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Research Notes

Changes in Lipids of Bengalgram *(Cicer arietinum)* on Heat Processing

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Manuscript received 9 *March* 1979; *revised* 3 *May* 1979

Heat treatment of Bengalgram during roasting and puffing resulted in a decrease of free lipids by 15 to 18 percent and an increase in bound lipids. In the five varieties of Bengalgram, the bound lipids constituted 31-44 percent of the total lipids. Both roasting and puffing retarded development of free fatty acids during storage over a period of 48 weeks. Puffing resulted in the retardation of the oxidation of unsaturated fatty acids, but roasting had no such beneficial effect.

Bengalgram (*Cicer arietinum*) is used in Indian dietary in a variety of ways. Mixed with edible oilseed meals, it is finding increasing use in protein supplements for growing children. For such uses the roasted and puffed *dhal* (split cotyledons) is preferred because of the pleasing aroma developed after roasting and puffing. The *dhal* contains 5-6 per cent lipids with linoleic acid constituting the major fatty acid[1]. Heat treatment during the puffing and roasting operations may bring about changes in the proportion of free and bound lipids. Other changes may also take place which have a direct bearing on the storage life of the product in terms of free fatty acid development and oxidative degradation of the unsaturated lipids. The present study was undertaken to study these aspects.

Materials and Methods

Five varieties of Bengalgram namely 'C-235', 'Hare Channa', 'L 550', 'G 130' and 'Jg 62' were obtained from ICRISAT, Hyderabad, IARI, New Delhi and PAU, Ludhiana. 'C-235' 'G-130' and 'Jg 62' are small and brown coloured, 'Hare Channa' is small and green coloured, whereas 'L 550' is bold and yellow.

Raw Bengalgram flour: Bengalgram *dhal* was purchased locally and the cleaned material was ground to pass through 60 mesh sieve (BS).

Roasted Bengalgram flour: Cleaned *dhal* was roasted in a coffee roaster for 30 min. The final temperature of the roasted material was 135°C. The cooled material was ground to pass through 60 mesh sieve (BS).

Puffed Bengalgram flour: The puffed *dhal* was a commercial sample from the local market. It was cleaned and ground to 60 mesh sieve (BS). During puffing Bengalgram is generally subjected to high temperature of 250°C for a short interval.

The flours were stored in 500 g capacity press-in-lid tin cans in duplicates at 37°C and also at room temperature (20–28°C). Samples were analysed at intervals of 8 weeks for development of free fatty acids, peroxides and thiobarbituric acid (TBA) reactive substances. Extraction and estimation of free and bound lipids were done according to the method of Suresh *et al.*[2] Peroxide value and free fatty acids were determined according to AOAC procedure[3]. TBA value was determined according to the method described by Mettler[4].

The five major varieties were analysed for nitrogen, ether extractives, ash and crude fibre according to the methods outlined in AOAC. Carbohydrates were calculated by difference. The values are given on moisture free basis in Table 1.

Results and Discussion

The protein content of 'Hare Channa' was highest (30.8 per cent) compared to the other four varieties in which it ranged from 21 to 24 per cent. Not much variation was found among the varieties with regard to ether extractives, ash and crude fibre. Bhandari *et al.*[5] studied the physical characteristics and fatty acid composition of the lipid extracts from two varieties of Bengalgram viz. Channa' and 'Kabulichana' and found no major differences. Ghirardi *et al.*[6] studied the lipid component of Indian and Italian varieties and found close similarity. Linoleic acid was the major fatty acid in all the varieties studied.

The free and bound lipids in the *dhal* together amount to more than 7 percent (Table 2). It is interesting to note that bound lipids form about 30-40 per cent of the total lipids. There does not appear to be much variation in the distribution of free and bound lipids among the five varieties studied.

TABLE 1. PROXIMATE COMPOSITION OF MAJOR VARIETIES OF BENGALGRAM (WHOLE)

Varieties	Colour	Size	Protein (N×6.25) (%)	Ether extractive (%)	Ash (%)	Crude fibre (%)	Carbohydrates (by diff) (%)
C-235	Brown	Small	21.0	4.7	3.1	3.9	67.3
Hare Channa	Green	Small	30.8	3.9	3.4	3.8	58.1
L 550	Yellow	Bold	22.2	5.1	3.0	3.7	66.0
G 130	Brown	Small	23.0	5.3	2.3	3.2	66.2
Jg 62	Brown	Small	24.9	4.1	3.3	3.5	64.2

During the processes of roasting and puffing Bengalgram is subjected to high temperatures. Changes brought about in the free and bound lipids are indicated in Table 2. There is a decrease of nearly 20 per cent in free lipid content with a corresponding increase in bound lipid content indicating the binding of some of the free lipids to other constituents, probably proteins, during the heat treatment.

The storage behaviour of lipids in raw, roasted and puffed Bengalgram flours were observed at intervals of 8 weeks by estimating the free fatty acids, peroxide values (P.V.) and by reaction with thiobarbituric acid (TBA).

Development of FFA during storage has been indicated in Figs. 1 and 2. The lipids in raw flour showed linear increase in FFA, the sample stored at room temperature increased from an initial of 0.8 to 7 per cent during 24 weeks to 10.8 per cent after 48 weeks. Increase in the case of samples stored at 37°C, was slightly lower (8 percent) at the end of 48 weeks. The development of FFA in roasted and puffed Bengalgram flour was much lower at room temperature as well as at 37°C, the FFA content being less than 4 percent after 48 weeks storage. This could possibly be due to partial inactivation of lipase during the heat treatment.

Oxidation of the lipids during storage was estimated by the measurement of peroxides and thiobarbituric acid reactive (TBA) substances. The former serves as an index of the primary stage of fat oxidation, whereas the TBA measures the carbonyls which are responsible for the off-flavours (Figs. 3 and 4). In raw Bengalgram flour peroxides rapidly increased till 16 weeks, reaching

TABLE 2. FREE AND BOUND FAT IN MAJOR VARIETIES OF BENGALGRAM AND EFFECT OF ROASTING AND PUFFING

	Free fat %	Bound fat %	Total fat %	Bound lipids (as % of total lipids)
C 235	4.7	2.4	7.1	33.8
Hare Channa	3.9	3.1	7.0	44.3
L 550	5.1	2.3	7.4	31.1
G 130	5.3	2.5	7.8	32.1
Jg 62	4.1	2.9	7.0	41.4
Raw Bengalgram flour	5.2	2.7	7.9	34.2
Roasted Bengalgram flour	4.2	3.1	7.3	42.5
Puffed Bangalgram flour	4.2	3.2	7.4	43.2

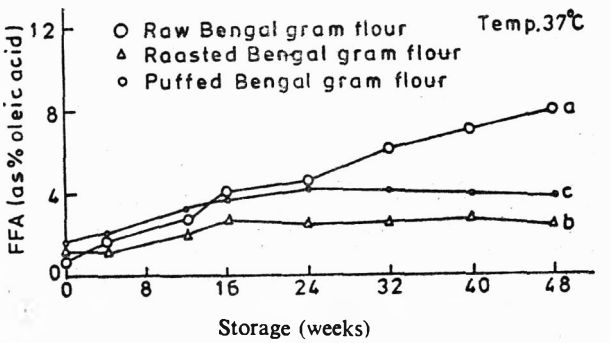

Fig. 1. Development of FFA during storage at 37°C

TABLE 3. CHANGES IN THIOBARBITURIC ACID (TBA) VALUES DURING STORAGE

Type of Bengalgram flour		Thiobarbituric acid value during storage for different weeks 0 wk	4 wk	12 wk	16 wk	24 wk	32 wk	40 wk	48 wk
Raw	37°C	0.045	0.040	0.050	0.060	0.020	0.020	0.030	0.035
Raw	RT	0.045	0.040	0.050	0.065	0.050	0.050	0.040	0.030
Roasted	37°C	0.060	0.065	0.087	0.095	0.090	0.080	0.080	0.050
Roasted	RT	0.060	0.065	0.065	0.087	0.105	0.100	0.085	0.060
Puffed	37°C	0.080	0.082	0.077	0.082	0.075	0.075	0.080	0.090
Puffed	RT	0.080	0.077	0.095	0.095	0.080	0.080	0.070	0.060

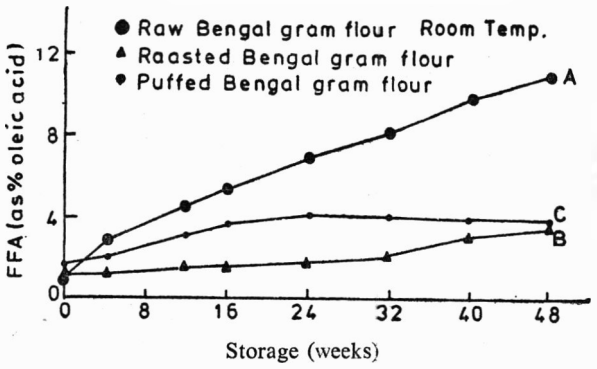

Fig. 2. Development of FFA during storage at room temp.

21 in the sample at 37°C and 18 in the sample at room temperature. Thereafter, no further increase, but a slight decrease was observed. Off-flavours typical of oxidative rancidity was noticed after 16 weeks storage. In roasted Bengalgram flour the increase in peroxide was somewhat lower, the maximum value obtained being 19 at the end of 24 weeks. Peroxide formation was still lower in the case of puffed Bengalgram flour. After 16 weeks storage the maximum value of 12 was reached. This rapidly came down to 5 and remained almost constant during further storage upto 48 weeks. Storage behaviour of roasted and puffed Bengalgram flours show considerable difference in respect of formation of peroxdies. The increased oxidative stability of puffed Bengalgram as compared to raw and roasted may be due to inactivation of the enzyme lipoxygenase at the high temperatures encountered during puffing similar to the dry heating-extrusion cooking process used for inactivation of soybean lipoxygenase[7].

The secondary stage of oxidation was followed by re-

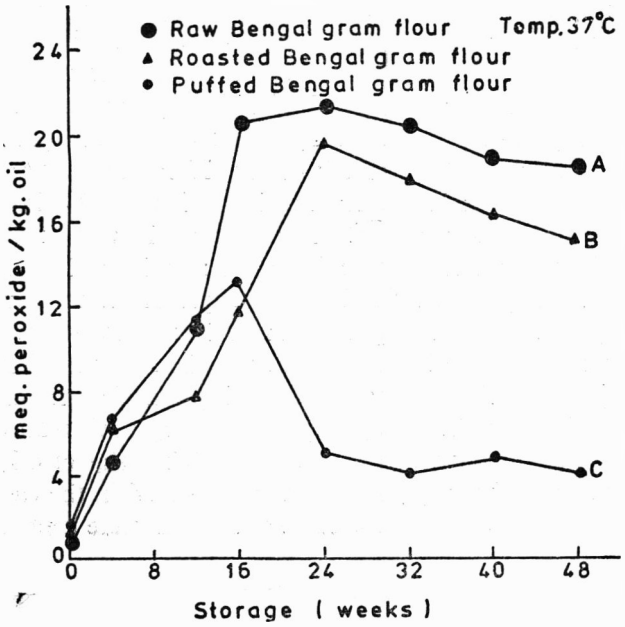

Fig. 3. Development of peroxide during storage at 37°C

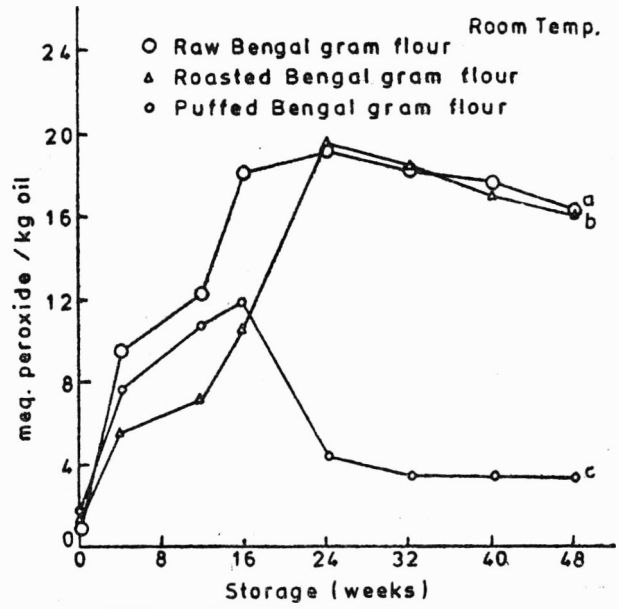

Fig. 4. Development of peroxide during storage at room temp

action with 2-thiobarbituric acid. The intensity of absorption of the TBA reactive substance at 450 nm is given in Table 3. In raw Bengalgram flours TBA values did not increase at room temperature and 37°C. Roasted Bengalgram flour showed slight increase from 4 weeks onwards till 16 weeks. TBA reactive substances did not form in puffed Bengalgram which observation was also made by St. Angelo *et al.*[8] They observed linear correlation between peroxide values and diene conjugation in peanut products. The TBA values were found valid for assessing the degree of oxidation of pure oils but not for oil bearing materials. In the latter case difficulties are encountered as a result of reaction of lipid oxidation products with proteins and possibly carbohydrates, rather than with the reagent. The storage studies indicate that the lipids in heat processed Bengalgram flours are fairly stable to oxidative changes upto six months. Roasting and puffing retard the development of FFA.

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Shelf-life of Sunflower Oil and Groundnut Oil*

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Raw sunflower and groundnut oils and refined groundnut oils produced in India and also imported were stored at ambient room temperature in mild steel and tin containers respectively. Raw sunflower oil and raw groundnut oil were stable hydrolytically and oxidatively for 1080 and 660 days respectively. Refined groundnut oils did not register increase in free fatty acid contents whereas peroxide values widely differed from each other. Refined groundnut oil even without added antioxidants stored in closed containmer without access to air showed low peroxide value at the end. Acceptability tests showed that the raw sunflower and groundnut oils were acceptable till they attained a peroxide value of about 25 and beyond this limit, there was rapid deterioration in the palatability of the preparations.

Autooxidation plays an important role in the shelf-life of edible oils. Variations in the type and origin of oil, refining practices, packaging methods, climatological conditions, presence of metallic centaminants, meal particles, moisture and other factors affect the stability and shelf-life of edible oils. Shortfall in the availability of edible oils, preference by hoteliers and manufacturers of fried snacks for refined oils as against raw oils because of the foaming tendency of the latter, increasing popularity of refined oils in the market and the policy of bringing non-traditional oils into the edible fold, have focussed the attention of researchers on the practical aspects of storage of edible oils, both raw and refined.

Extensive data exist on the theoretical aspects of autooxidation and the degradation products occurring in oils on storage and after deep frying, but practical aspects from the consumer point of view of storing oil in standard containers over extended periods have been inadequately studied under Indian conditions. Earlier studies at the Institute on the storage of crude, alkali-refined and bleached oils of watermelonseed oil[1], muskmelonseed oil[2] and *ambadi* oil, (unpublished work) indicated that alkali-treatment renders the oils more susceptible to oxidative rancidity. Narayana Rao and Swaminathan[3] showed that crude groundnut oil keeps well in glass, aluminium and tinned-brass containers. Srinivasa Gopal *et al.*[4] showed that for transport of refined groundnut oil, galvanized iron drums are preferable to square tin containers and high density polyethylene jerry cans. No work is available on the storage of sunflower oil under Indian conditions. Data in respect of refined groundnut oil are also lacking. Present studies were undertaken to record the changes in certain characteristics of raw sunflower oil and raw and refined (alkali-neutralized, bleached with earth and carbon, and deodourised with steam) groundnut oils when these were stored at ambient room temperatures.

Materials and Methods

Raw sunflower and raw groundnut oils freshly crushed from kernels in the pilot plant section of the Institute were selected for bulk storage. There was no time lag between the production of oils and commencement of storage studies. Raw sunflower and raw groundnut oils (170 kg each) were stored in clean mild steel barrels (200 capacity) leaving a head space of 15 cm. A screw lid closed a small opening (6.5 cm wide) at the top of the barrel air-tight. The opening facilitated sampling of oils. The two barrels were kept in a spacious room at ambient temperature (25-30°C). Refined groundnut oils of a popular indigenous commercial brand, and an imported (USA) refined peanut oil (the latter presumably containing antioxidants and synergists) supplied to the ready-to-eat (RTE) food factory of the Government of Andhra Pradesh were also kept for storage. A period of one month in the case of the indigenous refined oils, and of three months in the case of the imported refined oil, was presumed to have elapsed between their production at the factory sites and commencement of storage studies at the Institute. The imported refined peanut oil was kept in the original sealed tin of 3.8 l. capacity. The indigenous refined groundnut oils were kept in sealed original tins of 4-kg capacity. Two replicate tins were procured in the case of indigenous refined oil. All the three tins were kept on

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a laboratory shelf for storage. The lids of the indigenous and foreign tins were opened for periodical sampling of oils for analysis. One tin of indigenous refined oil was kept in a sealed state without opening. Practically no head spaces were noted when the tins were opened.

Samples of oils were drawn periodically and analysed for free fatty acid content, peroxide value, iodine value and Lovibond Tintometer colours as per the Official and Tentative Methods of the American Oil Chemists' Society. The oils were sampled from the barrels by dipping a small glass bottle attached to a string through the top of the barrels. The bottle was dipped in the middle of the barrel, where it got filled with oil. It was then lifted out of the barrel and the oil analysed. In the case of small tins, the tins were shaken well before sampling, the lid opened and a suitable quantity of oil poured out into a beaker for analysis. *Bajji* (a snack prepared by dipping green chilli (capsicum) or potato slice in a slurry of Bengal gram flour followed by deep fat-frying in oil) and potato chips were fried periodically in the stored raw sunflower oil and raw groundnut oils and were tasted by a panel of tasters. Results are given in Tables 1 and 2.

