empirical or frankly empirical correlations between V_0 (the initial volume of liquid in foam) and V , (the volume of liquid drained out in time, t) have been suggested¹. Liquid flow in circular capillaries was studied theoretically and experimentally⁵. Mathematical expressions for the drainage rate (dV/dt) in terms of V , V_0 , and t , have been assembled by Bikerman¹. The other theoretical model for drainage makes a number of simplifying assumptions such as interstitial flow through vertical channels⁶ with rigid walls. The channels might either be substantially circular^{6,7,8} or bounded by parallel planes⁹⁻¹¹.

About 700 ml of coffee extract were whipped into a foam and the different variables like soluble solids content (20-50 per cent), different foaming agents such as sodium carboxy methyl cellulose (1-3 per cent), guar gum (1-3 per cent) and glycerylmonostearate (1-5 per cent) and their various combinations, temperature variation (10-50°C), agitator speed (500-1000 rpm) and time of agitation (15-60 min) on foam stability were studied and the foam formed under optimum conditions was selected for the photographic study of drainage model for this particular case.

For a typical experiment, foam was formed by stirring about 700 ml of a coffee extract containing 30 per cent soluble solids and 3 per cent glyceryl monostearate (GMS), for 30 min at a speed of 650 rpm and at 30°C. Stirring was stopped and foam was transferred by a spatula into the square glass vessel and was immediately transferred to the oven at 50°C. Photographs of the foam were taken at regular intervals till 140 min. The photographs were developed, enlarged and the height of the foam and liquid settled out in the glass vessel, at a particular time was recorded.

A total of 20 observations were made. It was observed that till 70 to 80 min the amount of liquid draining through the foam is more and afterwards the amount of liquid draining through the foam is very small and the foam may be considered stable and used for drying. A plot of t vs. $1/(V_0 - V)^2$, is a straight line. It means that the mathematical expression for the volume of liquid, V , exuded through the foam during time t is

$$1/(V_0 - V)^2 - 1/V_0^2 = 2 kt \quad (a)$$

and drainage rate is given by:

$$dV/dt = k.(V_0 - V)^3 \quad (b)$$

For the stable foam of coffee extract so prepared, under given conditions of foaming at 50°C i.e. oven temperature,

$$k = 46.6 \times 10^{-6} \quad (c)$$

The experimental data fits the empirical equation (a) which makes drainage a third order reaction. It is valid for drainage proper, without bursting of bubbles. Thus the mechanism for drainage studied seems to be in agreement with the one proposed by Brady and Ross¹⁰.

Drying studies reveal that foam-mat drying of this foam after 30 min yields a fairly good product which is porous, can be easily reconstituted and fortified with flavour.¹²

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FRACTIONATION OF FATTY ACIDS OF SARDINE OIL BY UREA ADDUCT METHOD

An efficient method for fractionation of fatty acids according to degree of unsaturation is described.

Fatty acids of the sardine oil obtained from oil sardine (*Sardinella longiceps*) is a mixture of saturated to highly unsaturated fatty acids having carbon chain length of C_{14} to C_{24} . Gopakumar *et al*¹, have used gas liquid chromatography for qualitative and quantitative analysis of the components of fatty acids in lipids of oil sardine wherein oil was fractionated into phospholipids and triglycerides and then converted into methyl esters of fatty acids. Recently Kotwal *et al*², have split the oil and then distilled under vacuum to get two fractions; one is relatively

less unsaturated C₁₈ fraction and the other highly unsaturated fraction. These two fractions were subjected to gas liquid chromatography for further study of the fatty acids. Urea adduct method has not yet been used for fatty acids obtained from sardine oil. Hence an attempt has been made to apply this method for fractionating fatty acids of the sardine oil also.

Ahmed *et al*³, have tried segregation of fatty acids of codliver oil by urea inclusion method but the method followed by Mehta⁴⁻⁵ for fractionation of fatty acids of mustard oil and hydrogenated vegetable oil was found to be more convenient. Fatty acids of the sardine oil were prepared by alkali hydrolysis and subjected to fractionation by urea inclusion method. Mehta in his procedure has taken 50 g fatty acids, 10 g urea and 75 ml methanol, whereas in the procedure adopted here instead of 75 ml of methanol, 150 ml methanol was taken along with 12.5g urea.

Sardine oil weighing 460 g (500 ml) was taken in a pyrex beaker and was heated on water bath and stirred vigorously after adding 20 per cent caustic soda solution in small quantities at a time, till homogeneous solution was formed and was alkaline to litmus paper. To this enough quantity of common salt was added and soap was precipitated which was filtered through Buchner funnel. Soap was hydrolysed to fatty acids by using dilute sulphuric acid. Fatty acids were washed with water to make free from mineral acid and dried over anhydrous sodium sulphate. Yield was 81.95 per cent.

For fractionation of fatty acids, 150 ml methanol was added to 50 g fatty acids and then 12.5 g of urea was added and the mixture was warmed on water bath till homogeneous solution was obtained. This was kept aside overnight and solid formed was filtered through sintered glass funnel. Adduct was scraped into a beaker and was decomposed by hot water. Fatty acids liberated were extracted by petroleum ether and solvent was removed under reduced pressure and dried over anhydrous sodium sulphate.

On the first day when 12.5 g urea was added to the mixture of fatty acids and methanol there was no adduct formation but on the second day when additional 12.5 g of urea was added there was adduct which was separated as mentioned above. To the filtrate obtained once again 12.5 g urea was added and the procedure was repeated till 7 adducts were obtained. After 7th adduct, the filtrate was heated with 12.5 g urea and the mixture was kept overnight in the refrigerator (8-10°C) and 8th adduct formed was separated, and the remaining alcoholic solution was extracted by petroleum ether to separate the fatty acids which remained unreacted with urea.

TABLE 1. PER CENT YIELD, IODINE VALUE AND PHYSICAL PROPERTIES

Sl. No.	Description	Yield %	Iodine value	Condition at R.T.
1.	Fatty acids	—	150.3	Semi solid
2.	Adduct (1)	7.76	30.6	Solid
3.	Adduct (2)	12.42	48.3	„
4.	Adduct (3)	10.98	54.4	„
5.	Adduct (4)	13.34	90.0	„
6.	Adduct (5)	16.40	156.4	Liquid
7.	Adduct (6)	12.06	163.1	„
8.	Adduct (7)	1.20	—	„
9.	Adduct (8)	4.06	205.8	„
10.	Filtrate	10.52	304.9	„

R.T. = 30—35°C.

Per cent yield, iodine value and physical properties are given in Table 1.

This method, in general, is found to be very efficient in fractionating the fatty acids according to the degree of unsaturation. The iodine value of the fatty acids of the oil was 150.3, which when fractionated into 8 groups, the iodine value of each group ranged from 30.56 to 304.9. The first four adducts were solid at room temperature and had the iodine value 30.56, 48.30, 54.41 and 89.88 respectively. These may be considered as saturated fatty acids and their percentage yield in this particular oil was 44.5 per cent. Rest of the adducts were highly unsaturated and their iodine values were 156.4, 163.1, 205.8 and 304.9 respectively.