Results and Discussion

It is seen from Table 1 that the increase in free fatty acid of raw sunflower oil was from 1.8 to 4.7 per cent and the peroxide value was from 6 to 16 during 1080 days storage. No significant changes were observed in iodine value and colour. Kreis reaction gave a Lovibond colour of 2.2 red units in a 10-mm cell at the end of 1080 days.

The free fatty acid content of the raw groundnut oil increased from 1.2 to 2.7 percent and the peroxide value increased from 5 to 10 during 660 days of storage. But no significant change was noted either in iodine value or in visible colour of the oil. Kreis reaction gave a Lovibond colour of 4 red units in a 10-mm cell at the end of 660 days. While raw groundnut and sunflower oils of good initial quality keep well in storage, the peroxide value and Kreis colour of refined oils are influenced by conditions of storage and handling of oils during storage (Table 2).

The increase in free fatty acid content of refined groundnut oils, both indigenous and imported (Table 2) is not significant. The rise is from 0.08 to 0.18 for indigenous refined oil and 0.06 to 0.08 for imported refined oil at the end of 330 days' storage. During the same period, the peroxide values of indigenous and imported refined oils increased from 2 to 180 and 1.2 to 10 respectively. However the indigenous refined oil in the replicate tin which was kept sealed and opened only at the end of 330 days has shown a peroxide value of only 3 units. Thus non-exposure of the oil to air will not allow

TABLE 1. CHANGES DURING STORAGE OF RAW SUNFLOWER (SN) AND GROUNDNUT (GN) OILS

Storage period (days)	Free fatty acid (%)		Peroxide value (m. eq. of O_2/kg fat)		Lovibond Tintometer colour in 6.35-mm cell (Y+5R) units	
	SN	GN	SN	GN	SN	GN
0	1.8	1.2	6	5	3.5	3.0
30	2.0	1.3	5	6	3.8	3.1
60	2.1	1.3	4	6	4.2	3.0
90	2.3	1.4	4	6	4.6	3.5
120	2.4	1.4	4	6	4.7	4.1
150	2.5	1.4	4	6	4.7	4.0
180	ND	1.4	ND	6	ND	4.2
210	ND	1.5	ND	6	ND	4.7
240	2.5	1.6	4	7	5.8	4.2
270	2.6	1.6	4	7	6.1	4.7
300	2.7	1.6	5	7	6.8	4.8
330	2.8	1.7	7	7	6.8	4.7
360	2.8	1.7	8	7	6.0	4.8
660	2.9	2.7	10	10	6.4	5.8
1080	4.7	ND	16	ND	10.8	ND

ND: Not done

The iodine values of sunflower oil (119) and groundnut oil (93) remained unchanged throughout the storage period.

TABLE 2. CHANGES DURING STORAGE OF REFINED INDIAN AND IMPORTED GROUNDNUT OILS

Storage period (days)	Free fatty acid %		Peroxide value (m. eq. of O_2/kg fat)		Lovibond Tintometer colour in 2.54-cm cell (Y+5R) units	
	Indian	Imported	Indian	Imported	Indian	Imported
0	0.08	0.06	2.2	1.2	3.0	3·0
30	0.10	0.06	7.1	2.7	3.0	3.0
60	0.08	0.07	19	3.5	2.5	2.5
90	0.09	0.07	37	3.5	2.5	2.5
120	0.10	0.07	58	4.9	2.5	2.5
150	0.11	0.07	73	6.3	2.5	2.5
180	0.15	0.07	91	7.8	2.5	2.5
240	0.19	0.08	150	8.1	2.5	2.5
270	0.18	0.08	136	9.3	2.5	2.5
300	0.14	0.08	159	9.0	2.5	2.5
330	0.14	0.08	180	10.0	2.5	2.5

Indigenous oil was estimated to be about 30 days old and imported oil about 90 days old at the time of commencement of the studies.

the peroxide value to increase. The Kreis reaction in respect of imported refined oil gave a colour of 2.6 Lovibond red units (10-mm cell) at the end of 330 days whereas for the corresponding indigenous refined oil the value was 27 red units. The oil kept in the sealed tin and opened only after 330 days, gave a Kreis colour of 1.6 red. Refined groundnut oil even without added anti-oxidants would therefore, appear to keep well for about a year if the oil is stored in sealed containers without access to air or without air head-speaces.

Acceptability tests conducted periodically by preparing deep fat-fried snacks indicated that the raw sunflower and raw groundnut oils are palatable till the oils attain a peroxide value of 25, above this the palatability gets deteriorated.

Acknowledgement

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Digestibility of Proteins of Winged Bean Seed *(Psophocarpus tetragonolobus* L*)*

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The *in vitro* digestibility of the protein from the different parts of winged bean plant was estimated. Mature pods showed highest digestibility (73.8%) while raw seeds had the lowest (67.3%). No significant difference existed between time of soaking and cooking on digestibility. However, soaking of seeds in water prior to cooking improved digestibility. Autoclaving of seeds gave 84.3% digestibility followed by ether extracted seeds (72.2%), dry heat processed (71.6%) and raw seeds (70.7%). Elimination of proteinase inhibitors through heat treatment improved digestibility.

Soybean is the most important and widely used legume for processing[1,2]. Recently, Ekpenyong[3] has shown that the winged bean—a ropical legume, has a nutritional value similar to soybean. Although a number of investigators have attempted to develop simple and rapid *in vitro* methods for evaluating the nutritive value of proteins[4–6], recently, Hsu *et al.*[7] developed a multi-enzyme-automatic recording technique for *in vitro* protein digestibility which is less complicated, rapid but reliable and sensitive enough to detect the effects of processing and the presence of growth inhibitors.

Materials and Methods

The winged bean (*Psophocarpus tetragonolobus* L) samples ('PPt–1' and 'TPt–2' variety) were procured from International Institute of Tropical Agriculture in Ibadan, Nigeria. Soybean, Great Northern bean and Pinto bean were obtained locally. The winged bean obtained from Nigeria was grown in clay pots in the University of Nebraska greenhouse. Between planting and physiological maturity, samples of raw seeds, immature pods, mature pods, young and mature leaves, stem, the entire plant and roots were taken and stored in the cold room (5°C) until analyses were made. The enzymes used were trypsin (Type IX) with 14, 190 BAEE units/mg protein; bovine pancreatic chymotrypsin (Type II) with 60 units/mg powder and porcine intestinal peptidase (Grade III) with 40 units/mg powder. All the enzymes were purchased from Sigma Chemical Company, St. Louis, MO.

Legumes were finely ground to pass through an 80 mesh screen. The beans were also subjected to autoclaving (110°C, 15 psi, 30 min), and dry heating (110°C, 30 min) before determining the digestibility. Fat was extracted from the samples following AOAC methods[8].

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Fifty millilitre of aqueous protein suspension (6.25 mg protein/ml) in distilled water were adjusted to pH 8.0 with 0.1 N HCl and/or NaOH while stirring in a 37°C waterbath for 15 min. The multienzyme solution, consisting of 1.6 mg trypsin, 3.1 mg chymotrypsin and 1.3 mg peptidase/ml, was maintained in an ice bath and adjusted to pH 8.0 with 0.1N HCl and/or NaOH. Five millilitre of the solution was added to the protein suspension while stirring at a constant temperature of 37°C. The pH drop, on addition of the multienzyme solution, was monitored every 2 min over a 10-min period using a recording meter. The calculation was done as per Hsu *et al.*[7] which is

$$Y = 210.464 - 18.103X$$

Where X=pH of protein suspension after 10 min digestion with multienzyme solution.

Results and Discussion

Earlier it was shown that raw winged bean seeds contained protein, 36; oil, 16; and carbohydrate, 24 per cent while in dry roots it was 12, 1 and 21 per cent respectively[9]. Mature pods showed highest digestibility (73.8 per cent) while raw seeds were least (67.3 per cent) digestible (Table 1). It is also clear that the multienzyme system created the largest pH drop after 10 min of incubation. The advantage in the use of multienzyme system is that it avoids under predicting the digestibility of proteins containing the trypsin inhibitor.

While with a single enzyme system the magnitude of pH drop would be an indication of the activity of the respective enzyme, a multienzyme system as used here, reduces the effect caused by a specific enzyme inhibitor. The largest pH drop as shown in Table 1 was in mature pods while it was least in raw seeds. Raw seeds were the least digested while mature pods showed highest digestibility when compared with the different parts of the winged bean plant.

There was no significant difference between prolonged soaking (18 hr) and prolonged cooking (5 hr) when compared to short periods of soaking (5 min) and short cooking time (1 hr). Soaking for a short time prior to cooking was however, better than unprocessed raw seeds. The results in Table 2 suggest that unprocessed raw seeds had relatively low digestibility, while soaking prior to cooking for at least 1 hr, improved the protein digestibility by at least 20 per cent. These results agree with the earlier studies[10,11] wherein an improvement in digestibility of grain legumes with prolonged cooking was observed.

Processing of legumes before consumption is a common practice both in the food industry and in the home. Table 3 gives the effect of different processing conditions on the *in vitro* digestibility of the winged bean as compared to some other legumes. The 'TPt—2' variety of winged bean showed higher digestibility than 'TPt—1,

TABLE 1. *IN VITRO* DIGESTIBILITY OF THE DIFFERENT PARTS OF WINGED BEAN VARIETY TPT-1.

Sample	pH at different incubation time (min) 0	2	4	6	8	10	% digestibility
Raw seeds	8.00	7.95	7.94	7.93	7.92	7.91	67.3
Immature pods	7.99	7.85	7.79	7.74	7.71	7.66	71.8
Mature pods	8.00	7.74	7.67	7.62	7.58	7.55	73.8
Young leaves	8.00	7.00	7.84	7.80	7.76	7.73	70.5
Mature leaves	8.00	7.93	7.89	7.85	7.82	7.79	69.1
Shoot (no pods)	8.00	7.95	7.91	7.88	7.85	7.83	68.7
Entire plant	8.04	7.96	7.92	7.89	7.84	7.66	71.8
Roots	8.00	7.95	7.92	7.90	7.87	7.85	68.4

Regression equation: Y = 210.464—18.103X
where, X = pH of protein suspension after 10 min digestion with multienzyme solution.

TABLE 2. EFFECT OF SOAKING AND COOKING TIME ON *pH* AND *IN VITRO* DIGESTIBILITY OF WINGED BEAN AND SOYBEAN

Time (min)	*Soak* 18 *hr; cook* 5 *hr*		*Soak* 5 *min; cook* 1 *hr*		*Soak* 5 *min; cook* 30 *min*	
	Winged Bean	Soybean	Winged Bean	Soybean	Winged Bean	Soybean
0	8.00	8.00	8.00	8.00	8.00	8.00
2	7.20	7.50	7.21	7.39	7.49	7.70
4	7.11	7.36	7.12	7.29	7.39	7.61
6	7.06	7.28	7.07	7.20	7.33	7.54
8	7.03	7.32	7.04	7.15	7.30	7.50
10	7.01	7.18	7.02	7.11	7.27	7.45
% Digestibility	83.6	80.0	83.5	81.0	78.9	75.0

TABLE 3. EFFECT OF PROCESSING CONDITIONS ON THE *IN VITRO* DIGESTIBILITY* OF WINGED BEAN AND SOME OTHER LEGUMES

Processing conditions	% *Digestibility*							
	Winged Bean		Soybean		Great Northern		Pinto	
Raw seeds	70.71	(33.8)	71.61	(43.8)	77.58	(22.1)	74.87	(20.9)
Ether extracted seeds	72.15	(38.8)	72.70	(54.1)	75.77	(22.2)	73.42	(21.0)
Oven dried seeds	71.43	(34.9)	73.78	(40.7)	74.15	(25.1)	72.33	(24.3)
Dry heated seeds	71.61	(35.5)	74.87	(41.3)	73.42	(23.1)	71.70	(21.3)
Autoclaved seeds	84.28	(35.0)	82.29	(40.6)	79.76	(22.6)	79.58	(20.0)
Casein	93.15							

*Digestibility measured *in vitro* with the multienzyme solution. Values in parentheses indicate the percent protein content of the sample after processing.

Of the different processing methods, moist heat (autoclaving) yielded the highest percent protein digestibility in all the legumes studied. Besides, autoclaving was observed to remove the beany flavour from the meal. Autoclaving of winged bean meal yielded 84.3 percent protein digestibility although both contained almost the same amount of protein.

In Fig. 1 a comparison is made between raw and autoclaved samples of winged bean, soybean and casein. It is evident from the data that autoclaving was far superior to raw seeds in protein digestibility for both legumes (Table 3). When these legumes were autoclaved, winged bean showed the highest protein digestibility (84.3 per cent), followed by soybean (82.3 per cent), Great, Northern (79.8 per cent) and Pinto (79.6 per cent) respectively. As shown in Fig. 1 comparison of the two legumes with casein confirms that casein with the highest percent protein digestibility (93.2 per cent) created the largest pH drop.

Moist heat destroys proteinase inhibitors; enzyme activity, if previously present, is decreased or eliminated thereby permitting the opening of the protein structure through denaturation for hydrolysis by proteolytic enzymes. We suggest that the improvement in the protein digestibility of the winged bean is related to the destruction of the proteinase inhibitors (trypsin inhibitors) during proper heat treatment. Heat treatments must be applied with care because beyond a certain level of heat application, protein digestibility declines due to a non-enzymatic browing reaction, thermal cross-linking[12] and a possible disruption of the linkages between amino acids such that their release during digestion is delayed.

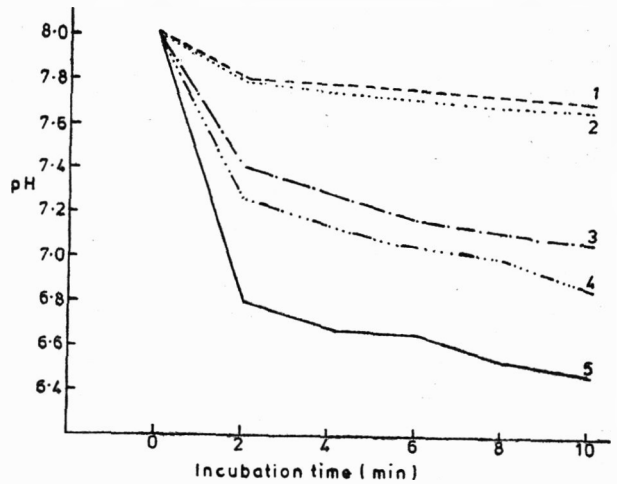

Fig. 1. Effect of heat treatment on *in vitro* digestibility

1, Raw winged bean; 2, Raw soybean; 3, Autoclaved soybean; 4, Autoclaved winged bean; 5, Casein.

A study was conducted to investigate the effect, if any, of fat content on protein digestibility. The *in vitro* digestibility of the raw seeds of winged bean and other legumes was compared to those of ether extracted meals. Results obtained are presented in Table 4. Both in winged bean and soybean, the ether extracted samples had a slightly increased digestibility over the raw samples, whereas the reverse was the case in Great Northern and Pinto beans.

Conclusions: On the basis of experimental data, mature pods with seeds intact yielded the largest pH drop after 10 min of incubation. This corresponded to a high percent digestibility of the protein of the winged bean. On the other hand, raw seeds had the least pH drop indicating a low digestibility.

Various methods of processing influence the digestibility of winged bean differently. Autoclaving (moist

TABLE 4. EFFECT OF FAT CONTENT ON PROTEIN DIGESTIBILITY

Sample	% Fat content (raw samples)	% Protein digestibility	
		Raw	Ether extracted
Winged bean	15.8	70.7	72.2
Soybean	16.1	71.6	72.7
Great Northern	2.4	77.6	75.8
Pinto	1.9	74.9	73.4

heat) was the best method that contributed to the improvement for the digestibility of the winged bean and hence its nutritive value. It eliminated the effect of trypsin inhibitors, removed the beany flavour and imparted an acceptable aroma to the autoclaved meal.

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Availability of Calcium and Phosphate in Calcium Caseinate

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Calcium and phosphate as present in calcium caseinate are physiologically fully available as determined by metabolic experiments in human subjects and absorption experiments in albino rats. The rate of absorption of calcium from calcium caseinate when administered via the stomach-tube to normal rats or injected into the small intestine in anaesthetised animals is comparable with inorganic calcium similarly administered. The trypsin-resistant phosphopeptides released during the digestion of casein in the gut do not seem to survive sufficiently long to interfere with the absorption of calcium.

Calcium caseinate is a ternary nutriment which provides sizable proportions of the valuable mineral elements, calcium and phosphate in addition to protein, all in a readily water dispersible combination. The nutritional adequacy of this preparation for application as a general protein supplement[1] and in the particular management of protein malnutrition and allied disorders has been demonstrated[2]. However, the physiological availability of the mineral elements contained in it has not been assessed. Such studies seemed quite essential and relevant in the context of the formation of trypsin-resistant phosphopeptides during the course of digestion of the protein in the gastro-intestinal tract, which have marked sequestering-action on calcium and phosphate[3-5]. Metabolic investigations on human subjects and absorption studies in albino rats were, therefore carried out to ascertain the extent of availability of calcium and phosphate in calcium caseinate.

Materials and Methods

Calcium caseinate: It was prepared according to the process developed at this Institute[6] and used in all the studies. It had the following proximate composition: moisture, 4.5; protein (N×6.38), 84; carbohydrate, 5.8; ash, 3.2; phosphorus, 0.8 and calcium, 1.0 per cent.

Analytical procedures: The Kjeldahl digestion procedure[7] was employed for the estimation of nitrogen, the oxalate method[8] for the estimation of calcium in human metabolism studies and the casein-EDTA-complexone titrimetric method adapted from Baron and Bell[9], for the determination of calcium in the absorption experiments with rats. Inorganic phosphate was deter-

mined by the Fiske-Subba Row[10] procedure after digestion with nitric and sulphuric acids[11]. Amino nitrogen was determined by the copper-phosphate method of Spies and Chambers[12].

All reagents used in the analytical procedures were of analytical reagent quality.

Human metabolism studies: After preliminary medical examination, seven healthy boys from a local orphanage in the age group of 8-11 years and weight range of 20-25 kg were chosen as the test subjects. They were de-wormed by oral santonin administration a month prior to the metabolic experiment.

During the metabolic period of ten days, the subjects received standardized meals at breakfast, lunch and dinner, which consisted mainly of rice and wheat preparation along with pulses, vegetables and small quantities of milk or buttermilk. The pattern was similar to the regular orphanage diet. The foodstuffs and nutrient content of the basal diet as computed from the data provided by Aykroyd[13] are given in Table 1. The first six days were treated as a preliminary period to adapt the subjects to the logistics of the feeding programme and to the equipment used for the collection of urine and faeces. Urine and faeces were collected during the last four days using the usual preservatives[14]. Strict record of quantities of food consumed were maintained. Duplicates of all dishes served to the subjects were collected daily, dried in an air-oven at 95-100°C and weighed for proximate analysis. Indigocarmine was used as a marker for faeces. Collection and estimation of urinary creatinine served as a check on completeness of urine collection. The diet and excretory products were analysed for calcium, phosphorus and nitrogen.

On completion of the first metabolic experiment on the basal diet, a second experiment was carried out during which each subject received the same basal diet along with a supplement of 40 g equivalent of calcium caseinate. The caseinate was made into a beverage by dispersing in water, milk or buttermilk (according to the subjects' choice) and served at lunch and dinner, 20 g equivalent being given each time. As before, the period of test-feeding was ten days, urine and faeces were collected during the last four days. The average food intake during the two periods did not differ by more than five per cent. The caloric intake was 1,400 calories per caput per day.

Intestinal absorption in rats: Preliminary orienting experiments indicated an absorption of 40-50 per cent of 10-20 mg calcium fed as calcium caseinate to adult male rats in 1 hr. Comparative tests were then carried out.

The absorption of calcium from calcium caseinate was studied in adult, male albino rats (250-300 g body weight) and compared with that from calcium chloride administered in equivalent amounts. Tests were made with both force-fed rats (stomach-tube fed) and amytal anaesthetized animals into whose small intestine ligated 3-4 cm away from the duodenum and also at a similar distance from the caecum, the preparations were injected. The procedure was essentially the same as that suggested by Magee[15] and Otinell and Hober[16]. The absorption was determined as the difference between the quantity of calcium administered and that left in the gut at the end of the period. All animals were fasted for 24 hr prior to the test. The calcium chloride solutions were prepared by dissolving pure calcium carbonate in hydrochloric acid.

TABLE 1. FOODSTUFFS AND NUTRIENT CONTENT OF CONTROL DIET (MEAN INTAKE PER DAY PER SUBJECT)

Foodstuffs	
Rice (raw, milled) (g)	350
Red gram (*Cajanus cajan*) (g)	20
Wheat flour (*Chapati*) (g)	10
Horse gram (*Dolichos biflorus*) (g)	20
Vegetables (Amaranth, ashgourd, brinjal, chocho, calabash, cucumber and onions) (g)	100
Condiments (red chillies, coriander, cumin, fenugreek, garlic, pepper and turmeric) (g)	5
Tamarind (*Tamarindus indicus*) (g)	5
Groundnut oil	20
Skim milk powder	5
Jaggery (crude cane-sugar) (g)	25
Common salt (crude salt) (g)	12
Supplement	
Edible starch (maize starch) or calcium caseinate (g)	45
Nutrients*	
Calories (k. cal.)	1400
Protein (N×6.25) (g)	36.2
Fat (g)	22.2
Carbohydrate (g)	335.2
Calcium (mg)	366.0
Phosphorus (mg)	645.3
Iron (mg)	15.1
Thiamine (mg)	0.4
Riboflavin (mg)	0.4
Nicotinic acid (mg)	5.6
Ascorbic acid (mg)	35.8
Vitamin A (I.U.)	1268

*All values were calculated from figures given by Aykroyd (1956), Health Bulletin No. 23, *The Nutritive Value of Indian Foods and the Planning of Satisfactory Diets*, 5th Ed., Published by The Manager of Publications, Government of India, New Delhi.

Results and Discussion

Human metabolism experiments: The metabolic balance data on human subjects presented in Table 2 make it obvious that nearly 95 per cent of the 400 mg of calcium supplement provided in the combined form as calcium caseinate is absorbed of which 60 per cent is ultimately retained. Of the phosphorus supplement of 312 mg provided by the caseinate, 75 per cent is absorbed of which 70 per cent is ultimately retained. In the case of protein[17], however, the digestion and absorption are well over 95 per cent of the supplement ingested but much of it appears to be catabolised and the end products excreted in the urine with only 20 per cent being retained. The retention on the basal regimen was also only about 32 per cent. This situation is probably due to the additional protein-load which considerably exceeds the recommended allowances for that particular age group[18].

The high degree of absorption and retention of calcium and phosphate of the calcium caseinate points to their almost complete physiological availability. The very efficient absorption of calcium appears to be particularly noteworthy. It may be ascribed to the favourable influence of the high protein supplement on the pH in the gut which may be maintained on the relatively acid side conductive to better calcium absorption[19] or the specific effect of certain amino acids like lysine, valine, etc. which may be released during digestion of the casein and which complex with the calcium effectively to increase its absorption[20-22]. The use of peptone and protein derivatives for increasing calcium absorption has been demonstrated[23]. Mellander[3] and Mellander and Olsson[4] claim that the phosphopeptides of casein which avidly combine with calcium promote better absorption of the mineral in the normal as well as the vitamin-D deficient state in humans[24,25] and experimental animals[26].

Absorption studies in rats: The absorption experiments in rats fully substantiated the findings on human subjects with regard to complete availability of both calcium and phosphate in calcium caseinate. The results pertaining to the absorption of calcium (Fig. 1) from the whole gastrointestinal tract in adult rats after administration of test doses of calcium chloride and calcium caseinate by stomach tube indicates that in the first 1-2 hr the absorption is the same in both cases but afterwards the calcium from the caseinate lags behind for another 2-3 hr, catching up with it again in 6 hr.

The picture of absorption when the test materials were instilled directly into intestinal loops was slightly different (Fig. 2). The rate of calcium absorption from calcium caseinate was relatively slower than that from calcium chloride. In contrast, in the force-fed experiments, absorption of calcium in the first hour was almost the same from either calcium caseinate or calcium chlo-

TABLE 2. AVAILABILITY OF CALCIUM, PHOSPHORUS AND PROTEIN OF CALCIUM CASEINATE

Human metabolism experiment: 7 young males, body weight 20-25 kg.

Diet	Daily intake	Daily excretion			Retention	% absorption
		Urinary	Faecal	Total		
			Calcium (mg)			
Control	314	37	119±38	156	158±46	62.2±8.1
Supplemented	714	159	140±38	299	415±46	80.4±8.1
			21±54 NS		257±64**	18.2±11.4 NS
			Phosphorus (mg)			
Control	728	174	370±27	544	184±26	49.2±3.3
Supplemented	1040	179	448±27	628	412±26	56.9±3.3
			78±38*		228±37***	7.7±4.7 NS
			Nitrogen (g)			
Control	5.90	1.97	2.06±0.05	4.03	1.87±0.31	65.1±1.1
Supplemented	11.40	6.49	2.23±0.05	8.71	2.69±0.31	80.5±1.1
			0.17±0.07*		0.82±0.43 NS	15.4±1.5***

All standard errors based on 6 df
NS, not significant ($P>0.05$)
Significance of difference from control values
*$P<0.05$; **$P<0.01$; ***$P<0.001$

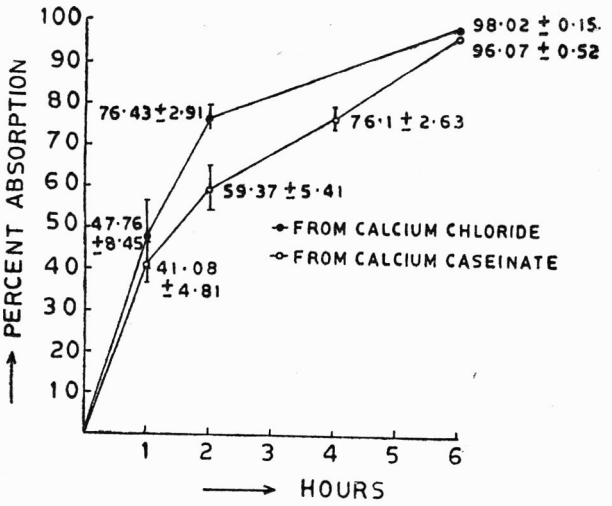

Fig. 1. Absorption of calcium caseinate

1, 2, 4 and 6 hr. absorption from G-I tract of groups of rats (5 for each interval) force fed 1 g of calcium caseinate in 5 ml aqueous dispersion. The calcium absorptions have been compared with that from an equivalent force fed dose of aqueous calcium chloride solution (3 ml of 0.11 M concentration).

ride and probably indicates that the acid-chyme from the stomach reaching the proxinal duodenum favourably influences the calcium absorption from caseinate.

The phosphate absorption from the caseinate was 40.0±12.7; 62.5±4.9 and 83.8±4.0 per cent of dose in 1.3 and 6 hr respectively, and was in general agreement with the absorption of calcium

Fate of the caseinate in the gastro-intestinal tract: At the end of a 1 hr of absorption, a good proportion of the protein was noticeable as a coagulum in the stomach

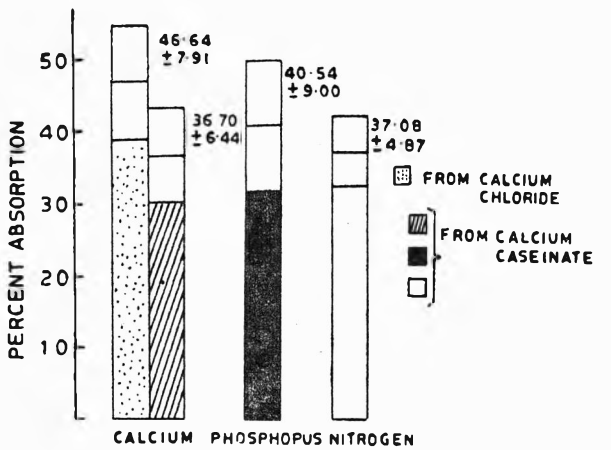

Fig. 2. Absorption of calcium, phosphorus and nitrogen from calcium caseinate in 1 hr.

500 mg calcium caseinate in 5 ml aqueous dispersion were injected into the small intestine of anaesthetized adult albino rats. The calcium absorption has been compared with that from an equivalent dose of calcium as aqueous calcium chloride solution (5 ml of 0.02 M concentration).

which, with the progress of time gradually disappeared. After 6 hr, when most of the calcium had been absorbed, the stomach was practically empty. The coagulum at the end of 3 hr amounted to 26.5 per cent of the ingested protein and had the same N/P ratio (40). On treatment of the soluble contents of the stomach with barium acetate in 50 per cent alcohol, no precipitate containing peptides was detected.

The situation with the intestinal lumen contents was different. At the end of 1 hr absorption only 13.9 per cent of the protein could be recovered by precipitation with acetic acid at pH 4.6 in the normal way. Even this precipitated protein had a different N/P ratio (98.6); which suggested that the protein was relatively phosphorus-poor. The soluble portions on treatment with barium acetate in 50 per cent alcohol, gave a precipitate that did not contain any peptide material, but was found to be barium phosphate. This at the end of 3 and 6 hr accounted for 15 and 10 per cent respectively of phosphate originally ingested. The relatively phosphorus-poor protein from the intestinal contents pointed to cleavage of phosphate from the protein, which is likely to occur in the intestines due to the action of phosphatases[27-29]. Incubation of caseinate suspensions (4-6 mg P equivalent) in loops of intestines at 37°C for 3 hr *in vitro* indicated a release of inorganic phosphate to the extent of 56 per cent.

The extent of proteolysis in the gastro-intestinal tract measured as the ratio of amino N to total N in the force fed experiments at the end of 1 and 3 hr was fairly uniform (47.2±7.0 at the end of 1 hr; 43.7±9.3 at the end of 3 hr). The absorptions for these periods were 26.6±6.6 and 71.9±4.8 per cent of the dose respectively.

Physiological degradation of casein phosphopeptides: In casein, it is known that mono-di-, and pyrophosphate linkages occur[30,31]. The wider specificity of intestinal alkaline phosphatase[32] and the more specific phosphoprotein phosphatase[28,29] which can act in phosphopeptones[33] and also partially dephosphorised casein[34] would be the enzymes involved in the cleavage of phosphate. However, the different forms of phosphate in casein may differ in their susceptibility to these phosphatases and rate of release of phosphate. This could possibly be the reason for the relative slowing down in the phosphate absorption rates from the gastro-intestinal tract between 2 and 3 hr as noted. The absence of typical phosphopeptides in the lumen, suggest that under physiological conditions, the enzyme-resistance of these peptides and their survival are probably governed by parallel activities of phosphatases. Here, it is worth recalling the experiments of Csopak *et al.*[34] and Donin[35] who have shown that dephosphorized casein is readily susceptible to proteolysis. Also, Theodoropoulos *et al.*[36,37] have actually prepared synthetic phosphopeptide sub-

strates suitable for tryptic-action and demonstrated the ready vulnerability of these peptides to acid phosphatases only after dephosphorization[38].

It is thus apparent that the pepsin/trypsin-resistant phosphopeptides of casein released during the physiological digestion of the protein in the gastro-intestinal tract are rapidly dephosphorized by the action of non-specific phosphatases (and also any specific phosphoprotein phosphatases) and subsequently further degraded by the proteolytic enzymes. Therefore, they do not have any opportunity to interfere with the absorption of either calcium or phosphate. Direct experimental tests employing the phosphopeptides in concentration in normal and rachitic animals would be required to reveal any possible influence they may exert on the rates of absorption of these inorganic elements. Such studies have been conducted and the results will be reported elsewhere.

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Survival of Microorganisms in Intermediate Moisture Foods

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Survival of *Staphylococcus aureus*, *Saccharomyces cerevisiae* and *Aspergillus flavus* in an IMF system has been studied. *S. cerevisiae* were the first to be destroyed followed by *S. aureus*. *A. flavus* survived longest. In addition to the reduced a_w, glycerol, potassium sorbate and potassium metabisulphite independently exert inhibitory effect on the growth and survival of these microorganisms.

In India, work has been initiated on intermediate moisture foods (IMF) to prepare a number of products for the use of the Defence Services[1-3]. The stability of the product is very much influenced by microorganisms[4], the activity of which is mostly limited by the water activity (a_w) of the food. The method of preparation involves lot of handling and the chances of microbial contamination are plenty. Therefore, studies were undertaken to know the fate of microorganisms which may get introduced into the product and to follow up the growth/survival of different types of microorganisms when inoculated into an IMF system and stored.

Materials and Methods

Three microorganisms belonging to bacteria, yeasts and moulds were selected for the study.

Bacterium: Staphylococcus aureus was selected as the test organism on account of its ubiquitous and enterotoxigenic nature[5]. Besides, amongst the non sporulating bacteria, this is the most resistant to salt, sugar and heat. Its presence in IMF is of significance for two reasons, (*i*) it can be an indicator of unhygienic practice in the preparation of the product, and (*ii*) it may contain the enterotoxin at levels which constitute a health hazard. The test organism was isolated in the laboratory from a raw mutton sample brought for processing.

Yeast: Saccharomyces cerevisiae isolated in the laboratory from a high sugar containing preserved fruit, was selected since yeasts tolerate high sugar concentrations.

Mould: Since moulds are known to grow at very low water activity as compared to yeasts and bacteria, *Aspergillus flavus* was also included in the study. It was also of interest to understand the survival of toxigenic moulds in IMF system. The organism was isolated from processing areas in the laboratory.

IMF system studies: The intermediate moisture food system used was IM banana made by the method of Jayaraman *et al*[3]. The IM banana was homogenised in a waring blender in order that the inoculum when introduced could be well mixed. Each batch was divided into 4 lots. One lot was inoculated with *Staphylococcus aureus*, second lot with *Saccharomyces cerevisiae* and the third with *Aspergillus flavus*. The 4th served as control. The cultures were grown and maintained on standard media[6]. *S. aureus* was grown on nutrient agar (NA) slants at 37°C for 16-18 hr and *A. flavus* on Potato Dextrose Agar (PDA) at 32°C for 72 hr. Cells/spores were washed into quarter-strength Ringer's solution and the suspensions were introduced by spraying them individually into each lot of the IM banana and mixed thoroughly with the product in a mixing bowl. A minimum quantity of water was used so that the water activity of the resulting product was essentially unchanged. Immediately after the inoculation they were analysed in triplicate to get the initial viable count using media and diluents as per standard methods[6]. The materials (100 g) were then distributed in paper/foil/laminated pouches and one half of each was kept at room temperature (RT) and the other at -5°C. Samples at RT were analysed at more frequent intervals initially and subsequently at monthly intervals along with those at 5°C.

The above IM banana had potassium sorbate (KS) and potassium metabisulphite (KMS) in the infusion liquid. To understand the role of KS and KMS separately and in combination, IM banana was also made with KS alone and KMS alone in the infusion liquid. Inoculation studies were carried out as in the earlier case along with sets having KS and KMS and both KS and KMS.