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A SPOT TEST FOR DETECTION OF ARGEMONE MEXICANA SEEDS IN MUSTARD SEEDS

A simple chemical test involving the detection of oil adulteration of mustard seeds is given

Mustard seeds are generally adulterated with the toxic seeds of *Argemone mexicana* plant which grows

wildly and abundantly. Although the argemone seed is similar in size and colour to that of mustard seed, it can be differentiated when examined under a magnifying glass by its roughness of surface in comparison with mustard seeds, which are smooth. Sometimes, further evidence based on chemical methods are needed when samples containing contaminated argemone seeds do not suffice for conventional chemical tests. The author suggests in this present communication a simple method which detects even a single seed of *Argemone mexicana* isolated from a suspected bag of mustard seed.

The method consists of placing the suspected seed of *Argemone mexicana* in between the two halves of a half folded filter paper Whatman No. I. The seed is pressed by means of a paper weight which bursts with a sound, leaving two stains of oil on the two sides of the filter paper.

One spot is soaked with concentrated HCl and tested with Dragendorff's reagent¹, the second spot with concentrated nitric acid² (A.R.). The spot tested with Dragendorff's reagent shows an orange red colour, while the spot tested with nitric acid shows an orange yellow to crimson colour due to alkaloids present in *Argemone mexicana* seeds. The positive results in both the tests³ performed simultaneously is confirmatory for the presence of *Argemone mexicana* seeds in mustard seeds. Mustard seeds do not give similar reactions.

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FEASIBILITY OF USING GERBER FAT TEST FOR RAPID ESTIMATION OF FAT IN KHOA

The Gerber method adopted for cheese can be utilised for routine fat estimation for *khoa* since the results are almost in close agreement with the Mojonnier gravimetric fat estimation method.

Conventional gravimetric method like Roesé—Gottlieb and Mojonnier¹ for determination of fat in *Khoa* are quite expensive and time consuming. It

was therefore, thought of investigating the possibility of using Gerber fat test commonly adopted for milk and some of the dairy products, for rapid estimation of fat in *khoa*.

Cow and Buffalo milk produced at National Dairy Research Institute, Karnal was used for these experiments. The bulk cow and buffalo milk was used as such separately for *khoa* making. In other lots, the same cow and buffalo milk was homogenised at 60°C at 200 kg/sq cm with Rannie homogenizer prior to preparation of *khoa*. *Khoa* was prepared by the method recommended by De and Ray².

Khoa from cow and buffalo milk, both homogenised and unhomogenised was weighed separately in 3 g quantity in Gerber cheese butyrometer. Eight ml of distilled water was added to each of the butyrometer. This was followed by very slow addition of Gerber sulphuric acid in 10 ml quantity (rapid addition results in charring of the contents). Next, 1 ml of amylalcohol was added to each butyrometer and the butyrometers were held in waterbath at 65°C for 30 min. Later, the butyrometers were centrifuged in Gerber centrifuge for 10 min and again held in waterbath at 65°C for 5 min before noting the readings. Simultaneously fat percentage was also determined on the corresponding lots of *khoa* samples by Mojonnier method.

From Table 1, it is clear that the Gerber method shows slightly lower results than the Mojonnier method of fat estimation both for homogenised and unhomogenised cow and buffalo milk *khoa*. Only one sample of unhomogenised buffalo milk *khoa* showed higher value of 0.3 per cent by Gerber method as compared to value obtained by Mojonnier method which may be attributed to experimental error. Except for two samples, difference between Gerber and Mojonnier methods was less than 1.00 per cent. The extreme difference by the two methods was only 1.4 per cent.

Homogenised cow and buffalo milk *khoa* showed lower fat values than the corresponding *khoa* prepared

TABLE 1. COMPARISON OF GERBER AND MOJONNIER FAT DETERMINATION METHOD ON HOMOGENISED AND UNHOMOGENISED COW AND BUFFALO MILK KHOA

Method	Cow milk	<i>Khoa</i>	Buffalo milk	<i>Khoa</i>
	(Fat%)	(Fat%)	(Fat%)	(Fat%)
	Unhomo-	Homoge-	Unhomo-	Homoge-
	genised	nised	genised	nised
Mojonnier	26.7	26.0	27.8	26.5
Gerber	26.5	25.0	27.0	26.0
Mojonnier	25.9	23.7	38.2	37.0
Gerber	25.5	23.0	38.5	36.5
Mojonnier	30.5	28.6	35.4	33.6
Gerber	30.0	27.5	34.0	33.0

from unhomogenised milk both by Mojonnier and Gerber method. The extreme difference was 2.5 per cent between homogenised and unhomogenised milk *khoa* fat.

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EFFECT OF CERTAIN PRESERVATIVES ON FOOD SAMPLES PRESERVED FOR ANALYSIS

Formalin was found to be an effective preservative for food samples used for analysis

Preservatives are generally used in food during its preparation to avoid spoilage. The food samples collected by food inspectors for analysis are required to be kept for a long time. The time varies from 3 to 6 months and sometimes even longer. The food samples, especially some prepared foods when thus reach the laboratory for analysis, are found to

be decomposed by bacteria or mold. Such samples are declared unfit for analysis and rejected.

So, for the proper maintenance of the perishable food samples, use of a suitable preservative is a must. For milk samples, addition of formalin is a common practice.

In this study, the preservative effect of several preservatives on food samples kept at room temperature is compared. The samples examined are bread, *channa*, *khoa* and cheese which are decomposed at a fast rate.

Bread, cheese, *channa* and *khoa* samples were collected from the market. Each sample was divided into seven parts. Each part was sprayed with different preservatives and bottled in a dry glass jar immediately after spraying. Then the bottles were stored at room temperature (25°-37°C) and examined visually every day for mold growth, change of color, production of oily substance, etc. Preservatives used are listed in Table 1. Results are summarised in Table 2.

TABLE 1. CONCENTRATIONS OF PRESERVATIVES USED IN FOODS

Preservatives	Concentration
Sorbic acid	0.5 per cent (alcoholic)
Acetic acid	4% (aqueous)
Formalin	as it is
Formalin	4%
Mercuric chloride	0.5% (alcoholic)
Sodium benzoate	0.5% (aqueous)
Hydrogen peroxide	3% (aqueous)

TABLE 2. PRESERVATIVE EFFECT OF DIFFERENT CHEMICALS

Preservative used	Keeping quality			
	Bread	<i>Channa</i>	<i>Khoa</i>	Cheese
Sorbic acid	>10 months	1 month	2 days	1½ months
Acetic acid	20 days	5 months	7 days	7 days
Formalin	>10 months	>10 months	>10 months	>10 months
Formalin 4%	5 months	4 months	9 days	7 days
Mercuric chloride	>10 months	10 days	5 months*	About 2 months
Sodium benzoate	1 month	20 days	3 days	3 months
Hydrogen peroxide	4 days	2 days	3 days	7 days
Dried bread (at 100°C for 4 hr.)	>10 months			

* After that the colour changed to light brown which lasted for more than 10 months.

From the experiment, formalin (0.1 ml per 25 g) is found to be the best preservative for food samples. Dried bread does not require any preservative. So, surface spraying of the collected samples with formalin and immediate bottling will preserve the samples for a considerable length of time.