Results and Discussion

The changes in the microbial population on storage at both the temperatures are given at Tables 1, 2 and 3 alongwith the initial count. It may be seen that the

TABLE 1. CHANGES IN *Staphylococcus aureus* DURING STORAGE

Storage period (days)	Storage temp. (°C)	Number of organisms in Control	KS+KMS	KS alone
0	—	79.5×10^6	78.5×10^6	85.4×10^6
1	37	12.5×10^6	14×10^1	45×10^4
2	37	20×10^5	9×10^1	22×10^1
5	37	69.5×10^2	—	—
15	37	47.5×10^1	6.5×10^1	11.5×10^1
120	37	Nil	Nil	Nil
	-5	28×10^4	15×10^3	61.5×10^3

KS - Potassium sorbate; KMS - Potassium metabisulphite

TABLE 2. CHANGES IN *Saccharomyces cerevisiae* DURING STORAGE

Storage period (days)	Storage temp. (°C)	Number of organisms in Control	KS+KMS	KS alone
0	—	71.5×10^5	60×10^5	96×10^5
1	32	30×10^4	7×10^1	89×10^4
3	32	73.5×10^3	6×10^1	73.5×10^3
30	32	1×10^1	Nil	1×10^1
120	32	Less than 10	Nil	Nil
	-5	22×10^4	79×10^1	35×10^4
180	32	Nil	Nil	Nil
	-5	20×10^4	Nil	15.5×10^4

TABLE 3. CHANGES IN *Aspergillus flavus* DURING STORAGE

Storage period (days)	Storage temp. (°C)	Number of organisms in Control	KS+KMS	KS alone
0	—	74×10^6	90×10^6	62×10^6
3	32	21×10^4	2×10^2	20×10^2
30	32	86×10^3	Nil	8×10^1
	-5	20×10^4	,,	17×10^1
60	32	40×10^3	,,	<10
	-5	11×10^4	,,	Nil
180	32	65×10^2	,,	,,
	-5	86×10^3	,,	,,

TABLE 4. CHANGES IN *Staphylococcus aureus* IN IM MANGO ON STORAGE AT RT.

Initial	15 days	60 days
73×10^{10}	2×10^1	Nil
52×10^9	9×10^1	,,
31×10^9	5×10^1	,,
33×10^9	2×10^1	,,

microbial load showed a progressive decrease with storage time at RT. This was all the more pronounced in the case of *S. aureus* and *S. cerevisiae*. However, in the case of *A. flavus* the decline was comparatively slower, probably because *A. flavus* can grow at lower a_w ranges than the other two organisms. An inoculum calculated to have a cell concentration of 10^{12}/g on the product basis had to be added in order to have an initial load of 10^6/g as their number gets drastically reduced immediately on introduction into the IMF atmosphere. Table 1 shows that the number surviving after two days' storage is 10^4 in the presence of KS whereas without KS it is 10^6. The controls which had no KS or KMS became visibly mouldy in 15-18 days. Labuza *et al.*[7], however, observed that they disappeared immediately after preparation. The reduction is faster in the presence of KMS. Hence, although KMS is added primarily to control non-enzymatic browning, the results show that it has a microbicidal action. KMS releases molecular SO_2 during dissociation of the complex[8].

The number of staphylococci recovered at the end of four months of storage was extremely low. Thus, although sorbic acid and its salts are normally incorporated as mould inhibitors in products, they could also inhibit staphylococci when there is no competition with other microorganisms. Hollis *et al.*[9], also founds significant decrease of staphylococci in the food systems (4 log cycles in 3 months). In addition to the reduced water activity, the antimicrobial action of KS, glycerol and KMS also appear to add to the inhibitory effect on the growth and survival of these microorganisms. Perhaps there may be a synergistic effect also. Compared to staphylococci, the effect on *S. cerevisiae* is much higher as all were destroyed by the end of one month storage period. This indicates that staphylococci can survive in IMF for much longer periods than yeasts. Similar results were obtained with IM mango also (Table 4).

The studies indicate that addition of KS and KMS is necessary and microorganisms cannot grow or proliferate in IMF system in their presence. As *S. aureus* is a ubiquitous organism occurring in persons handling foods, the risk of enterotoxigenic staphylococci occurring in IMF can be expected as with many other manufactured foods of composite nature if proper precautions are not taken. The greatest single protection thus lies in maintaining highest standards of hygiene at all stages of production. The studies further confirm the earlier observation that the IMF systems not only prevented the growth of pathogens but caused their reduction[9].

Acknowledgement

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Changes in Pectic Substances of Citrus Fruits Infected with Different Citrus Pathogens

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Penicillium digitatum, *Penicillium italicum*, *Diplodia natalensis* **produced soft rot and** *Alternaria tenuis* **produced firm rot in citrus fruits. The firm rot fungi reduced total pectic substances by 10-13% and soft rot fungi by 49-55%. The soft rot fungi had little effect on alkali fraction, whereas the firm rot fungi caused substantial increases. Soft rot fungi produced pectin methylesterase, polygalacturonase, and macerating enzyme in culture, but in firm rot fungi, the polygalacturonase enzyme is inactivated.**

Infection of fruit by fungi may cause different degree of changes in fruit texture. Two types of rots, soft and firm, have been distinguished in peaches[1] and apple[2] infected with different fungi. Some relationship was suggested between the type of rot which occurred and the nature of changes in pectic substances in infected fruit. Akinrefon[3], Cole and Wood[4] and Cohen *et al.*[5] reported that losses in pectic material were greater in soft rot as compared to firm rot. The importance of pectolytic enzymes in pathogensis has long been recognized[6].

The changes in pectic substances in albedo of citurs fruits infected with *Penicillium digitatum*, *Diplodia natalensis Pencillium italicum* and *Alternaria tenuis*, and production of pectolytic enzymes *in vitro* by pathogens have been described in this paper.

Materials and Methods

Water soluble, acid soluble and alkali soluble fractions of pectin in lime (*Citrus aurantifolia*), *mosambi* (*Citrus sinensis*) and orange (*Citrus reticulata*) inoculated with *P. digitatum D. natalensis*, *P. italicum* and *A. tenuis* were determined. Fruits were stored at 25°C for 7 days. Peels from healthy and infected fruits were dried at 60°C and finely ground. Dried peel (100 mg) powder was taken in a glass tube and pectin fractions were extracted progressively with water, HCl (N/75), and NaOH (N/75). Pectin content in the fractions was analysed by the colorimetric method of Dietz and Rouse[7].

To produce pectolytic enzymes, citrus pathogens were grown on asparagine pectin medium consisting of 2 g asparagine, 10 g pectin, 1 g KH_2PO_4 and 0.5 g Mg $SO_4.7H_2O$, a trace amount of an orange peel extract, and the volume made to one litre. The medium was dispensed in 100 ml aliquots into 250 ml Erlenmeyer flasks and sterilized by autoclaving at 15 psi for 20 min. Flasks were inoculated with 5 mm diameter disc cut from the edge of potato dextrose agar culture. After 5 days of incubation in shake culture, mycelium was removed and the filtrate centrifuged at 9,000 rpm for 10 min to make it cell free. Solution was stored at 20°C until required.

Pectinesterase activity was measured by titrating the

TABLE 1. PECTIC SUBSTANCES IN ALBEDO OF LIME, MOSAMBI AND ORANGE INFECTED WITH CITRUS DECAY PATHOGENS AS MG ANHYDROGALACTURONIC ACID PER G DRY WEIGHT OF ALBEDO

Albedo	Lime pectic fraction soluble in			Mosambi pectic fraction soluble in			Orange pectic fraction soluble in		
	H_2O	HCl N/75	NaOH N/75	H_2O	HCl N/75	NaOH N/75	H_2O	HCl N/75	NaOH N/75
Uninfected	159.00	167.00	22.00	186.00	205.00	31.00	172.40	183.50	26.30
Infected with *P. digitatum*	55.65	84.83	20.00	70.00	97.10	27.60	62.20	86.94	22.40
D. natalensis	60.20	97.30	22.00	74.00	94.30	26.00	71.20	95.40	24.00
,, *P. italicum*	71.78	78.00	19.00	86.40	104.30	29.00	68.00	99.50	23.10
A. tenuis	130.25	142.51	28.00	158.00	184.00	38.00	150.60	160.00	29.40

carboxyl groups liberated from a 1.0 per cent solution of pectin[8].

The polygalacturonase (PG) activity was determined by the loss of viscosity of pectin solution, and macerating activity by the disc method[9]. PG activity has been expressed as 100 divided by the time taken for the enzyme to reduce 50 per cent of the total possible loss of viscosity. The macerating activity is given as the time in min taken for the disc to lose coherence.

PE activity was determined in the pH range of 3.5 through 6.6 and of PG in the range of 4.5 through 6.0.

Extracts from sound and infected fruits were examined by descending chromatography on Whatman No. 1 paper using *n*-butanol, acetic acid and water as solvent. Chromatograms were developed with aniline acetic acid reagent[10].

Results and Discussion

In the albedo of lime infected with *P. digitatum*, the loss in total pectin was 53.8 per cent (Table 1). The water soluble, acid soluble and alkali soluble fractions were reduced to 65.0, 49.5 and 9.10 per cent respectively of the original value. When infected with *D. natalensis* and *P. italicum*, the pattern was similar except that the value differed (Table 1). When infected with *A. tenuis*, losses in total pectin and solubility in different fractions were small. Similar changes occurred in orange and *mosambi* fruits infected with these fungi.

The results show that in citrus fruits infected with *P. digitatum*, *P. italicum* and *D. natalensis* which produce soft rot, there is a marked derease in all pectic fractions. Water soluble fraction of pectin reduced considerably than the insoluble fraction. Very little change occurred in the NaOH soluble fraction. *A. tenuis* produced firm rot and caused substantial increase in NaOH soluble fraction (11.78-27.27 per cent).

Each of the fungi reduced the quantity of pectin fraction, insoluble in water but soluble in dilute acid. The pectin fraction insoluble in water amounts to 56 per cent, which is expected to contain protopectin. The firm rot fungi, *A. tenuis*, caused relatively little change in this fraction, but the soft rot fungi reduced it by 41-56 per cent. The fraction insoluble in acid, but soluble in dilute alkali consists of mainly pectic acid and insoluble pectates. It is present in the middle lamella. In soft rot, this fraction is less in comparison to that in healthy tissue, but it is substantially higher in firm rot. The firm rot fungi is expected to alter the solubility of some of the acid soluble fraction of sound citrus fruits, probable by demethylation of pectinic acid to give pectic acid.

Effect of pH on PE and PG activity: PE activity was found to be maximum at pH 3.5 in culture filtrate of *P. digitatum*, 4.0 in *P. italicum* and 5.1 in *A. tenuis* and *D. natalensis*, (Fig. 1, 2, 3 and 4). The optimum pH for PG activity was 5.0 in *P. digitatum*, *D. natalensis* and *A. tenuis* and 4.5 in *P. italicum* (Table 2). Macerating

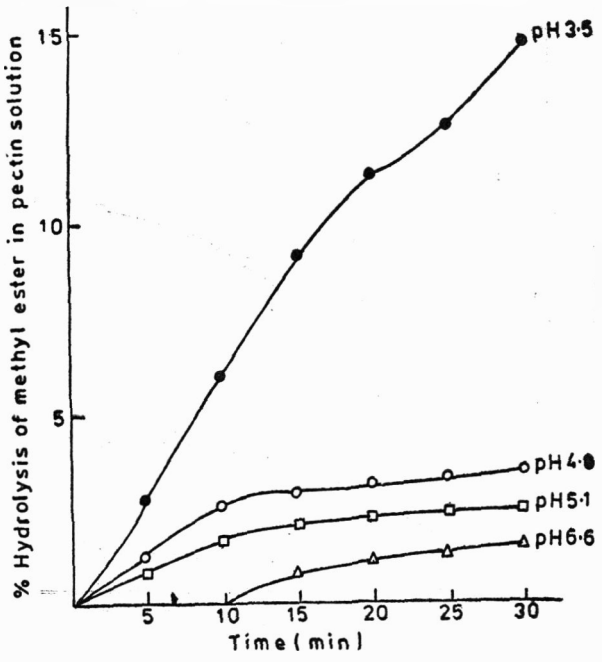

Fig. 1. Effect of pH on pectin methylesterase activity of culture filtrate of *Penicillium digitatum* grown on pectin.

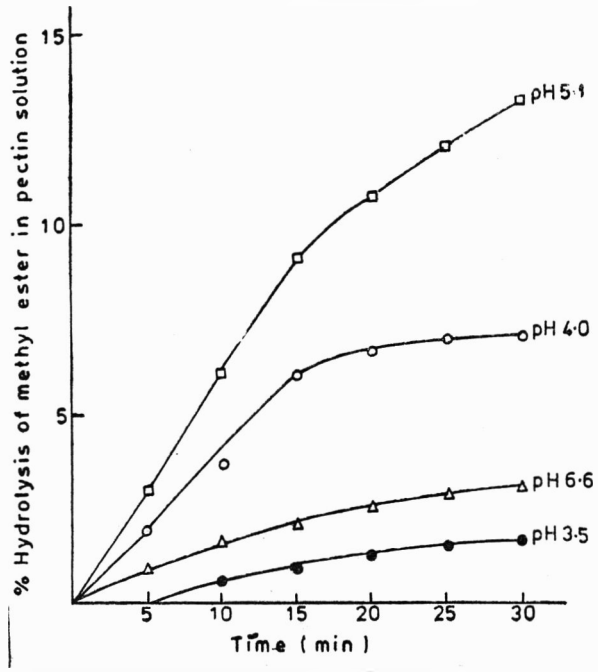

Fig. 2. Effect of pH on pectin methylesterase activity of culture filtrate of *Diplodia natalesnis* on pectin.

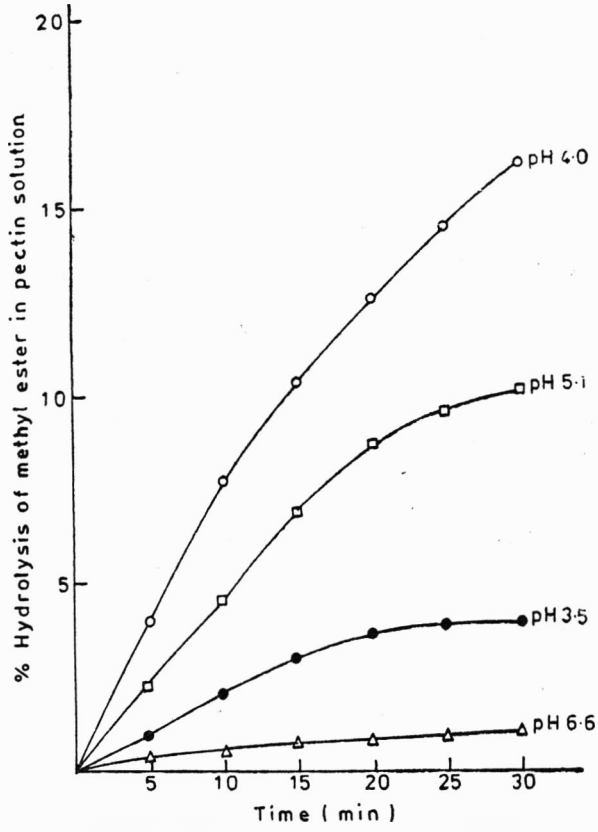

Fig. 3. Effect of pH on pectin methylesterase activity of culture filtrate of *Penicillium italicum* grown on pectin.

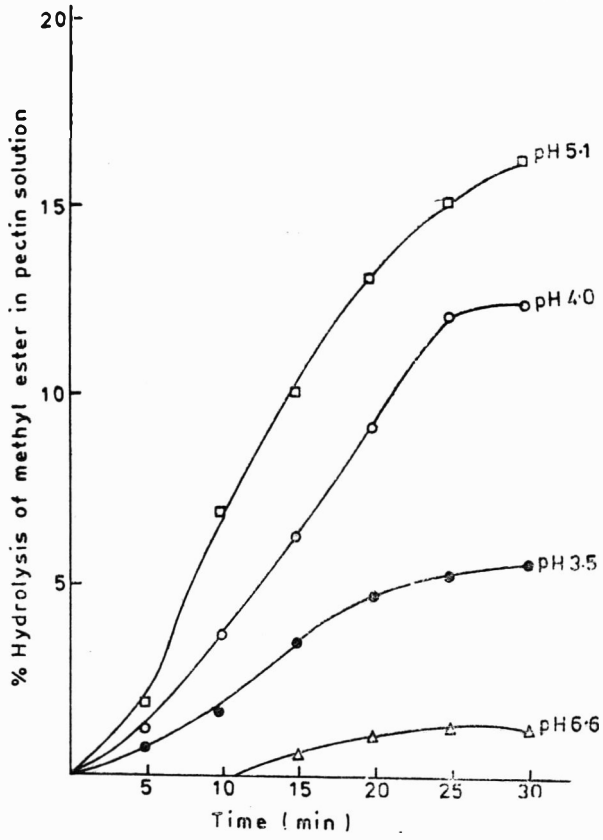

Fig. 4. Effect of pH on pectin methylesterase activity of culture filtrate of *Alternaria tenuis* grown on pectin.

TABLE 2. EFFECT OF PH ON POLYGALACTURONASE ACTIVITY OF CULTURE FILTRATES OF FUNGI

Microorganism	PG activity at different pH			
	4.5	5.0	5.5	6.0
P. digitatum	5.6	6.5	5.0	3.6
D. natalensis	4.2	5.0	3.2	3.0
P. italicum	6.1	5.0	4.0	2.8
A. tenuis	2.8	4.8	3.0	4.0

TABLE 3. MACERATING ACTIVITY OF CULTURE FILTRATES OF PATHOGENS

Microorganism	Macerating activity (100/time in min.)
P. digitatum	2.7
D. natalensis	2.0
P. italicum	2.6
A. tenuis	1.8

TABLE 4. PAPER CHROMATOGRAPHY OF PEEL FROM HEALTHY AND INFECTED TISSUE

	Galacturonic acid	Lower mol wt. breakdown products of pectin
Healthy fruit	no spot	no spot
P. digitatum	+ + +	+ + +
D. natalensis	+ +	+ + +
P. italicum	+ +	+ + +
A. tenuis	traces	no spot

Increase in spot size is indicated by more + signs.

activity was greater in culture filtrate of *P. digitatum* and lower in *A. tenuis* (Table 3).

Paper chromatography of extracts from healthy and infected tissues from citrus fruits showed three reddish brown spots of Rf 0.24, 0.10 to 0.11 and 0.04 to 0.05 in the infected fruits but no spot was visible in case of healthy fruit. Rf value of 0.24 corresponded to Rf of standard D-∝-galacturonic acid. The other two spots having lower Rf values may be said to be due to pectic acid units having more than one galacturonic acid units. These results indicate that the three pathogens studied, viz., *P. digitatum*, *D. natalensis* and *P. italicum* produced breakdown products of polygalacturonic acid. Trace of spot was observed in *A. tenuis* rot indicating a little breakdown of pectic substances. As the time of reaction increased, the intensity of the spot corresponding to galacturonic acid increased and the two others corresponding to Rf 0.10 and 0.04 decreased. It is believed that this is a characteristic feature of polygalacturonase activity.

The most important result from the analysis of water extracts of healthy and rotted tissues by paper chromatography was the apparent absence of low molecular weight polymer of galacturonic acid in *A. tenuis* (Table 4) and its presence in greater amount in *P. digitatum* as it degraded the insoluble pectic materials most extensively.

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Effects of Carriers on the Oral Toxicity of Lindane (γ-BHC) to Albino Rats

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Several formulations of Lindane are being used for the protection of food grains from insect attack. While formulating, it is essential to minimise the hazards and risks to non-target species. Lindane (γ-BHC) carried in various oils of both vegetable and mineral origin was orally administered to adult albino rats at 125 mg/kg body weight. Higher mortality was obtained with Lindane carried in coconut, groundnut, and mustard oils. All the animals recovered with castor oil, SAE-90, and Dutrex-10. It appeared that the toxicity of Lindane depended on its solubility in the carriers and viscosity of carriers used. It is suggested that the incorporation of mineral oils in Lindane formulations is advantageous in promoting mammalian safety.

Generally oils are used as solvents and carriers in pesticidal formulations. It is well established that the insecticides are more potent in mineral oils than in vegetable oils[1-3].

While applying the pesticide formulations, it is imperative to protect the non-target animals, live stock and humans from their toxicity hazards. The currently used Lindane formulations are no exceptions. It has

been reported that the acute oral LD_{50} of Lindane to albino rats varied from 125 to 225 mg/kg body weight[4,5]. Considering the efficiency, persistence on jute bags, availability and cost, a standard oil-based formulation known as Durobase oil containing a mixture of vegetable and mineral oils, was formulated[3]. The acute oral LD_{50} value of Lindane in Durobase oil established was 115 mg/kg body weight[6] and hence this dosage was selected.

As the oral toxicity profile of Lindane was found to be highly variable due to the impurities, carriers and other substances in the formulations[7], attempts were made to study the influence of carrier oils (both of vegetable and mineral origin) on the toxicity of Lindane to albino rats. Another objective was to select a suitable carrier for Lindane ensuring mammalian safety.

Materials and Methods

Animals: Adult female albino rats (*Rattus norvegicus*, Wistar-CFT strain) weighing 150-200 g were grouped by randomised block design. Individually caged rats were conditioned for two days by feeding with cooked basal diet. Water was provided constantly.

Carrier oils: All the oils screened were of commercial grade (Table 1). Durobase oil consisted of groundnut, castor, and jutebatching oil. The mineral oils were obtained from Indian Oil Corporation. They were petroleum products obtained from crude oil as per the specifications of Society of Automotive Engineers (SAE).

Technical Lindane (99.9 per cent) was ground to a fine powder in a pulveriser and solutions of suitable concentration were prepared with each oil (Table 1). Lindane was fed to the animals at 125 mg/kg body weight. The actual doses were calculated according to the body weights of rats and the total volume of solution intubated ranged from 0.9 to 1.5 ml per animal. Specific gravity and viscosity (in Brooksfield viscometer expressed as centipoise units) for all oils were measured. Solubility of Lindane in different oils was also determined.

The animals were partially starved prior to the intubation of chemical. Ten rats were used in each test. Animals intubated with carrier oils alone (at 1.5 ml/animal) served as controls. Symptoms and mortality were noted for four weeks. The survivors were maintained with cooked basal diet and water, and weekly body weighs were recorded. At the end of fourth week autopsies of all survivors were conducted. After recording the fresh weights of organs like liver, kidney, heart and spleen, they were fixed in formalin and processed for histological observations.

Results and Discussion

Mortality: Animals intoxicated with Lindane were hypersensitive, excited, lost equilibrium and breathed rapidly. Convulsions occurred before death. Maximum number of animls died with coconut oil and groundnut oil-Lindane solutions (Table 1). No death occurred when Lindane was carried in castor oil, SAE-90 or Dutrex-10. None of the carrier oils (controls) were toxic to rats.

TABLE 1. CARRIER OILS USED TO DETERMINE THE TOXICITY OF LINDANE IN FEMALE ALBINO RATS

Carrier oil*	Mortality** (%)	Sp. gr.	Viscosity (centipoise units)	Lindane solubility (g/100 g carrier)
Groundnut oil	70	0.91	60	15.10
Coconut oil	70	0.92	48	15.12
Mustard oil	60	1.01	64	9.62
Durobase oil	50	0.96	47	9.98
Jute batching oil	30	0.86	10	8.73
Olive oil	20	0.91	66	10.46
Cotton seed oil	10	0.91	53	10.25
SAE-30	10	0.89	181	5.48
Castor oil	0	0.94	465	7.07
Dutrex-10	0	1.03	300	6.98
SAE-90	0	1.90	420	4.19
Water	10	1.00	1	10 ppm.

*The carrier oils without Lindane were nontoxic

**All the test animals were dosed at 125 mg per kg body weight.

The time-response (mortality) curves (Fig. 1) indicated that the maximum death period was 144 hr with the exception of water. Thirty to forty per cent of the animals died within 24 hr when mustard, coconut and groundnut oils acted as carriers. In contrast, there was only 10 per cent mortality within 24 hr with SAE-30. The death time ranged from 96 to 120 hr whenever higher mortalities were noted.

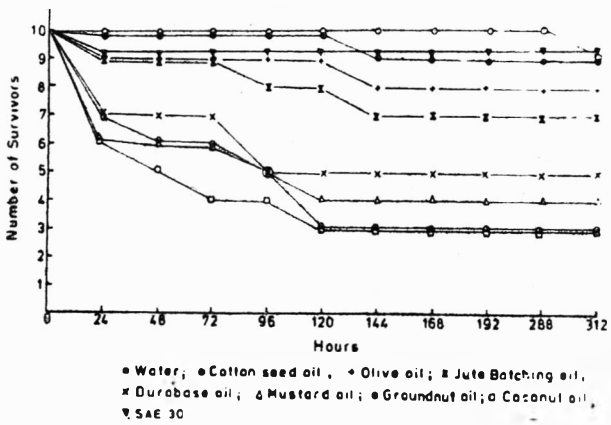

Fig. 1. Mortality profile of albino rats treated with Lindane at 125 mg/kg body weight carried in different oils.

Food intake: Treated animals consumed only 25-50 per cent of the total food provided on 2nd and 3rd day after intubation. Food consumption was resumed to normal within 6 days. The rate of recovery and food consumption were faster and more in animals fed with castor oil, SAE-90 and Dutrex-10-Lindane solutions.

Body and organ weights: There were no significant changes in the body weights of rats irrespective of the carriers used. Animals lost weight at the end of first week after intubation and gradually gained weight. The weights of liver, kidney and heart of animals did not show any significant change.

In general no histopathological changes were observed in liver, heart and spleen of the treated animals. However, varying degrees of haemorrhages were observed in kidneys.

As could be seen from the mortality profile (Table 1), Lindane carried in vegetable oils like coconut and groundnut in which its solubility is comparatively more, resulted in high mortality, their viscosity being quite low compared to mineral oils. Though mustard oil had the same range of viscosity and Lindane solubility, the higher mortality obtained could be perhaps due to inherent toxic property of the oil whose chief constituent is allyl isothiocyanate. (The control mustard oil did not elicit any lethal effect). The recovery of all animals in case of castor oil is due to the quick elimination of the toxicant rendered possible by the high viscosity and purgative effect.

However, in case of mineral oils tested, the low Lindane solubility coupled with high viscosity of the carriers resulted either in low or no mortality. The recovery of animals in mineral oils could be due to the poor rate of hydrolysis and absorption in the small intestine thereby resulting in quick elimination from the system. Starek and Zabinski[8] while studying the toxicity of Lindane, have proved that there is a clear relationship between the time of maximum hydrolysis of fats carrying Lindane in small intestine and the degree of absorption into the blood stream.

Our observations clearly point out that the toxicity of Lindane is highly influenced by its solubility in the carriers used and the viscosity of carriers. Further, they indicate the advantage of mineral oils in the development of pesticidal formulations for effective insect control measures. The use of mineral oils decreases the toxicity hazards and risks to non-target species and thus promote primate safety. Hence mineral oils have greater scope in developing effective and safer pesticidal formulations.

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Performance of A Through Circulation Type Casein Dryer

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An electrically heated air circulation type dryer was tested for drying of wet casein with different levels of casein loading in the trays, namely 25, 38, 50 and 63 mm. (The temperature of air was maintained at 60-65°C). The casein was made from skimmed milk by acid coagulation and the wet casein had an initial moisture content of 155-160% (on dry basis). The rate of drying was highest both in constant rate and falling rate periods, when the wet casein was filled to a height of 38 mm in the trays. At this level of loading, the drying time was minimum and it took 5 hr to reach a final moisture content of 8%. The dryer description, tray size and the output of dry casein per batch were also determined.

Of the 2.3 million tons of milk per year handled by the organised sector of the dairy industry in India, about 5 to 10 per cent of the milk is received in a sub-standard condition. Such milk has either a high lactic acidity or contains low milk solids. The utilisation of such milk is an economic necessity for the plants. Usually such a milk is separated to recover the fat as cream and the skimmed milk is utilised for conversion to commercial casein, particulrly by the medium and small capacity dairy plants. However, in the absence of a suitable mechanical dryer the casein curd is usually sun dried which results in a sub-standard product. Many times difficulties are experienced in such drying, especially on rainy days.

Reinikovs *et al*[1] reported on the performance of a fluidised bed dryer for casein drying. The mechnical and heating procedures employed in commercial drying of casein are discussed by Kajda[2]. An indirect oil fired straight through type dryer was described by Vickers[3]. Filonenko *et al.*[4] studied a vortex type dryer and Cesul *et al*[5]. investigated the applicability of vortex drying technique for casein. However, these refer to casein dryers of large capacities involving heavy capital outlay. It was in this context that a small through circulation electrically heated dryer was fabricated at this Institute to meet the needs of small or medium sized dairy plants. The present communication is a report on the performance of this equipment for casein drying.

Materials and Methods

As illustrated in Fig. 1, the dryer is made of 20 gauge mild steel sheet welded to 25 mm angle iron frame. The chamber is open at the top to receive on suitable angle iron supports, one beside the other, three wooden frames each of size 480×730×100 mm with 24 gauge wire netting bottom to support the ground wet casein. A three blade blower, directly coupled to a single phase 0.5 H.P. motor is fixed on the side end of the chamber, to provide 70-75 cubicmeters of air at 12.5 mm of water pressure. A set of seven finned electric heaters, each of 1 KW capacity and 600 mm length are fixed horizontally on two vertical supporting frames, with four heaters on one and three heaters on the other to uniformly heat the air to 60-65°C. The heaters are so arranged that the free space between any two consecutive heaters is the same and the two banks of heaters are separated by a centre to centre distance of 210 mm. Inside the chamber, a sloping floor is provided to facilitate the collection of fine particle of casein passing through the wire mesh and to give positive direction to the movement of hot air.

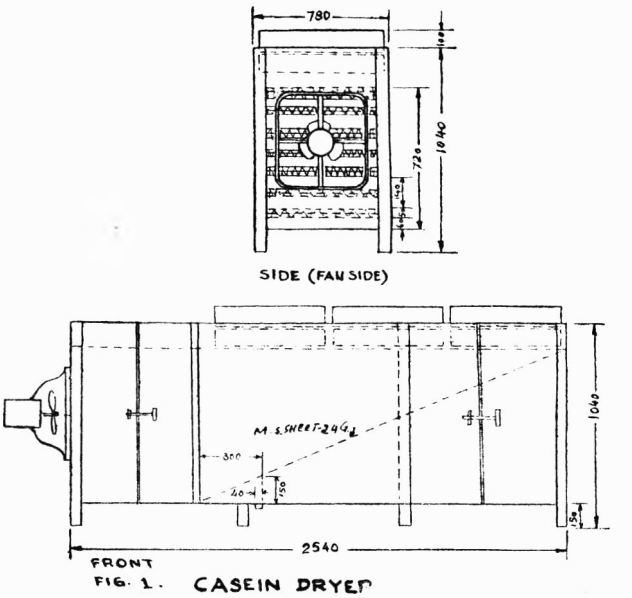

FIG. 1. CASEIN DRYER

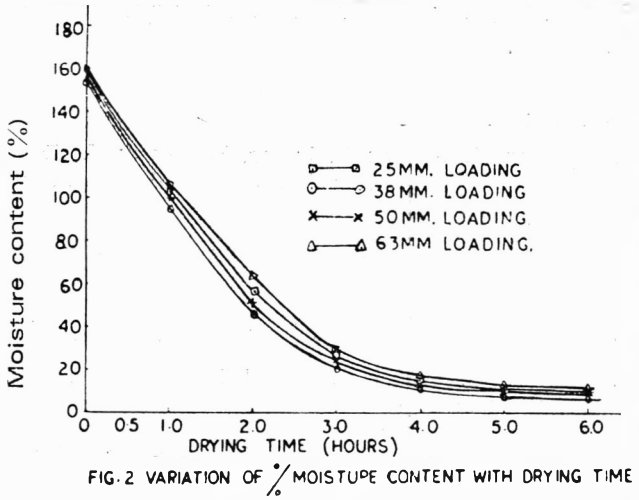

FIG. 2 VARIATION OF % MOISTURE CONTENT WITH DRYING TIME

Batches of casein were made from skimmed milk adopting the standard procedure of acid coagulation. The wet casein in the different batches had an initial moisture content of 155-160 per cent on dry basis. The curd was ground to 3-4 mm size particles in a casein grinder and spread evnely on the trays to depths of 25, 38, 50 and 63 mm for four levels of loading. The loosely packed trays for each trial were placed in the drier and power supply to blower and coils was switched on. During drying, the wet casein was uniformly mixed twice at 2-hr intervals. The moisture content of representative samples of casein initially and at hourly intervals over a period of 6 hr was determined by gravimetric method. The average moisture content for four trials on each level of loading calculated on dry basis (bone dry solids basis) is given in Fig 2. The wet and dry bulb temperatures of atmospheric air were 22-27°C and 34-38°C respectively during the trials.

Results and Discussion

The drying rates for the four levels of loading were evaluated graphically by the method of tangents. The plots of drying rate as grams of water removed per hour per hundred grams of dry solids against per cent moisture content (on dry basis) are shown in Fig 3 which indicates the effect of bed thickness on the drying rate as a function of the moisture content of the curd.

From the curves in Fig 3, it is observed that at the four levels of wet casein loading (25, 38, 50 and 63 mm) the drying of the wet casein is characterised by constant rate of drying and falling rate of drying conditions as a function of moisture content of the casein. The rate of drying during the constant rate period was highest at 1.33 g/hr for 100 g of dry product when the wet casein was filled to a height of 38 mm, whereas it decreased with increased height of wet casein bed. This could be expected because of increased resistance to heat and mass

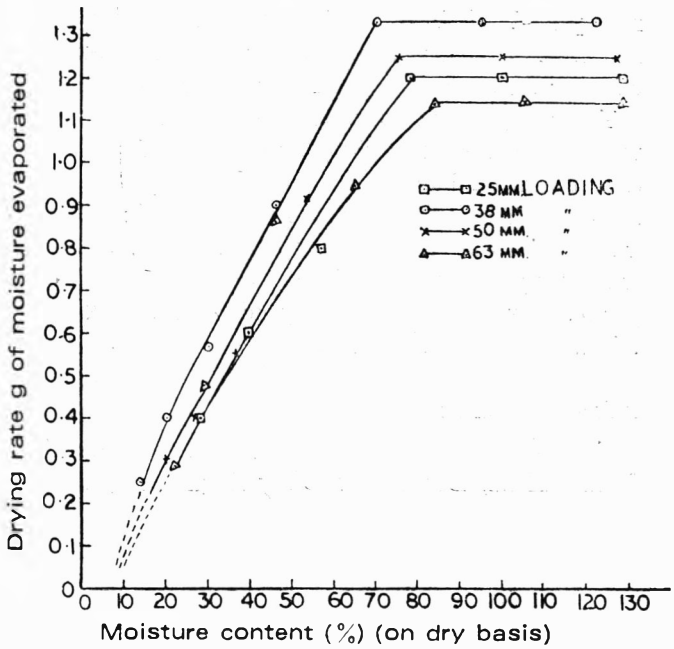

Fig 3. Plots of drying rate *vs* moisture content for different heights of loading.

transfer. The drying rate was also lower when the wet casein bed was only 25 mm thick because of insufficient material for uniform spreading on the tray with the consequent short circuiting of the hot air through areas of minimum bed resistance. It was observed that although the wet casein could be spread uniformly on the tray, on partial drying the casein curd particles shrunk leaving areas of minimum or no loading of casein on the tray. In the falling rate of drying also, similar trend was noticed although the differences in the rate of drying at the four levels of loading were progressively lower with decreasing moisture content in the wet casein being dried.