Central Food Laboratory
Calcutta
15 May 1973

SMT G. MUKHERJEE
T. V. MATHEW

ANTI-OXIDANT EFFECT OF A SPICE MIXTURE ON SARDINE OIL

The antioxidant effect of a spice mixture and its oleoresin on sardine oil was studied in model system. It was found that both of them have antioxidant effect, the latter being more effective.

Non-defatted fish flour for human consumption with 5-6 per cent fat content, prepared from oil sardine fish (*Sardinella longiceps*) when mixed with a spice mixture was found¹ to be comparatively free from rancid odour and flavour both initially and also during storage for about six months at 28-33°C. Against this background and in the absence of any reported study on the efficacy of spices in retarding the development of rancidity in fish oils, the antioxidant effect of the spice mixture with respect to sardine oil was studied.

Good quality spices namely cinnamon leaves, cinnamon bark, red chillies, turmeric, dried ginger, black pepper, clove, cumin and mustard seeds were powdered and mixed in equal quantities. For preparing the oleoresin, 5 g of this spice mixture was taken in 100 ml of 95 per cent ethyl alcohol, heated to boiling, kept overnight at room temperature, filtered and washed thrice with 20 ml alcohol each time. The solvent was removed by distillation. The oleoresin thus extracted was 15.7 per cent of the weight of the spice mixture taken.

Sardine oil was prepared according to Sen and Padival². Before using it for these experiments it was heated to 212°C for five minutes to destroy built up peroxides.

The spice mixture at 0.5 per cent and 2.0 per cent levels by weight of the sardine oil was taken in cuvetts containing the oil. In another set, the same quantities of spice mixture was added to sardine oil, warmed at 40-50°C for 3 hr, filtered and aliquots of filtered oil was taken in cuvetts for the study. In a third set only the oleoresin at different levels were tested.

The weighing method of Olcott and Einset³ as modified by Sen and Padival² for measuring autoxidation of the oil at 50°C was followed. The time

required to have 0.5 per cent increase in weight, worked out graphically, was taken as the induction period.

For corroborative evidence, thiobarbituric acid (TBA) value⁴ and peroxide value⁵ of the samples stored at 50°C with 1 per cent oleoresin were determined.

In each set of experiments butylated hydroxy anisole (BHA), 0.02 per cent along with citric acid, 0.001 per cent was used as a reference antioxidant.

The induction periods of the systems with spice mixture and its oleoresin at different levels are reported in Tables 1 and 2. At 0.5 per cent level the antioxidant effect of the spice mixture was very limited but was more at 2.0 per cent level. The components for antioxidant effect appear to be soluble in sardine oil, for the induction periods either with (i) oil containing the spice mixture or (ii) oil treated with spice mixture and filtered, were more or less the same.

The oleoresin constituted 15.7 per cent by weight of the spice mixture. However, 1 per cent of the oleoresin in sardine oil gave an induction period of 207 hr as compared with an equivalent quantity of the spice mixture which was calculated to be 162 hr. Thus the oleoresin appears to be more effective than the spice mixture. This could be due to the spice mixture containing pro-oxidants insoluble in alcohol.

TABLE 1. INDUCTION PERIOD OF SARDINE OIL WITH SPICE MIXTURE AND ITS ALCOHOLIC EXTRACT

Description	Induction period (hr)
Sardine oil	108
" +BHA+CA	186
" +0.5 % Spice mixture	113
" +2.0 % Spice mixture	123
" +0.5 % Spice mixture, heated in oil	114
" +2.0 % " "	124
" +0.1 % oleoresin	140
" +1.0 % oleoresin	207

CA=Citric acid

TABLE 2. INDUCTION PERIOD OF SARDINE OIL WITH OLEORESIN OF SPICE MIXTURE

Description	Induction period (hr)
Sardine oil	74
" +BHA+CA	122
" +0.1 % oleoresin	92
" +0.3 % oleoresin	122
" +0.5 % oleoresin	136
" +1.0 % oleoresin	170

TABLE 3. PEROXIDE VALUE AND TBA VALUES OF THE SARDINE OIL MIXED WITH BHA AND OLEORESIN OF SPICE MIXTURE

Period of storage (days)	Sardine oil		S.O+0.02% BHA		S.O+1.0% oleoresin	
	PV	TBA	PV	TBA	PV	TBA
1	0.02	1.37	—	—	—	—
2	0.03	31.0	0.03	20.0	0.03	32.7
3	0.07	47.8	0.07	35.8	0.04	12.6
4	0.16	251.1	0.09	117.1	0.09	37.5
5	0.24	323.3	0.17	152.0	0.12	95.1
6	0.32	465.8	0.32	214.8	0.12	205.1
7	0.68	958.3	0.48	562.0	0.25	629.2
8	1.11	879.6	0.53	539.5	0.38	601.2

* P.V.=O.D./mg of oil TBA=TBA/kg of oil

It may also be seen that 1 per cent oleoresin and 0.02 per cent BHA have equivalent effect.

The changes in peroxide and TBA values determined daily with oil samples containing the oleoresin and stored at 50°C shown in Table 3 confirm the above trend of results.

From this study it appears that the spice mixture used has antioxidant action on sardine oil which is considerably more in the oleoresin derived from it.

CFTRI Fish Technology
Experiment Station
Mangalore
9 May 1973

G. D. REVANKAR
D. P. SEN

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BOOK REVIEWS

Food For Peace: by ROBERT G. STANLEY, Gordon and Breach, Science Publishers, Inc. New York, Paris and London, 1973, pp. xiii+355, Price £4-15.

The book aims at clarifying one aspect of the U.S. foreign aid program by evaluating the role and reviewing the history of U.S. agricultural commodities in world development. It analyses current distribution methods, problems and potential future programs involving U.S. agricultural exports under donation or concessional sales agreements. The book discusses the origin, functions, successes and failures of P.L. 480 which though initially a vehicle to help the United States dispose of its agricultural surpluses is at present much broader in scope covering aspects like fertilizers, tools, seeds, education and means of population control. Its economic consequences both in U.S.A. and abroad are far reaching and diverse covering stability of local currencies, foreign exchange reserves, inflation, commodity prices, incentives to increase agricultural productivity, population control and general overall economic growth.

Because of disposal of its surplus agricultural produce, savings of storage space, dollar savings in provisioning for its diplomatic missions abroad and dollar earnings in freight charges, P. L. 480 arrangements certainly conferred distinct advantages to U.S.A. At the same time, to the extent it made developing countries slack in efforts to attain self-sufficiency in food, the arrangement was not beneficial to them.

The book is a good attempt to review several aspects of world food problem with emphasis of U.S. role in it.

B. S. BHATIA

The Proceedings of the 5th International Colloquium on the Chemistry of Coffee by Association scientifique internationale du café (ASIC) 34, rue des Renaudes, 75017 Paris.

In this Colloquium participants from Germany, Austria, Belgium, Brazil, Columbia, Spain, U.K., France, U.S.A., Italy, Norway, Portugal, Sweden, Switzerland and other countries took part and the papers read have been documented under the heads, —Information and Documents, Methods of Analysis and Chemical Composition, Chemistry and Techno-

logy, Organoleptic and Physiological aspects. Details of 50 papers presented on the above colloquium are presented in this compilation.