It was also observed that when the moisture content in the product reached 10 per cent on dry basis (9.1 per cent on wet basis) the rate of drying by extrapolation would be 0.12 g/hr/100 g of dry casein for a bed thickness of 38 mm and 0.085, 0.05 and 0.05 for bed thicknesses of 50, 63 and 25 mm respectively. Thus it would be seen that the rate of drying, both in the constant and falling rate conditions was maximum at a bed thickness of 38 mm. In order to reach any desired moisture level, minimum time was required when the height of wet casein bed was 38 mm followed by beds of 50, 25 and 63 mm thicknesses as indicated in Fig. 2. In regular plant practice, where the moisture content of casein is maintained at 7 to 8 per cent, it takes much longer time than needed to reach the 10 per cent moisture level, when the loading levels are more than 38 mm since the rate of drying falls off rapidly as function of both increasing bed height and decreasing moisture content in the falling

rate period of drying. The economic advantage of increased through put per batch by higher thickness of casein bed in the trays is offset by longer drying times.

The best performance of the casein drier was achieved when the bed thickness was 38 mm and in 5 hr the product attained a final moisture content of 8 per cent from an initial moisture content of 154 per cent (both on dry basis). The output of the dryer under these conditions was 12 kg of dry casein (8 per cent moisture content) per batch which would be the yield from approximately 350 l. of skimmed milk.

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RESEARCH NOTES

DESTRUCTION OF AFLATOXIN IN RICE BY DIFFERENT COOKING METHODS

Rice naturally infested with moulds, and rice to which mouldy rice or aflatoxin B_1 has been added were subjected to normal cooking, cooking with excess water or pressure cooking. Normal cooking destroys 49 per cent aflatoxin. Pressure cooking and cooking with excess water destroys aflatoxin by 73 and 81.6 per cent respectively. Pressure cooking of rice seems to be an effective measure of reducing aflatoxin content.

Aflatoxin has been detected in rice by six countries and the highest level of 0.6 ppm is reported from Thailand[1]. The incidence of primary liver cancer correlated with the aflatoxin content of processed foods[1]. An epidemeological survey conducted by Central Food Technological Research Institute, Mysore, in coastal areas of Mangalore showed correlation between the presence of aflatoxin in rice and the incidence of hepatomegaly in children[2].

Several methods like autoclaving moist groundnut meal[3], heating at 100°C for 2 hr[4] or roasting peanut at 150°C for 30 min[5,6] have been found to lower the aflatoxin content in groundnut and its meal. Similarly, roasting of pecans at 191°C for 15 min has been found to reduce aflatoxin by 80 per cent[7]. An attempt has been made to find out the effect of domestic cooking on the aflatoxin content of rice.

Preparation of mouldy rice: Fifty gram samples of rice were taken in six 250 ml Erlenmeyer flasks along with 20 ml of distilled water and sterilized at 120°C for 20 min. The flasks after cooling were inoculated with *Aspergillus flavus* CFTRI strain (IMI 138325) and incubated at 28°C for 7 days and inactivated by adding 50 ml of acetone to each flask and kept for one hour. Later the mouldy rice was air dried under shade for 3 hr and dried in a hot air oven at 60°C for 5 hr. The mouldy rice was then added at various levels to samples of fresh rice free from aflatoxin.

Addition of pure aflatoxin to rice: Known quantities of aflatoxin B_1 in chloroform were added to uncontaminated samples of both whole and powdered rice in aluminium containers. The chloroform was removed on a water bath. These samples were subjected to various cooking methods.

Naturally contaminated rice: Rice samples collected from the coastal areas of Karnataka State containing aflatoxin B_1 from 0.002 to 0.08 ppm were subjected to different cooking procedures.

Ordinary cooking: Twenty five gram of each sample of rice was mixed with 100 ml of water in aluminium containers (500 ml capacity) and cooked by direct heat with frequent stirring. In about 30 min, cooking of rice was completed.

Cooking with excess of water: Twenty five gram of rice was cooked as above with 200 ml of water. Gruel was decanted and aflatoxin was estimated in both cooked rice and gruel.

Pressure cooking: Twenty five gram samples of rice as indicated above along with 100 ml of distilled water were taken in aluminium container of 500 ml capacity and cooked in a pressure cooker at 15 lb psig for 5 min. It took 20 min for cooking.

Method of extraction and estimation of aflatoxin: The method of B.F. procedure[8] was modified and adopted for the analysis of aflatoxin in both cooked and uncooked samples of rice. In order to determine the initial aflatoxin content of rice samples before cooking, 25 g of powdered (36 mesh British Standard) rice containing aflatoxin was blended with 125 ml of 55 per cent aqueous methanol in an electric blender for 3.5 min and filtered. The filtrate (25 ml) was defatted with hexane (50 ml). The aqueous methanol layer after removing traces of hexane on a water bath was extracted three times with 25 ml of chloroform at room temperature. It was passed through a bed of anhydrous sodium sulphate and evaporated to dryness on water bath. A known quantity of chloroform was added to the extract and aflatoxin was estimated on silica gel G TLC plates by Dilution to Extinction Method[9].

The cooked samples were weighed and the increase in weight over and above 25 g indicated the water absorbed by rice during cooking. Based on this, the volume of methanol was adjusted to give the required 55 per cent concentration and other operations continued as above.

The aflatoxin contents of all the three types of rice samples were estimated before and after cooking by (*a*) ordinary cooking, (*b*) cooking rice with excess of water followed by decanting the gruel, and (*c*) pressure cooking.

The results in Table 1 show the analysis of 59 samples of rice before and after normal cooking. Forty six samples of normal rice to which mouldy rice had been added contained aflatoxin ranging from 0.2 to 3.3 ppm. They were subjected to ordinary cooking and it was found that the cooked rice samples had an aflatoxin content ranging from 0.1 to 1.6 ppm. This corresponds to a 40 to 58 per cent destruction with a mean value of

TABLE 1. DESTRUCTION OF AFLATOXIN IN RICE BY ORDINARY COOKING

Type of sample	No. of samples analysed	Initial aflatoxin (ppm)	Residual aflatoxin in cooked rice (ppm)	Destruction (%)
Rice + mouldy rice	46	0.20-3.30	0.10-1.60	40-58 (48.3)
Rice + aflatoxin B_1	9	0.04-4.00	0.02-2.10	46-50 (49.0)
Mould infested rice	4	0.02-0.08	0.01-0.04	45-50 (47.5)

Figures in parentheses indicate the mean values; rice to water ratio is 1:4.

TABLE 2. DESTRUCTION OF AFLATOXIN IN RICE BY COOKING WITH EXCESS OF WATER

Type of sample	No. of samples analysed	Initial aflatoxin (ppm)	Residual aflatoxin (ppm)		Destruction (%)
			Rice	Gruel	
Rice + mouldy rice	14	0.40-3.30	0.12-0.77	0.14-0.87	70-88 (81.6)
Rice + aflatoxin B_1	6	0.44-2.00	0.11-0.33	0.1-0.7	67-87 (75.7)
Mould infested rice	4	0.02-0.08	0.005-0.02	0.005-0.02	70-79 (74.5)

Figures in parentheses indicate the mean values; rice to water ratio is 1:8.

TABLE 3. DESTRUCTION OF AFLATOXIN IN RICE BY PRESSURE COOKING

Type of sample	No. of samples analysed	Initial aflatoxin (ppm)	Residual aflatoxin in cooked rice (ppm)	Destruction (%)
Rice + mouldy rice	43	0.20-3.30	0.06-0.80	63-87 (73.0)
Rice + aflatoxin B_1	12	0.04-4.00	0.013-1.3	68-76 (70.7)
Mould infested rice	4	0.02-0.08	0.006-0.02	66-75 (70.5)

Figures in parentheses indicate the mean values; rice to water ratio is 1:4.

48.3 per cent. Similar results were obtained when 9 normal rice samples to which pure aflatoxin had been added and 4 natural mould infested samples were subjected to the same treatment.

Fourteen samples of normal rice to which mouldy rice containing aflatoxin ranging from 0.4 to 3.3 ppm were used. The residual aflatoxin ranged from 0.12 to 0.77 ppm in cooked rice and 0.14 to 0.87 ppm in gruel depending upon the initial toxin content (Table 2). As the gruel is normally discarded, the amount of aflatoxin ingested through rice is further reduced. Therefore, the amount of aflatoxin that is removed by this practice of cooking ranged from 70 to 88 per cent with a mean value of 81.6 per cent. Similar results were also obtained with samples of normal rice to which pure aflatoxin was added and naturally mould infested rice samples. The practice of cooking rice with excess of water and discarding the gruel would reduce the aflatoxin content but there would be a loss of some nutrients.

The results of the pressure cooked rice samples are presented in Table 3. The average per cent destruction of aflatoxin in all the three types of rice samples ranged from 70.5 to 73.0 per cent. This shows that irrespective of the type of sample of rice containing aflatoxin, almost same amount of aflatoxin is destroyed by pressure cooking.

In the above experiments, both whole rice and powdered rice samples yielded identical results when subjected to cooking methods.

Since the levels of aflatoxin encountered in rice are not very high and as nearly 70 per cent of this can be destroyed by pressure cooking, the possible health hazard is reduced to minimum. To achieve maximum destruction of aflatoxin present in rice without loosing much of the nutrients, pressure cooking of rice may be followed in households.

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STUDIES ON THE OCCURRENCE OF OCHRATOXINS IN FOODGRAINS

Foodgrain samples (180) were collected in the city of Mysore and analysed for ochratoxins. Only ochratoxin A was found in 6% of the samples, comprising wheat, ragi, sorghum, corn and groundnuts. It ranged from 30 to 70 ppb. One sample of groundnut was contaminated with 2000 ppb of ochratoxin A. Rice, coffee and samples of pulses examined contained no ochratoxin.

Ochratoxins are highly toxic metabolites produced by moulds such as *Aspergillus ochraceus* and *Penicillium viridicatum* and are known to cause nephropathy in animals and possibly in man. Ochratoxin A and B have been encountered as naturally occurring contaminants in food and feed such as corn, wheat, oats, barley, peanuts, rye, white beans and grain feed[1]. Although much work has been carried out on analytical methods, toxicology and microbiology of the toxin, information on the occurrence of ochratoxins in foods are lacking, particularly in India. This note presents data on the occurrence of ochratoxin A in 180 samples of food from the market in the city of Mysore.

Samples of wheat, ragi (finger millet), sorghum, corn, groundnut, rice, paddy, coffee seed, black gram, green gram, Bengal gram, horse gram, broad bean and field bean were purchased from various grocery shops in the city of Mysore. The samples were ground to pass through 20 mesh sieve (British Standard) and 50 g samples were taken in 500 ml Erlenmeyer flasks and 25 ml of 0.1 M H_3PO_4 as well as 250 ml chloroform were added and extracted by shaking on a rotary shaker for 30 min[2]. The slurry was filtered through a fluted filter paper covered with 10 g of diatomaceous earth. The extract (50 ml) was mixed with hexane (40 ml) and was passed through a column (70 × 1.7 cm) containing 2 g of diatomaceous earth mixed earlier with 1 ml of 1.25 per cent $NaHCO_2$ solution. The column was eluted with 75 ml of $CHCl_3$ and the eluates were discarded. Ochratoxin A was eluted with 75 ml of freshly prepared solvent of formic acid and chloroform (1:99), collected in 250 ml Erlenmeyer flask and immediately evaporated to dryness on water bath[2]. The residue was dissolved in appropriate proportions of acetic acid to benzene (1:99) and graded amounts were spotted on silica gel G TLC plate. The plates were developed using methanol to acetic acid to benzene (5:5:90) in unlined unequilibrated tank[2]. The toxin was estimated quantitatively by Dilution to Extinction Procedure.

Ochratoxin A was identified by its typical R_f value, bluish green fluorescence under UV light along with standard ochratoxin A and confirmed by spraying the spots with NaOH solution which changes ochratoxin A fluorescence to intense blue[2].

Recovery experiments to assess the efficiency and validity of the method were carried out with powdered samples of wheat, corn and sorghum added with known quantities of standard ochratoxin A.

Standard samples of Ochratoxin A, B and C were obtained from Dr. S. Nesheim, Division of Chemistry and Physics, Food and Drug Administration, Washington DC 20204. Ochratoxin A was also prepared in pure form by growing an isolate of *A. ochraceus* on yeast extract-sucrose medium[3]. Its spectral and chromatographic characteristics were found to conform with those of standard ochratoxin A. The identity was further confirmed by treatment with NaOH solution. This ochratoxin A was used in all the recovery experiments.

Of the 180 food samples analysed, only 11 contained ochratoxin A (Table 1). Ochratoxins B and C could not be detected in any of the samples. Ochratoxin A was detected and confirmed in three out of 21 samples of corn (30-50 ppb), two out of 24 samples of wheat (30-50 ppb), three out of 24 samples of sorghum (50-70 ppb), two out of 18 samples of groundnuts (50-2000 ppb) and one out of 15 samples of ragi (70 ppb). None of the other food samples such as 33 samples of rice, 10 samples of paddy, 11 samples of coffee seed, 6 samples of black gram, 3 samples of green gram, 7 samples of Bengal gram, 3 samples of horse gram, 4 samples of broad bean and 1 sample of field bean examined showed the presence of ochratoxin. The number of samples of other commodities analysed in this preliminary survey are perhaps not large enough to establish the probable occurrence of ochratoxin.

The modified B. F. procedure[4] was used in the initial stages to screen some of the samples since the method has been found to be simple and rapid but the results

TABLE 1. OCCURRENCE OF OCHRATOXIN A IN FOODGRAINS

Food item	Samples analysed (No.)	Samples contaminated (No.)	Ochratoxin A (ppb)
Corn	21	3	30
			30
			50
Wheat	24	2	50
			30
Sorghum	24	3	50
			70
			70
Groundnut seed	18	2	50
			2000
Ragi	15	1	70

were not reproducible due to interfering substances. Even the recovery experiments with this method yielded only 60 per cent recovery. Hence, the method of Nesheim *et al.*[2] was adopted for further analysis. This method yielded satisfactory recoveries ranging from 89 to 98 per cent and interference with other compounds was negligible.

So far ochratoxin A was reported as a contaminant in five species of cereals, green coffee and peanuts[5]. Residues of ochratoxin have also been found in bacon, poultry and in pork meat[6]. In the present studies we have not found ochratoxin in green coffee which may perhaps be due to the limited number of samples analysed. The percentage occurrence of ochratoxin in the food samples analysed so far in the present survey is only 6 as compared to about 20 in a random sample survey[7]. The percentage occurrence has been found to be high (58 per cent) when epidemiologically designed sampling plan was used[7]. The concentration of ochratoxin A occurrence in foods in the present survey has been very low which ranged from 30 to 2000 ppb as compared to 0.02 to 27.5 ppm reported in literature[8]. Though the cultures of *A. ochraceus* group isolated from food materials produced both ochratoxin A and B, the food materials showed only the presence of ochratoxin A as observed by others[8]. It is of interest to note that a sample of ragi and a few of the infested sorghum contained only ochratoxin A.

The toxicological effects of ochratoxin to farm animals and human beings are well known[8]. The present preliminary survey has shown the occurrence of ochratoxin A in agricultural products like wheat, ragi, corn, sorghum and groundnuts. Moreover, ochratoxin A has been found to be fairly stable in cereal products[9]. These observations suggest the need for a systematic survey on the occurrence of ochratoxin A in major food commodities in India.

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SUITABILITY OF LACQUERED CANS FOR CANNING MANGO JUICE

Two types of imported lacquered cans were compared with the indigenous lacquered cans for canning mango juice. Imported white lacquered cans based on polyester gave satisfactory results.

The use of lacquered cans for canning food products has increased after the introduction of electrolytic tinplate with thinner tin coating and other advantages. In India, lacquered cans are being used mainly for canning fruits containing anthocyanin pigments (acid resistant lacquer) and sulphur containing products such as meat, fish and vegetables like peas, cabbage, etc (sulphur resistant lacquer). Indigenous lacquers are not suitable for canning acid products such as mango, pineapple and citrus. This note presents the results of preliminary studies carried out with imported lacquered cans to find their suitability for canning mango juice and compare it with that of indigenous lacquered cans.

Lacquered cans: Two batches of lacquered cans (401 ×411) were received from M/s. Schmalbach-Lubeca, West Germany. The details are as follows:

Inside white coating: Ends are coated inside with vinyl type of lacquer—film coating weight 25 g/m^2, internal surface of the body is coated with polyester; film coating weight 10 g/m^2.

Inside gold lacquered: Internal surface of the body and ends are coated with epoxy phenolic resin (roller-coated plus spray lacquered); film coating weight 8-12 g/m^2.

Inside gold lacquered indigenously available can is used as control.

Canning of mango juice: 'Badami' ('Alphonso') mango juice containing 35 per cent mango pulp with Brix, 20° and acidity, 0.3 per cent was prepared. The juice was heated to 85°C, filled hot into cans, sealed and processed in boiling water for 30 min and then cooled. Filled cans were stored at room temperature (25-30°C) and at 37°C and analysed periodically for 12 months. Organoleptic evaluation of canned mango juice was carried out by a panel of judges. The results of the cutout analysis at the end of 12 months storage are given in Table 1.

TABLE 1. CUTOUT ANALYSIS OF CANNED MANGO JUICE AFTER 12 MONTHS OF STORAGE

Type of can	Storage temp. (°C)	Vacuum (inches of Hg.)	Colour	Taste	Flavour	Can interior
White coated (imported)	Room temp. (25–30°)	9	Yellow	Satisfactory	Normal	Normal; no peeling of lacquer
Gold lacquered (imported)	,,	12	,,	Slightly bitter and lacquer taste	Lacquer flavour	,,
Gold lacquered (indigenous)	,,	6	,,	Bitter and lacquer taste	,,	Few black spots on sides; few black vertical streaks
White coated (imported)	37	8	Yellow with light brownish tinge	No lacquer taste	Normal	Normal; no peeling of lacquer
Gold lacquered (imported)	,,	10	,,	Slightly bitter	Lacquer and off-flavour	,,
Gold lacquered (indigenous)	,,	4	,,	Bitter	,,	More black spots; few vertical balck streaks

During storage, slight decrease in vacuum was observed in all the cans. As indicated in Table 1, except for the colour, the quality of the canned mango juice was quite satisfactory in cans coated with white lacquer, both at room temperature and at 37°C. Light brownish tinge was, however, noticed in the juice stored at 37°C. In the case of mango juice canned in imported as well as indigenous yellow lacquered cans, bitter taste with lacquer flavour was noticed. This was not acceptable to the majority of the judges in the panel. There was no change in the acidity, pH and Brix of the juice during storage.