The following are some of the subjects covered under methods of analysis and chemical composition. Chlorogenic acid and substitutes, analysis of some characteristic of raw coffee from Angola, mineral constituents of coffee in Angola, caffeine content of Mozambique coffee, analytical studies of coffee cultivars in Angola, variations in coffee ash content with roasting, influence of roasting on sulphur amino acids and amino acids in coffee. In one paper caffeine chlorogenate complex of coffee has been studied using NMR. Hydrolysis coffee grounds has been found to be a good media for culturing of fungi.

A study of the coffee drying floors indicated that ripeness of cherry and drying method are more important than types of drying floors used. The chlorophyll content of integument has been related to the final raw bean colour in coffee. The study on fermentation using different methods indicated that alkali treatment and soaking under sodium metabisulphite was found to be best followed by mechanical removal and under-water treatment in the case of robusta coffee. The loss of dry matter from parchment during processing of coffee has been reported to be 1 per cent in 48 hours dry-fermentation. The loss in under water soaking is as much as in dry fermentation.

In one of the papers the quality has been correlated with physical properties of roasted coffee. An interesting paper on histological studies on structure of coffee during roasting has been presented. It was reported that pressure roasting of coffee gives less roasting loss and higher water extract.

A series of papers are presented on physiological aspects of coffee consumption in vascular disease, diabetes mellitus and effect of chlorogenic acid on the growth rate. In one paper it was shown that etiology of cancer is not related to caffeine. No significant variation was noticed in free fatty acid and free glycerol, triglycerides and blood glucose with and without caffeine. Only in high doses of caffeine over 1000 mg in 10 hours seem to influence the metabolism. No clear indication of metabolic rearrangement due to coffee ingestion has been indicated.

In one of the papers the presence of carboxylic acid hydrotryptamides in surface layer has been reported and it was concluded that the content of the above is an indication of the freshness of coffee bean.

From the above it can be seen that there has been a considerable research activity connected with coffee. The Organisers-Association Scientifique Internationale du Cafe, Paris should be congratulated for arranging such an International gathering and presenting useful information on various aspects of coffee research that are being undertaken in different parts of the world.

This publication will be of great interest to all workers in the various coffee research institutes.

C. P. NATARAJAN

Evaluation of Certain Food Additives and the Contaminants Mercury, Lead, and Cadmium: Sixteenth Report of the Joint FAO/WHO Expert Committee on Food Additives. World Health Organization Technical Report Series, 1972, No. 505; 32 pages. price: 40p, \$1.00, Sw. fr. 4.-. French, Russian, and Spanish edition in preparation. Available through WHO Regional Office, Ring Road, New Delhi.

The contamination of food by certain metals is causing increasing concern to health authorities in many parts of the world. This latest report of the Joint FAO/WHO Expert Committee on Food Additives deals with the problems of contamination by mercury, lead, and cadmium. These are major pollutants, and the report points out that if pollution with these metals is allowed to continue it is likely to result in the loss of large sources of food in the foreseeable future.

Taking each of the metals in turn, the report examines the sources from which it may be derived, the levels in different foods, and the potential health hazards. It takes into account significant levels in other media, such as air and water, but occupational exposure is not discussed except in a few special cases.

For a variety of reasons, which are explained in the report, the Committee considers it inappropriate to attempt to set acceptable daily intakes for heavy metals; instead it allocates a provisional tolerable weekly intake for each of the metals considered. The intakes were set on a weekly basis because these metals accumulate in the body and because of their uneven distribution in the daily diet. In view of the paucity of data on the possible health effects

of the metals at the levels considered, figures could be established.

Monographs containing summary biological data and toxicological information with comments on levels of the metals and methods for their analysis are published separately.

The report also considers benzo(a)pyrene, amaranth and reviews the use of sodium benzoate and octyl gallate in the tea industry in the light of new evidence which casts doubt on their safety. The acceptable daily intake recommended for amaranth earlier was replaced by a smaller, temporary acceptable daily intake, pending a review of additional data. It is recommended that octyl gallate should not be used in beverages and that diethylpyrocarbonate should be used only in soft drinks and under specified conditions. In addition, a specification was prepared for caramel colours (ammonia process) setting limits for the impurity 4-methylimidazole.

1971 Evaluation of Some Pesticide Residues in Food, Geneva World Health Organization, 1972 (WHO Pesticide Residues Series, No. 1), 345 pages. Price: £1.90, \$4.50, Sw. fr. 15.-. French edition in preparation. Available through WHO Regional Office, Ring Road, New Delhi.

This first volume in the *WHO Pesticide Residues Series* contains, in the form of monographs, the evaluations prepared by the 1971 Joint Meeting of the FAO Working Party of Experts on Pesticide Residues. The pesticides considered are: chlordane, dieldrin, chlorfenvinphos, 2,4-D, endosulfan, fenitrothion, omethoate, thiabendazole, trichlorfon, trichlorfonate, carbon disulfide, carbon tetrachloride, 1,2-dichloroethane, ethylene oxide, hydrogen phosphide, and methyl bromide.

Each monograph contains, as appropriate, information on the identity of the pesticide, summaries of biochemical and toxicological studies, a toxicological evaluation, an account of residues in food and their evaluation, recommendations for tolerances, temporary tolerances or practical residue limits, and bibliographical references. Annexes to the volume include an index to documentation and a summary of recommendations concerning acceptable daily intakes; tolerances and practical residue limits, as of November 1971; the 1971 additions and amendments to the 1967 glossary last published in its entirety in the report of the 1969 joint meeting (*Wld Hlth Org. techn. Rep. Ser.*, 1970, No. 58); an excerpt on fumigants from the 1971 report; and an evaluation of lindane.

Physics and Chemistry of the Sugar Beet as the Basis of Processing Methods, by Vukov, K., (Translated into German by Dr Alfred Falvay). Akademiai Kiado, Budapest, 1972, pp. 458.

This monograph represents a compilation of the author's researches which began nearly twenty years ago in a Hungarian sugar factory, and were later continued in the Research Institute for the Sugar Industry at Budapest.

In the first part of the book, the most important physical and chemical properties of the sugar beet have been selected after a statistical evaluation, then defined unambiguously, and measured by modern methods. The data so obtained have been used to characterize the technical quality of the sugar beet, and to work out (in the third part) the most appropriate processing technology for any given quality of beet.

In the second part of the book, the influence of various varietal and environmental factors on the physical and chemical properties of the sugar beet has been described in detail. Information has been collected from the technical literature on the different climatic and ecological characteristics of different areas of cultivation, as well as prevalent agricultural and storage practices, and the effect of all these on a variety of parameters such as resistance of cutting, modulus of elasticity, diffusion constant for saccharose, beet yield and sugar content, mineral substances, nitrogen compounds particularly amino-nitrogen

content, invert sugar content, content of other components, non-sugar content in raw beet juice, and pith content.

The third part of the book describes the various technological operations, and how they can be adjusted to maximize sugar recovery. The author has also worked out the effect of size of the apparatus on sugar recovery, and how to arrive at the optimal size in each case. The fourth part is really a series of appendices describing eleven little known methods of measuring the various physical and chemical properties of sugar beet. A list of 1349 literature references, another list of symbols, and a subject index complete the book.