In the imported lacquered cans, the can interior was normal, and black spots or peeling of lacquer were not noticed. In the case of indigenous lacquered cans, however, black spots and vertical black streaks were noticed in the can interior.

In view of the encouraging results obtained with the imported white lacquer cans, based on polyester coating for canning mango juice, it is proposed to extend the studies to other fruit products.

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CHANGES IN PECTIN AND PECTINESTERASE ACTIVITY IN DEVELOPING GUAVA FRUITS

Pectin content and pectinesterase (PE) activity were determined at immature, mature, ripe and over ripe stages in seven varieties of guava. Pectin content was maximum in Allahabad Safeda, Banaras and Red Flesh' in ripe fruits and in all the other four varieties when immature. PE activity in all the seven varieties was high at immature and ripe stages.

The progressive softening in fruits while ripening is attributed to changes in pectic constituents. Close association between textural changes and pectinesterase (PE) activity while ripening has been reported in a number of fruits[1-5]. Changes in pectin content and PE activity in guava fruits during ripening are reported in this paper.

Five guava varieties and two hybrids grown at the Experimental Station, Hessaraghatta, Bangalore (India) were collected at four stages of growth and analysed for pectin and PE activity. Pectin was estimated by the AOAC[6] method. PE was extracted by the method of Kertez[7] as modified by Rouse and Ackine[8] and assayed by the method of Ratner *et al*[9]., using citrus pectin as substrate.

Pectin content showed three, distinct patterns (Table 1). "Allahabad Safeda" and 'Red Flesh' showed an increase in pectin content upto ripe stage which decreased at the over ripe stage. The varieties 'Beaumont', 'Hybrid I' and 'Hybrid II' recorded a decrease in pectin content from immature to over ripe stage. However, the varieties

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TABLE 1. PECTIN CONTENT AND PECTINESTERASE ACTIVITY IN GUAVA FRUITS DURING GROWTH AND DEVELOPMENT

Variety	Immature				Mature				Ripe				Over ripe			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Allahabad Safeda	53	240	127.2	1.00	97	260	252.2	0.38	162	325	526.5	0.58	169	260	439.4	0.85
Banaras	50	230	115.0	0.60	98	200	196.0	0.30	150	270	405.0	0.33	141	260	366.6	1.00
Beaumont	61	500	305.0	0.58	108	370	399.6	0.17	128	260	332.8	0.42	115	240	276.0	1.54
Red Flesh	48	230	110.4	0.92	85	380	323.0	0.50	123	385	473.6	0.79	118	310	365.8	1.03
Triploid Guava	30	270	81.0	1.67	63	235	148.1	0.83	93	250	232.5	1.00	65	220	143.0	1.50
Allahabad Safeda × Banaras (Hybrid I)	56	450	252.0	0.78	110	320	352.0	0.70	151	310	468.1	0.99	145	290	420.5	1.13
Allahabad Safeda × Red flesh (Hybrid II)	62	440	272.8	0.73	96	310	297.6	0.46	160	250	400.0	0.51	152	200	304.0	1.05

A = Average weighting of one fruiting; B = Total pectic substances (as calcium pectate) on fresh weight basis (mg %); C = Total pectic substances (as calcium pectate) per fruit (mg); $D = \frac{\text{Pectinesterase activity}}{\text{PE. U} \times 10^2 \text{ per g}} \left\{ \frac{\text{ml of NaOH solution required} \times \text{Normality of NaOH}}{\text{g of the sample} \times \text{Titre period in min}} \times 10^2 \right.$

'Banaras' and 'Triploid' showed a decrease from immature to mature stage, an increase upto ripe stage and again a decrease at over ripe stage. Abrupt decrease in total pectin content after attaining full maturity has been recorded in earlier studies on guava[5]. Total pectin available per fruit at different developmental stages was calculated from pectin per cent and average weights of fruit. Except "Beaumont', the other varieties registered maximum pectin per fruit at ripe stage.

The pattern of PE activity was similar in all the seven varieties studied. The enzyme activity was fairly high at immature stage, decreased considerably at mature stage, again increased as the fruits became ripe and was maximum at over ripe stage. In our earlier study with two grape varieties a similar trend in PE activity was recorded[10]. Increase in PE activity during ripening period has been reported in a number of other fruits also.[1,3,4].

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SEPARATION, PROCESSING AND UTILIZATION OF RICE GERM

Rice germ (embryo), mostly gets crushed during the milling and gets mixed up with the bran. Simple equipment for its separation from brown rice has been designed and applied successfully on a small scale. The separated germ possess high nutritive value which can be further subjected to solvent extraction of oil. Storage results in a rapid increase in free acidity of the oil.

The germ is the most nutritious and valuable part of the grain, being a rich source of oil, protein and vitamins (Table 1) and an excellent article of human food.[1-3] Due to lack of efficient methods for its separation, the germ gets disintegrated and becomes a part of the bran after which, fractionation is not easy.

TABLE 1. PROXIMATE COMPOSITION OF RICE GERM (RAW RICE)

Moisture, (%) ..	About 8.0
Oil (on moisture free basis) (%) ..	35–37
Acid-insoluble ash*, (%) ..	0.6
Crude fibre,* (%) ..	1.0
Crude protein* (N 6.25), (%) ..	30 – 32
Thiamine,* (mg/100 g) ..	5–7

*Moisture and oil free basis; rice variety; ADT 8

The germ content of brown (dehusked) rice ranges from 2 to 4 per cent depending on the variety, and the size of the grain.

During parboiling, the germ looses a good part of its solubles, shrinks in size and looses about 30 to 40 per cent of its weight. A nearly proportionate (about 45 per cent) increase in oil content occurs. Being sensitive to heat, rice germ turns reddish brown on parboiling, but is still nutritionally good.

During the polishing process of rice, the germ, along with the outer membrane and a part of the aleurone layer, gets separated even by mere friction. It then gets mixed up with the grain, and is subject to rubbing action against the abrasive surface of the polisher. Consequently, it gets pulverised, and becomes a part of the bran. In the Schule—Dandekar polisher, for instance, 30-40 per cent of the germ is removed during the polishing in the first cone. Only a small part of this, especially in the case of raw rice, escapes crushing. This undamaged germ, together with brokens, husk and grit is collected in the mill as the germ fraction. This fraction is fit only for poultry feeding, though through aspiration, a small quantity of germ can be separated from it.

When the partly polished rice passes into the second cone, the remaining germ is fully removed, gets crushed, and passes along with the bran fraction through the sieves. In this condition, it is subject to quick microbial spoilage and it rapidly becomes unfit for human consumption. The presence of the germ contributes however, to the oil content of the bran. In modern rice mill, against a germ yield of 2.1 per cent, actual germ recovery was only 0.87 per cent or 41 per cent of the total germ content (Table 2).

TABLE 2. GERM RECOVERY IN THE KYOWA MODERN RICE MILL, CHINGLEPUT

Stage of polishing	Yield of bran (%)	Yield of germ (%)	Brokens (%)	Degerming (%)
1st	1.8	0.110	21.1	30.0
2nd	2.0	0.350	27.1	52.0
3rd	1.6	0.405	31.3	82.0
Total	5.4	0.865	31.3	82.0

Rice variety; Co 19 (raw rice)

TABLE 3. RECOVERY OF RICE GERM IN THE PPRC DEGERMER UNIT USING MODIFIED CONDITIONS

Variety and type of brown rice	Brown rice taken (g)	Germ yield (g)	Bran yield (g)	Broken yield (g)
ADT-8 Raw	4000	105	143	1120.0
Parboiled	4000	40	120	120.0
IR 20 Raw	3500	55	108	770.0
Parboiled	3500	19	87	133.0

To overcome these difficulties, a simple equipment has been designed and fabricated at the Paddy Processing Research Centre. The clean brown rice is fed into this unit and kept in a state of suspended motion so as to avoid any abrasion or other mechanical damage to the grain. Friction caused between the grains results in some polishing and separation of the germ. The walls of the chamber are perforated so that as soon as the germ gets detached, it is blown out by air through the perforations. Some degree of polishing also occurs at the same time, with the result that what emerges is a mixture of germ and fine bran in approximately equal proportions, from which the germ can be separated by sieving and winnowing. In the case of rawrice, if the total polish is about 5 per cent on the weight of brown rice, 2.0—2.5 per cent is obtained as germ and the rest will be bran. In the parboiled rice because of the low germ content, only about 0.8 to 1.2 per cent is obtained as germ and the rest will be bran. Table 3 shows illustrative data from the PPRC degermer unit. If the husk is not fully removed at an earlier stage of dehusking, it gets distributed between the germ and the bran fraction. It is important therefore, that only clean brown rice be

TABLE 4. YIELD OF RICE AND OTHER PRODUCTS OBTAINED IN THE LABORATORY MCGILL POLISHER AND THE DEGERMER

Equipment	Brown rice taken (g)	Polished rice (g)	Bran (g)	Germ (g)	Brokens (g)	Oil from bran (g)
Laboratory McGill polisher	750	700	48	—	187.5	53.44
Degermer (PPRC)	4000	3700	153	110	1120.0	295.68

Rice variety; ADT 8' raw rice

TABLE 5. GERM STORAGE AND FFA CONTENT

Storage period (days)	% F. F. A. in oil
0	3.2
3	6.8
6	11.2
9	17.4
15	27.4
26	31.2

Variety: ADT-8 raw rice

used for such processing (Engineering details for the machine are available with the author).

In the PPRC trials, over 90 per cent degerming was possible for raw rice, and ever 95 per cent for parboiled rice. Breakage of 20-30 per cent is largely because of sun-cracks in raw rice; this cannot be avoided in any of the present polishers. Trials with the same brown rice samples in the laboratory McGill Polisher also gave the same percentage of brokens in the polished rice (Table 4). In processing parboiled rice, breakage is negligible. Even by the fluidised friction PPRC method, some percentage of the germ is lost by crushing between the grains, and there is scope for further improvement.

Of the germs thus separated, about 25 per cent are viable and capable of germination. This needs to be improved upon by ensuring quick separation of the germ after it is loosened from the grain. At least 50-60 per cent germinating capacity in the germ might be taken as a test of efficient separation of the germ without damage.

The fresh germ is quite tasty and can be eaten as such without any cooking. As flaking helps in the better extraction of oil during solvent extraction the rice germ can first be flaked, oil removed by solvent extraction and the deoiled flaked germ can be used for edible purposes. Rice germ would be an excellent material for incorporation into any high class protein rich food formulation, either as such or after deoiling[4].

The free fat acidity of the oil in the germ, especially that derived from raw rice, tends to increase in the same way as in raw bran (Table 5). Strong heating will cause discolouration. The germ, which for practical purposes is a small, light sack, can be very easily dried in a current of warm air and this should help to keep it reasonably stable. Since germ will get easily spoiled every precaution has to be taken for safe handling.

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SHELF-LIFE STUDIES ON A FLOUR BLEND BASED ON MAIZE AND PULSES

The equilibrium moisture content—relative humidity relationship studies were conducted at 27°C on a flour blend based on maize and pulses. The critical moisture content for the blend from the point of free flowing property and development of off-flavour was found to be 12.2% corresponding to a relative humidity of 65%. The storage studies carried out in one kg product packed in high density polyethylene woven sack material at 27°C with 65% RH and 38°C with 90% RH have indicated shelf life of about 130 and 25 days respectively.

At the instance of a private organisation, packaging and storage studies were undeertaken on a flour blend produced by them which was based on maize and pulses. The requisite shelf-life was 5-6 months at 30°C and 70 per cent RH storage. The product, as received, had 10.8 per cent moisture (wet basis) and 3.3 per cent fat.

Moisture sorption studies of the flour blends were carried out at 27±1°C by exposing the sample to 11-90 per cent RH using appropriate salt solutions[1]. Before storing, the blend was exposed to carbon dioxide for 7 days to ensure freedom from insect infestation. Individual pouches of the size 20×23 cm made of high density polyethylene (HDPE) woven sack material (HDPE filament woven material laminated to 150 gauge low density polyethylene liner) were filled with about 1 kg of the flour blends. The pouches were folded with an overlap of 3 cm and double stitched in a machine. The filled pouches were stored at two conditions (*i*) 27°C with 65 per cent RH (normal) and (*ii*) 38°C with 90 per cent RH (accelerated). The stored product was analysed at intervals for moisture pick-up and for free fatty acids and peroxides by standard methods[2].

Moisture sorption studies: The results of the equilibrium moisture content-relative humidity relationship studies are presented in Table I. It is observed that the flour blend with an initial moisture content of 10.8 per cent equilibrated to 58 per cent RH. The product was free-flowing upto a moisture level of 11.8 per cent.

TABLE 1. EQUILIBRIUM MOISTURE CONTENT—RELATIVE HUMIDITY OF THE FOOD BLEND AT 27°C

Relative humidity (%)	Equilibrium moisture content (% wet basis)	Quality
11	4.7	Free-flowing
22	6.4	,,
32	7.8	,,
44	9.1	Good
56	10.5	,,
64	11.8	Acceptable
75	14.5	Stable odour, unacceptable
86	18.3	Mould growth
90	23.9	,,

At a moisture content of 14.5 per cent (E.R.H.—75 per cent) the product was slightly soggy and developed a stable odour. Mould growth was observed at 86 and 90 per cent R.H. The moisture sorption isotherm was a sigmoid one, typical of a high starchy food product and the inflexion occurs at about 65 per cent RH beyond which the curve becomes very steep indicating that moisture pick-up is considerable. The inflexion corresponds to a moisture content of 12.2 per cent. The formation of free fatty acids due to hydrolysis would be very pronounced[3,4] above this moisture level. Thus, the critical moisture content for acceptability was fixed at about 12.2 per cent.

Storage studies: The results of the storage studies on the maize-pulses blend are presented in Table 2.

TABLE 2. CHEMICAL CHANGES IN THE FOOD BLEND STORED AT TWO ENVIRONMENTAL CONDITIONS

Storage period (days)	Moisture (%)	F. F. A. (% oleic acid)	P. V. (m. eq. of peroxide/kg fat)
Normal condition (27°C, 65% RH)			
Initial	10.8	12.7	35.3
30	11.2	13.9	50.2
50	11.4	16.0	75.0
75	11.8	21.1	103.2
105	12.0	22.0	209.2
131	12.2	41.0	744.8
151	12.4	51.0	269.7
Accelerated condition (38°C, 90% RH)			
Initial	10.8	12.7	35.3
30	13.1	30.1	85.5
50	13.9	41.7	125.1

Under the normal environmental conditions of storage, the increase in the moisture content steadily increased upto $4\frac{1}{2}$ months (12.2 per cent) and at the end of 5 months, it was 12.4 per cent. The initial value of free-fatty acids was 12.7 per cent (as oleic acid) and it increased gradually and at the end of 5 months it was 51.0 per cent. The initial peroxide value of 35.3 milli equivalents per kg of fat increased rapidly after $3\frac{1}{2}$ months of storage reaching a maximum of 745 at the end of $4\frac{1}{2}$ months, but later decreased to 270 at the end of 5 months of storage. At moisture levels above about 12 per cent, the liberation of free-fatty acids was rapid, indicating further that the critical moisture content was about 12 per cent. The product at the end of $4\frac{1}{2}$ months had attained the critical value and was just acceptable from the point of free flowing property and off-flavour development.

The storage studies conducted under the accelerated condition of 38°C and 90 per cent RH showed that the product had a moisture content of 13.1 per cent at the end of 30 days. But after 50 days the moisture was 13.9 per cent, and the product had turned soggy and unacceptable. Thus, it was observed that the shelf-life of the product at 27°C and 65 per cent RH was about $4\frac{1}{2}$ months whereas under 38°C and 90 per cent RH, it had a shelf-life of only about 25 days when the product was packed in 1-kg HDPE woven sack. The effective area of the pouch was 920 sq. cm and the water-vapour transmission rate of the material was 3.7 g/m^2. d at 38°C and 90 per cent RH gradient. As it was envisaged to package 25 kg of the product in HDPE woven sacks of 50 × 75 cm size with an effective area of 7500 sq. cm, based on storage studies on model packages and on the water vapour permeability figures, it could be concluded that the product would have a shelf-life of about 6 months at 30°C and 70 per cent RH when packed in 25 kg bags. Further, if the initial moisture content were to be reduced to about 8-9 per cent, this would ensure the desired shelf-life to the product.

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BOOK REVIEWS

Food Quality and Nutrition: Research Priorities for Thermal Processing: edited by W. Downey, Proceedings of COST seminar, Dublin Applied Science Publishers Limited, London, 1977, pp. 712.

This book is based upon a series of papers presented by eminent scientists at the COST (European Cooperation in Scientific and Technical Research) Industrial Food Technology seminar held in Dublin, Ireland in Nov. 1977. The contributors have reviewed the current developments in the sphere of quality production of various food items viz., meat, poultry, dairy, fish, cereals, fruits and vegetables, etc. as affected by thermal processing.

Session 1 is devoted mainly to the current status of research and development of specific food commodities. Sessions 2 to 6 deal with processing procedures. The research priorities in the areas of pasteruization, blanching, sterilization, dehydration, chilling, freezing, thawing and cooking have been identified and brought out very lucidly. The subjects have been dealt both from the point of view of industrial processing as well as domestic utility.