Though the author himself would not like to claim that his book is a compendium of industrial sugar chemistry or sugar technology, still, he has succeeded in covering all the technical information that has accumulated in the past twenty years, and presents new aspects and inter-relationships that have contributed so much to the recent advances of beet sugar technology. The book, therefore, should be of great help, as regards both theory and practice, to all those whose work is connected with the quality of sugar beets, viz., plant breeders, beet growers, chemists, technologists, processing engineers, designers, etc.

So far as India is concerned, this book will arouse only a limited interest, because of the very small area under sugar beet. Would it be too idle, perhaps, to hope for the appearance of a similar work on sugar-cane?

K. M. DASTUR

NOTES AND NEWS

Salt treatment for paddy

Studies undertaken at the Paddy Processing Research Centre, Tiruvarur (Tamil Nadu) have shown that salt can withdraw water from paddy without entering the kernel, can prevent absorption of water by the paddy and can also arrest sprouting without reducing the ultimate germinating capacity. Application of the findings to parboiling and drying of paddy has shown reduction in cost of fuel and prevention of spoilage of high moisture paddy. Also, a spray of salt solution on the ears of mature paddy ripened, reduced in moisture and got them ready for harvest within two days after application.

First All India Carcass Utilisation Conference

The first All-India Conference on Carcass Utilisation was held from 7th to 9th November, 1973 in the Animal By-products Plant, Gannavaram, Andhra Pradesh, organized by the Ministry of Agriculture, Government of India in collaboration with the Govt of Andhra Pradesh. It has been pointed out that there are nearly 343.7 million livestock population in the country (1966) and it has substantially increased by now. The average death rate amounts to 8 per cent in case of large animals and 4 per cent in case of sheep and goats. Therefore, it may be stated that nearly 28.8 million carcasses of the fallen animals are available in the country per year and most of these carcasses are not properly utilized. Economic loss to the country due to improper utilisation of these dead animals appears to be nearly Rs 500 million. If these carcasses are effectively utilised, it can produce about 3 million tonnes of meat-cum-bone meal with the available protein of nearly 50 per cent valued at Rs 300 million, apart from other by-products like tallow, hides, horns, hoof, etc. This can very well serve as a cheap source of protein supplement for poultry and pig feed and would help the rearing of the calves. The Conference focused attention of all concerned on the economic importance of this programme and made concrete recommendations to highlight the importance of setting up more carcass utilisation centres in different parts of the country to generate wealth from waste.

Sea wealth of Karnataka

Wealth is floating along the 320 km. coastline of Karnataka. But at present only a modest amount

of it reaches the shores. In hundreds of hamlets dotting the sea-front of Karnataka, over a lakh of people depend on fishing for a living.

A little less than 10 per cent of the country's marine fish catch (11.5 lakh tonnes) is contributed by the State (1970). It has vast potential for inland fisheries too. To develop fisheries, both marine and inland, in the recent past significant initiatives have been taken. A deep-sea fishing centre is being established at Mangalore. The State Fisheries Development Corporation located here is having plans to extend large scale assistance to the fishermen. Besides selling, purchasing and distributing fish, the Corporation's functions include manufacture and hire of fishing boats, fishing in high seas, storage and transportation of fish.

Since 1951 to 1969 over five crores of rupees have been invested for developing fisheries in Karnataka. Started as a minor scheme of inland fisheries development at Soraba, Shikaripura, at a cost of Rs 3.3 lakhs in 1951, in the current plan this sector attracted as much as three crores of rupees. With the advent of mechanised fishing, fish catch has increased by 30,000 tonnes a year. About 6,000 people have got employment as a result. The change this has brought about in the fish exports has been sizeable. From Rs 4 lakhs it has shot up to Rs 4 crores in 1972-73.

Toned and Double Toned

Is toned milk another name for watered milk? Actually it is a mixture of whole milk, skim milk powder and water. The skim milk powder 'tones up' the non-fat solid level to the original level of cow's milk. It also reduces the fat percentage.

The term toned was adopted by the Bombay Milk Scheme where the milk was introduced for the first time in 1946. A special nomenclature was necessary as the product could not be described as whole milk or standard milk. Soon the product was introduced in Calcutta, Delhi and Madras.

Toned milk is one of the answers to the increased demand in big cities as such milk doubles the volume. Toned milk will continue to be supplied to large metropolitan cities until milk production increases enormously, when whole milk can be supplied.

Double toned milk is a product of a deal between India and the UNICEF. Double toned milk has

a fat content of 1.5 per cent or half the fat content of toned milk. Its solid content is higher than that of toned milk. Milk is valued for its proteins and both toned and double toned milk are no longer as cheap as they used to be. Skimmed milk powder which is mainly imported, has gone up in price.

Composite Fish Culture

The frugal Chinese were the first to experiment with composite fish culture—the combined farming of compatible fish species. Now composite fish culture has become fashionable in most Asian and Far Eastern countries on the lines of the Chinese system.

The association of species with non-competitive feeding habits results in the optimum utilization of fish food and space available in the ponds. The most favoured species in India for composite fish farming are the Indian major carps; the Catla which feeds on plankton, the Rohu which feeds on algae and the Mrigal which subsists on semi-rotted vegetable matter and detritus. Recently, the common carp, the silver carp and grass carp have also been included in the mixed fish farming.

Experiments on composite fish culture have been going on for the last 23 years at the pond culture division of the Central Inland Fisheries Research Institute.

The Indian Council for Agricultural Research is following up the work done by the Central Inland Fisheries Research Institute by breeding Indian and exotic fishes under different soil conditions. Six centres, one each in Andhra Pradesh, Haryana, Maharashtra, Tamil Nadu, Uttar Pradesh and West Bengal have begun to function.

Controlling the Desert

The Indian Desert is three and a half times the area of Punjab and Haryana put together. Twenty-million Indians depend on the desert for sustenance. They are out-numbered by their cattle by three million. Research has established that the desert is partially man-made. The scarcity of water, vegetation and fuel leads to the over exploitation of natural resources leading to soil erosion and infertility.

Contrary to popular belief the desert is not 'marching' although there have been local changes in the volume of sand in the desert. Scarcity of water is the main insurmountable factor for agricultural production in the arid zone. Therefore, the Central Arid Zone Research Institute, Jodhpur has evolved

methods for predicting the soil moisture under native grasses and the rate at which water penetrates the sandy soil. The Institute conducts investigations on the dispersal of rainwater. Since the infiltration rate is very high in sandy tracts, the runoff is barely 5 per cent of the rainfall. Detailed work is done on preventing water losses through evaporation and seepage. Surveys have located areas where new 'Nadis' can be constructed for storing the rainwater. The Institute has also developed a technique which enables geologists to locate shallow underground water resources from an interpretation of aerial photographs of buried drainage systems.

Techniques of desert control: Afforestation, establishment of pastures and grasslands, shelter belts, wind-breaks and sand-dune stabilisation increase the yields of food, fodder and fuel up to 300 per cent. Modern agricultural techniques have also been evolved to aid the farmer: contour bunding, subsoiling, fallowing, strip cropping and water harvesting.

Developing Grasslands

The country has embarked on a massive programme for increasing the supply of milk. For this, exotic dairy cattle: the Jersey, the Brown Swiss and the Red Dane are being imported. The cross-breeds have to be fed adequate quantities of nutritive fodders to maintain the remarkable increases in milk production. This has shown up the shortages of concentrated feeds and green fodders. India produces a third of its green fodder and a quarter of its crop residue requirements and only a seventh of the necessary feed concentrates. No wonder malnutrition of cattle is so rampant that it is not even noticed. It is the major reason for their poor performance.

During the Fourth Five Year Plan a scheme has been launched for the establishment of seven regional stations for forage production and demonstration in the different agro-climatic zones of the country. The primary objective of the stations is to bring the latest research findings along with their package of practices to the knowledge of the farming community. The United Nations Development Programme has agreed to assist in the early establishment of these stations by supplying modern equipment and some technical know-how. The project is expected to commence from April 1974.

A Portable Grain Drier

Mountains of grain are transported after every harvest season for drying. It costs Rs 40 per ton to dry the grain. Now Dr S. K. Pingle and Shri N. V. K.

Rao have invented a portable grain drier which uses paddy husk for generating heat. As a result, drying rates have been reduced to Rs 6 per tonne and transport costs have become negligible. The new blower is versatile. It can dry grain which has already been stacked in bags by changing an attachment. A grain purifier has also been developed. The purifier has a 3-ton per hour capacity which can be operated on electricity or petrol. The purifier can be docked with the grain-drier. This composite unit which consists of one hot air-blower and two purifiers, dries and purifies 3 tonnes of grain per hour and costs only Rs 12,000.

The scientists of the Food Corporation of India have been working on the 'Foodcrop Purifier and Foodcrop Drier' for the last two years. The two machines have been assigned to the National Research and Development Corporation which will enter into agreements with commercial manufacturers for producing the equipment for procuring agencies, rice-millers, co-operative societies and farmers.

Indian Scientists Develop Space Age Food In Australia

An Indian scientist is working with a group of Australian researchers on the development of space age food at a unique establishment in Australia's island State of Tasmania.

Dr Subbaiah Venkataraman is head of the food technology section at the Armed Forces Food Science Establishment at Scottsdale, Tasmania. Dr Venkataraman is engaged in research and development in dehydration, compression and packaging of food for the Australian armed forces. However, work at the establishment also has important significance for civilians in many countries, particularly the developing nations.

Recently, Dr Venkataraman head a paper on different aspects of his work to more than 70 representatives of major Australian food manufacturing companies and the armed services during a three-day convention on the freeze drying of food.

Australian Scientists Develop Space Age Food

Research scientists in Australia are developing space age food processing techniques with important significance for countries hit by natural disasters such as floods, earthquakes and famine.

The work is being done at the Armed Forces Food Science Establishment at Scottsdale, in the State of Tasmania, and it is concerned with the dehydration, compression and packaging of food.

Established by the Australian Government in 1958, the Armed Forces Food Science Establishment is controlled by a Director, Dr R. C. Hutchinson, who is also the Defence Food Science Adviser to the Australian Government.

While the prime objective of the establishment is to produce improved types of food for the Australian armed forces, work being done by its researchers has wide civilian significance.

Food dehydration techniques of world importance are being developed at the establishment, and scientists believe that much of this space age food will be of immense value in the future to medical teams combating floods, earthquakes, famines and similar disasters.

Because of reduced weight and bulk, compressed dehydrated food with a high nutritional value is easily transportable and has a long storage life even under the most extreme conditions.

A marked trend towards dehydrated food was already evident in many countries, and forecast that within 10 years they would form a substantial part of family diet.

The Australian Government intends to allow the products and results from the establishment to become available to a much wider group of Australians than the armed services.

A New NMR Book of Polymers Published by Sadtler

Sadtler Research Laboratories, Inc., Philadelphia, announces the publication of a new book on the analysis and classification of polymers by nuclear magnetic resonance spectroscopy. It is entitled *The Sadtler Guide to the NMR Spectra of Polymer* and is written by W. W. Simons and M. Zanger. The price is \$ 24.50.

New Infrared Spectra Collection of Polymer Additives

Sadtler Research Laboratories, Inc., Philadelphia, announces the publication of a new infrared reference spectra collection of polymer additives. The collection contains 300 spectra of various types of commercial products which are used as additives in the production and processing of polymers. Included are materials which are used as accelerators, antioxidants, antistatic agents, stabilizers and UV absorbers.

New Infrared Spectra Collection of Toxic Chemicals

Sadtler Research Laboratories, Inc., Philadelphia, announces the publication of a new infrared reference

spectra collection of toxic chemicals. The collection contains 300 spectra of substances which are used in industrial processes and which are considered to be health hazards. The compounds have been selected from various sources including the Toxic Substances List, 1972 published by the U.S. Department of Health, Education and Welfare. The collection is intended to provide the toxicologist with a single source of infrared spectra of toxic substances which are frequently encountered in analytical studies.

Indian Standards Institution

The following standards have been published:

IS:1320-1972 Baker's Yeast	Rs. 6.00
IS:[5701 (Part V)-1972] Code for Breeding, Care, Management and Housing of Laboratory Animals	Rs. 4.00

IS:3633-1972 Tea	Rs. 5.50
IS:2128-1973 Parathion Ethyl, Technical	Rs. 4.00
IS:2569-1973 Malathion Water Dispersible Powder Concentrates	Rs. 5.00
IS:6690-1972 Rotavator Blades for Power Tillers	Rs. 5.00
IS:1668-1972 Losenges	Rs. 3.00
IS:6840-1972 Code of Practice for Preventive Maintenance of Agricultural Wheeled Tractor.	Rs. 6.00
IS:6747-1972 Chewing Gum and Bubble Gum	Rs. 3.00
IS:1682-1973 Cuprous Oxide, Technical (Fungicidal Grade)	Rs. 5.50
IS:2568-1973 Malathion Dusting Powders	Rs. 4.00

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ASSOCIATION NEWS

Gardners Award

The paper on 'Dissipation of Malathion Residues on Maize Grain in Relation to Dosage—Storage Conditions and Baking' by O. S. Bindra and T. S. Sidhu, as given on page 29 of Volume 9, No. 1 of March 1972 has been selected for the Gardners Award for 1973, by the Selection Committee.

Seminar at CFTRI

A Seminar on 'Problems of Technology Transfer in Developing Countries' by Dr H. A. B. Parpia, Senior Officer of FAO was held on 22nd November 1973 at CFTRI.

Two Seminars on 'Enzymes in Brewing' and 'Continuous Fermentation in Brewing' by Dr Olga Bendova, Director, Malting and Brewing Research Institute, Prague, Czechoslovakia, were held on 28th November 1973 and 3rd December 1973 respectively at CFTRI.

Seminar on Food Machinery

The Association (Western Region) held a seminar in Bombay on 'Food Machinery' on 19th and 20th January 1974. It arranged an exhibition of the range of food machinery that is presently being fabricated in the country. The seminar focused attention on the problems facing the industry and suggested possible remedial measures that would enable the industry to play an active role in the years ahead.

The objectives of the seminar were as follows:

(1) To assess the present and anticipated demands of the food processors for machinery and equipment catering to their specific need.

(2) To focus attention on the problems involved in the fabrication of conventional and sophisticated equipment as required by the food industry.

(3) To stress the need for establishment of indigenous design facilities for development of types of food machinery and equipment and modify existing units.

(4) To evaluate the performance of the industry so far, to iron out its shortcomings and promote a smooth and rapid development in the future.

There was active participation in the seminar by authorities, leading industries, technologists, engineers, R and D Laboratories, the trade and other agencies.

Formation of Bangalore Chapter

The Bangalore Chapter of the Association has been formed with Mr M. R. Chandrasekhara, Project Administrator and Coordinator, Miltone Project, Bangalore Dairy, Bangalore as the Convener of the *Ad hoc* committee.

New Members

Mr. Dalpat Singh Khurdiya, Asstt. Fruit Preservation Officer, Divn. of Horticulture and Fruit Technology, I.A.R.I., New Delhi-12.

Mr. Lalit Kumar Sarada, Director, Sarada Food Processing Ent. Pvt. Ltd., P-18, Green Park Ext., New Delhi-16.

Mr. K. K. Achankunju, Technologist, Kerala Food Packers, Palluruthy, Cochin-6.

Mr. N. T. Kuruvilla, 1/58, Shanti, Garodia Nagar, Rajawadi, Bombay-400 077.

Mr. V. W. Padhye, Ulhas Darshan, Deral (C.Rly), Dist. Thana, Maharashtra State.

Mr. Gopal Kumar Srivatsava, 122, New University Hostel, Matunga, Bombay-19.

Mr. Godbole Gopal Trimbak, Room No. A-20, V.J.T.I. Hostel, Matunga, Bombay-19.

Mr. Lambert Rodrigues, Foods and Fermentation Section, Dept. of Chemical Technology, Matunga, Bombay-19.

Mr. M. M. Alexander, Dept. of Chemical Techy, Food Technology Section, Matunga, Bombay-19.

Mr. Rajendra Y. Angle, B/8, Union House, L.J. Cross, Road No. 2, Mahim, Bombay-400 016.

Mr. Arun Kumar Bhattacharyya, Food Section, U.D.C.T., Matunga, Bombay-19.

Mr. Pushkar Singh Bora, 140, New University Hostel, Matunga, Bombay-19.

Mr. Upkar Singh, A/4, V.J.T.I. Hostel, Matunga, Bombay-19.

Mr. Ishwara Chandra Shukla, A/32, V.J.T.I. Hostel, Matunga, Bombay-19.

Mr. Pulak Sen, D-1713, I.I.M. Hostel, I.I.M. Vasatrapur, Ahmedabad-380 015.

Miss Ofelia A. Cipriana, A/18, International Hostel, FAO IFTTC CFTRI, Mysore-570 013.

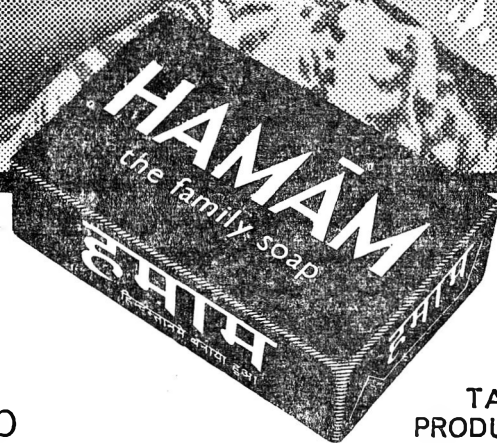
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- Mr. G. S. Sannabhadti, I, Raju Mansion, Vallabh Baug Lane, Garodia Nagar, Ghatkopar (East) Bombay-75.
- Mr. R. H. Suryawanshi, D-80/794, M.I.G. Colony, Gandhinagar, Bandra (East), Bombay-51.
- Vijay V. Kamat, Sheshank Sea Foods Pvt. Ltd., Kasargod, (N. Kanara), Karnataka State.
- Mr. M. N. Saikia, Govt. Fruit Preservation Factory, Department of Agriculture, P.O. Vairengte, Mizoram, U.P.
- Smt. Shanthi Narasimhan, CFTRI, Mysore-13.
- Mr. K. R. Aggarwal, House No. 14, Street No. 15, Jullundur Cantt., Punjab.
- Mr. S. K. Patankar, C/o M/s. K. C. Patankar, 1486, Deshpandegalli, Belgaum-590 002.
- Mr. J. B. Patnaik, C/o Mr. R. B. Pattanaik, Reba Colony, Kafa Bazaar, P.O. Cuttack-2, Dist. Cuttack (Orissa).
- Mr. D. N. Kulkarni, C/18, International Hostel, CFTRI, Mysore-570 013.
- Mrs. A. V. Save, 11/234, J. S. Road, Bombay-4.
- Mr. V. K. Patil, Sarthak Building, Pandurang Wadi, Dombivali (East) Dist. Thana, Tel: Kalyan, Maharashtra State.
- Miss L. Jhangiani, No. 23, 2nd Floor, 'Sangita', Colaba, Bombay-5.
- Miss I. M. Tahiliani, Park View, 7/29A, 'West' Sion, Bombay-22.
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- Mr. P. T. Davis, Andhra Pradesh Small Scale Industrial Development Corporation, I-B-174, Fatel Maidan Road, Shankar Bhavan, Hyderabad-4.
- Mr. P. V. Parameswaran, 3-6-14F/2/1, Himayat Nagar, Hyderabad-29.
- Dr. N. Chandrasekhara, Scientist, CFTRI, Mysore-570 013.
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- Mr. V. V. L. Narasimhan, D-13, International Hostel, CFTRI, Mysore-570 013.
- Mr. Sanjiv Raghunath Marathe, Block No. 2, Bldg. No. 1, Jai-Vijay Housing Society, Express Highway, Vile-Parle (East), Bombay-400 057.
- Mr. R. N. Dixit, Project Chemist, Bangalore Dairy, Hosur Road, Bangalore-560 029.
- Mr. Ashok Kumar Kanchan, S/o Shri Chhate Lal Khothari Mohalla Ganj, P.O. Talbehat, Dist. Thana, Maharashtra State.
- Mr. Charles A. Wesley, Principal, Food Craft Institute, Bangalore-1.
- Mr. S. Bhaskar, No. 78, "Sree Sudha", 37th Cross, 9th Block, Jayanagar, Bangalore-11.
- Mr. U. V. Sulladmath, Professor of Horticulture, Division of Horticulture, University of Agril. Science, Hebbal, Bangalore-24.
- Dr. N. C. Ganguli, Dairy Chemist and Head, N.D.R.I., Karnal, Haryana State.
- Mr. Mohan Singh Ahuja, 7, R. B. Rattan Chand Road, The Mall, Amritsar.
- Mr. Morde Chandrakant Eknath, 55, Victoria Building, Near Rani Ganj, Dr. Ambedkar Road, Bombay-27 DD.
- Mr. Kanetkar Gajanan Vaman, C/o Nila Products Ltd., 98, Dadar Main Road, Bombay-14.
- Mr. Yashawantha Kumar J. Asher, P.O. Box No. 3116, Mississippi State, Mississippi-39762, U.S.A.
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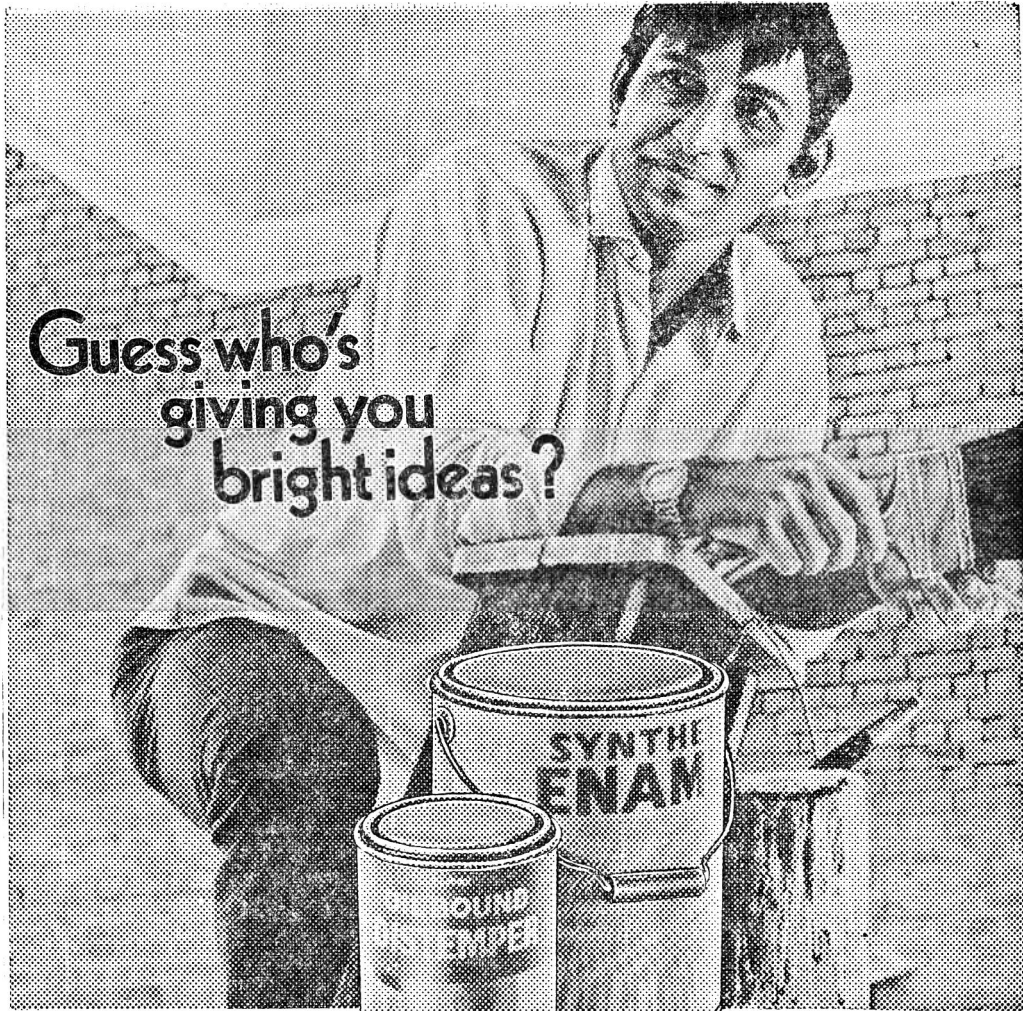
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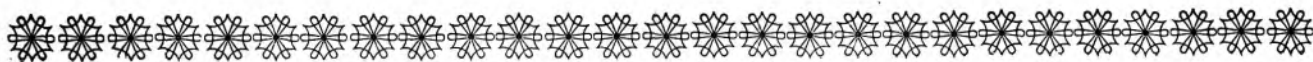
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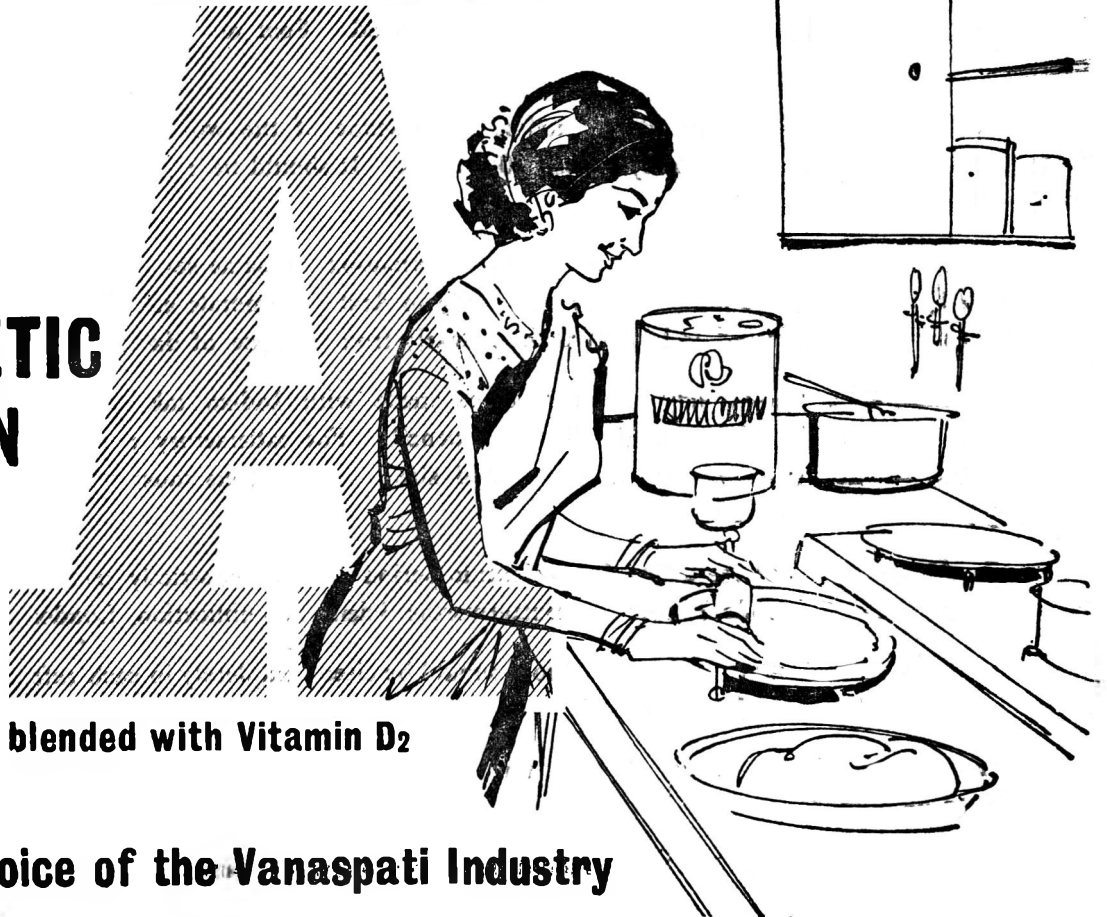
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(c) *References to article in a book*: Joshi, S. V. in *The Chemistry of Synthetic Dyes*, by Venkataraman, K., Academic Press, Inc., New York, 1952, Vol. II, 966.

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

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