The commodity panels have laid special emphasis on the future needs of research and development—particularly the effect of heat treatment—on various commodities. The concluding session is the gist of the book and the summary of the points put forward in earlier sessions has been made with meticulous attention to all research needs on all the commodities covered in the compilation.

Though the book has been written with the specific aim of serving the COST countries (Keynote address page 5-13) yet the scope of the book has been extended so as to be useful for the entire R & D effort on thermal processing of foodstuffs throughout the world. The effect of thermal treatment on the nutrient content of various food products has been reviewed before stressing the future priorities for research in that direction. It has been recommended that on the basis of the results available and the future research data, cooking procedures or holding conditions which would largely guarantee the preservation of nutritive value should be standardised and knowledge about the effect of processing variables on the quality of food products should be improved.

In a multi-disciplinary subject such as Food Science and Technology, it is always difficult to identify grey areas and define criteria for formulation of new projects with appropriate priorities. The "Commodity" and "Processing Dimensions" thereof have been very well linked and the aspects requiring further R & D effort in this field clearly pinpointed.

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Structure—Activity Relationships in Human Chemoreception: by M. G. J. Beets, Applied Science Publishers, London; 1978: Pp. XII+408; Price: $ 50.00

Study of olfaction and gustation and relating structure of stimuli to the response has attracted many research groups in the last three decades. Inspite of a large number of papers it became obvious that the simplistic approach of relating individual or a few physico-chemical parameters of the stimulants to the response did not have general predictive capability in human chemoreception. Developments in this time in electron microscopy. electro-physiology, neural mechanisms and psychophysics have made multi-disciplinary approach possible. Such studies established the complexity of the sequence of events in stimulant-receptor interaction, information generation, transfer to higher centres for recognition, storage and retrieval. Dr. Beets has approached the problem from a total consideration of these processes of information organisation and saw the necessity of a generalised concept as opposed to the specialized concepts of shape and structure of molecules contacting complementary sites in specific receptors. The varied dimension of odour, intrinsic intensity, concentration. unimodel quality of many different stimulant and also odour profile of a single chemical stimulant, all point to a pattern of information rather than highly specialized single informational mode. Dr. Beets' ideas, developed in publications since early sixties has crystallized into this scholarly treatise. It is a serious book for the specialists. The first part of the four part book is difficult but a pleasure to read the reasoned derivation of the generalized concept which considers the simulant molecules and their reaction at a number of potential receptor sites in a statistical sense. The terminal quality of an odour is the summation of the effects of whole population of molecules contacting a collection of polar epithelial locations. The odour is identified with an epithelial area and the firing frequency the bit of infort mation. These consequences of the generalized concepis radically different from a combination of earlier theories all of which requires specailized receptor sites.

The second part reviews the esperimental approaches available, the anatomy of chemoreceptary systems, and the extensive data in literature on the use of subjects with sensory deficiencies for identifying the primary odours, adaptation and cross-adaptation phenomena, chirality and other structural features related to olfactory and gustatory modes. The generalized concept acknowledges the effect of shape, size, stereochemistry, functional groups but lays greater emphasis on the oriented profiles of the collection of stimulant molecules at the time of contact with a variety of epithelial sites containing varying concentration and types of receptors.

The third and fourth part of the book discusses the highly documented studies on the musk and few other olfactory modalities and the four basic gustatory modalities, each of which forms individual monographs.

The extensive literature on the natural and synthetic compounds used as sweeteners and the studies on carbohydrates, amino acids and peptides which evoke sweet and bitter sensations are discussed in the light of the Shallenberger-Kier HA-B-X concept. The sweet-bitter sensations of a number of compounds are explained under the generalized concept by a pattern of reactions of the polyfunctional compounds with different epithelial areas located in varying complex topology. The HA-B-X concept has been extended to sour and salt modalities and it will be interesting if it can be extended to pungency which also shows some structural requirements in the corresponding stimuli.

The amount of literature covered, their critical analysis are very impressive. The many suggestions of model compounds, for synthesis which will prove the predicitive capability of his theory, should be of great value to synthetic organic chemists, Dr. Beets', book will be a reference volume, and an exciting experience to the physiologist, flavour chemists, and food technologists to understand the mechanism of perception and flavour selection. The book is well produced, with a pleasing format, plenty of explanatory figures and molecular structures and with an excellent index. This important book should find a place in all libraries of research and advanced level teaching institutions.

V. S. GOVINDARAJAN
C.F.T.R.I., MYSORE

Food Packaging: by Nicholas D. Pintauro, Food Technology Review No. 47, Noyes Data Corporation, Park Ridge, New Jersey, U.S.A., 1978; Pp. 414,

With rapid growth in food industries, the demand for various processed foods in a functional attractive package is ever increasing. The present book aims in arriving at protective packaging for food products against chemical deterioration arising out of entry of oxygen and mositure, insect infestation and contamination, etc. Special emphasis is laid to instant foods such as precooked, pre-mixed, no-bake, ready-to-serve items. Suitability of both conventional packaging consisting of mainly paper based materials, glass, metal etc., and plastic films, rigid containers and foils etc., have been discussed for each food product. Further, use of non-returnable beverage container and use of package as cook and serve utensil, and application of microwave techniques for cooking in package, which are prominent in packaging research in United States in recent years, have been covered in depth. Important feature of this book is its presentation of detailed descriptive information on U.S. Patents dealing with food packaging. Also, it presents an advanced, technically oriented review on food packaging.

This book contains eleven topics, in which advanced packaging aspects and design and functional aspects of representative package systems for different food categories are covered. Further, detailed information on package design, composition, construction and fabrication in relation to product protection and shelf-life are discussed. Three dimensional drawings of almost every package are given so that the reader gets better understanding and view of the package. At the end of the book, company inventor and U.S. Patent number indices have been provided.

The important topics covered are: returnable and pressurized beverage containers; package with utensils and containers for reconstituted dried foods; dairy products; use of packaging trays, plastic over-wraps; vacuum packaging, shrink, etc., for fresh meat, poultry and seafoods; and blister packaging, saran cooking bags, etc., for processed meats; use of plastics as cook in pouches, tray packages and packages for microwave oven; bakery products, designs to reduce bruising, plastic foam protection and in package atmosphere control for fruits and vegetables; coffee packaging.

This book contains very useful information based on U.S. Patents on current food packaging methods and is a desirable addition to library as reference book.

N. BALASUBRAHMANYAM
C.F.T.R.I., MYSORE

Dairy Products and Eggs—Recent Development: Food Technology Review No. 48 by Marcia Gutcho. Noyes Data Corporation, Park Ridge, New Jersey, USA, 1978, Pp. 352; Price:

The information is based on U.S. Patents issued since January 1976. The first part covers recent advances in the technology of milk and milk products. The second part which is only of 33 pages as compared to 315 pages on dairy products, covers egg products.

Among the egg products covered in addition to conventional usages, are developments in the field of frozen omlets, low-cholesterol products, egg yolk extenders, egg white substitutes, etc. Of particular interest to food technologists in the field of egg substitutes, is the section on whey as an egg white substitute and use of soybean protein for obtaining egg yolk extenders and substitutes.

The major portion of the book, dealing with milk and milk products, is divided into seven sections—Cheese, whey, milk, yogurt, ice-cream, whipped toppings and coffee whiteners and margarine. The latest developments as judged by patent literature in the field are described under each section. For example under milk, modification of the conventional spray driers as well as techniques for two stage drying, i.e., the first by spray-drying and the second by fluid bed drying are described. Techniques for production of condensed milk with non-calorie sweeteners like lacitol and high purity maltose are given.

In addition, various improvements in the preparation of yogurts, patents relating to reconstitutable acidophil fermented milk powders, protein stabilized yogurts sterilized at pH_4 and soybran yogurts are also described.

By far the largest section of the dairy products is, covered by patents relating to cheese. The coverage is extensive and include all aspects, both traditional and recent innovations. Microbial enzymes for coagulating of milk, freeze stabilized bacteria for fermentation, freeze dried cottage cheese blend and soy cheese, are some of the less familiar aspects, patents on which are detailed.

Noyes publications have some unique features. They provide up-to-date information regarding US Patents in particular fields. At the same time, they give enough descriptive information to stimulate the interest of the food technologists, to undertake research work in those fields. The information contained will provide the background on which further research could be built.

The trends in food processing are fast changing in India. What is today in U.S.A., will certainly be here in the next few years. An insight into what is happening elsewhere, will help the processers in India to shape their future programmes.

The book under review has, as in the case of all Noyes publications, contents organized in such a way as to serve as a subject index, index of companies who hold the patents, index of the inventors and US patent number index.

The book is recommended for all dairy and food technologists and for libraries of research and teaching instituttes.

M. R. CHANDRASEKHARA
PROTEIN FOODS AND NUTRITION DEVELOPMENT ASSOCIATION OF INDIA, BOMBAY.

Chemistry and Specifications of Pesticides: Second report of the WHO Expert Committee on Vector Biology and Control, World Health Organisation Report Series, No. 620, 1978, Price: 36; Sw. fr. 5; US $ 2.50.

This is the second report of WHO Expert Committee on Vector Biology and Control. Chemical pesticides continue to hold the filed in vector control programmes and a periodical review is essential for laying down and modifying recommendations regarding their use. Thus, this small book forms a very essential guideline in this area.

A considerable part of the discussion is on the serious problem of malathion poisoning to humans. The principal potentiator of toxicity is isomalathion formed after manufacture and during shipment and storage. The Committee has laid down a practical limit. of 1.8 per cent of the nominal malathion content as the acceptable value.

New insecticides discussed by the Committee include synthetic pyrethroids and insect growth regulators. Chlorpyrifos and pyrimiphos methyl are being developed as encapsulated formulations.

The report has two annexes on the recommended changes in specifications and in the methods employed for testing the compounds.

The report is a worthwhile addition to libraries and pesticide laboratories.

R. RADHAKRISHNAMURTY
C.F.T.R.I., MYSORE

Multilingual Dictionary of Fish and Fish Products: by Organization for Economic Co-operation and Development, Paris—XVI; Fishing News Books Ltd., Farmham, Surrey, England. 2nd Ed.; 1978; p. 430.

This dictionary contains 1117 words and terms used in the fish trade. The main entries are serially numbered, and are arranged in their alphabetical order in English.

A brief explanation of the term in English and French is followed by its equivalents in 14 other languages—11 West European languages as well as Greek, Serbo-croat, and Turkish. The scientific name of each species of fish is also given. There is an alphabetical index in each of the 15 languages and also an index of scientific names, which refer the reader to the main entry by means of the serial number.

Every effort has been made to standardize the names of various types of fish and to arrive at a unified nomenclature. Similarly, much effort has gone into finding the correct equivalents of the terms used in the processing and marketing of fish. The printing is very neat and clear-a strong point in any dictionary. All in all, an excellent work of reference.

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

ASSOCIATION NEWS

OBITUARY

Dr V. Subrahmanyan

Dr V. Subrahmanyan, Founder-President of the Association of Food Technologists (India) passed away following an operation at Thiravarur on 30 January 1979, aged 77.

Dr Subrahmanyan began his scientific career in 1922 in the Department of Biochemistry, Indian Institute of Science, Bangalore. Two years later he went to England to work at the Rothamsted Experimental Station on the biochemistry of swamp soils in reation to the nutrition of the rice plant, which earned him in 1926 the Research Medal of the Royal Agricultural Society of England. He returned to Bangalore in 1927 with the D. Sc. degree of the University of London, to continue his research, first as a lecturer and then as Professor of Biochemistry, for the next two decades. His research interests in Bangalore included soil productivity, the treatment and utilization of sewage and solid wastes for agricultural production, and food processing and nutrition. During World War II he undertook several schemes of research and development that were of immediate practical utility.

His growing interest in the technological aspects of food led the Council of Scientific and Industrial Research in India to invite him in 1948 to plan, start and direct the Central Food Technological Research Institute at Mysore. He organized this into one of the largest and best-equipped research institutions in the country, and indeed into perhaps the most comprehensive food institute in the world. A great deal of work was undertaken in such areas as the processing of cereals, pulses and oilseeds, and the preservation of fruits, vegetables and fish. Special mention should be made of his work on the development of the baby food industry based on buffalo milk.

Dr Subrahmanyan published over 600 papers in various areas of food technology. Several awards paid formal recognition to the merit of these contributions: the Rafi Ahmed Kidwai Prize of the Indian Council of Agricultural Research first in 1960 for "outstanding contributions in dairy science", and again in 1963 for "outstanding contributions in horticulture'. in respect of the preservation of fruits and vegetables; the Padma Shri of Government in 1960; the Sen Medal of the Institution of Chemists of India for "distinguished contributions in chemical technology" in 1959; the K G Naik Medal for "distinguished contributions in biochemistry" in 1964; the Babcock-Hart Award of the Institute of Food Technologists, USA in 1962 for "outstanding contributions in food technology" resulting in the betterment of human health through nutrition; the Friesland Award of the Netherlands Association for the Advancement of Dairy Science in 1963 for "outstanding contributions on the role of milk and milk products in tropical nutrition"; awards of recognition from the Philippine Association of Nutrition and the Philippine Association of Food Technologists in 1966 in appreciation of contributions leading to the advancement of the coconut industry, in particular the yield and quality of copra; and the B. C. Guha Memorial Lecturership of the Indian National Science Academy for 1969.

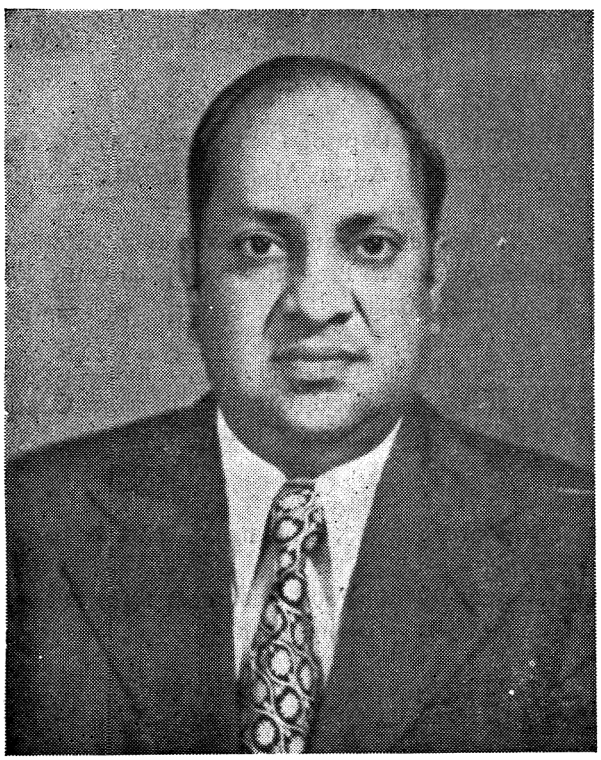
After retirement from Mysore, he was appointed Emeritus Scientist of the Council of Scientific and Industrial Research. During 1964-66, he was in the Philippines as FAO Food Technologist, followed by three years (1966-69) as Adviser, Subsidiary Foods and Nutrition at the Ministry of Food, Agriculture, Community Development and Cooperation, New Delhi.

Since 1969, he had acted as Honorary Adviser to Government, and was Officer-in-Charge of the Government of India project for improvement of the storage, processing and milling of rice.

The Paddy Processing Research Centre, Thiruvarur, which Dr. Subrahmanyam helped to start during 1968-69, has since attained an authoritative position in the pre-and post-harvest technology of rice and its authoritive position in the pre- and post-harvest technology of rice and its associated products. The use of salt spray for the quick ripening and increased yield of paddy, the elimination of smell from and an increased yield of parboiled rice, the production of oil-rich bran and the isolation and utilisation of rice germ have all found extensive application.

Even at the time of his death he was launching, through the Indian Council of Agricultural Research, a new National Centre for the Rice-based Farming System, and an Operation Research Project for Landless Labourers and Marginal Farmers.

Dr. Subrahmanyan founded the Society of Biological Chemists (India) during his stay in Bangalore. The Association of Food Scientists and Technologists (India) was launched in Mysore and nurtured through its infancy to sustained growth: the highest honour of the Association is the institution of V. Subrahmanyan Industrial Achievement Award. In his death the country has lost an eminent scientist, a dedicated worker and an inspiration to many. May his soul rest in peace.



Dr D. V. S. K. Rao

Dr Devarakonda Venkata Seshachela Kameswara Rao expired of burn injurues at Jaslok Hospital in Bombay on 20 December 1978, the only victim of an air crash in Hyderabad that had occurred two days earlier. He was just short of 52, and left behind four children, his wife having predeceased him by a decade.

Dr. Rao was a chemical engineer with bachelor's and master's degrees from Andhra University. This was followed by short spells of teaching and in the sugar, paper and chemical industries, before he went on to London University to earn a doctorate in 1955. Thereafter he joined Unilever in the UK as process development engineer, first at Port Sun-light in the oil, soap and detergent areas (resulting in a patented continuous process for neutralisation of alkyl benzene sulphonic acids), and then at Colworth House (where a fluidised-bed process for dehydration of vegetables was likewise patented). In 1960, Dr. Rao was transferred to Hindustan Lever Ltd., in Bombay for development work, from where he moved on as Food Development Manager to the Lever Factory in Ghaziabad, UP, dealing with cattle feed, convenience foods, baby foods and dehydrated peas.

Six years later, he joined the Britannia Biscuit Co. Ltd., Bombay as R & D Head, involving himself in enriched bakery products, frozen foods and the like. This led to an interest in applied nutrition, and in 1974 to his joining UNICEF, New Delhi as Project Officer for the South-Central Asia Region. He turned his considerable energies to devising means of producing and distributing low-priced nutritive foods to vulnerable groups of the population, and to such ancillary measures as improving water supplies.

Simple and approachable, there were few programmes in food and nutrition in the last decade with which he was not associated. He was at various times a representative of the Federation of Biscuit Manufacturers Association of India and of the Federation of Indian Chambers of Commerce, a member of several executive and advisory committees (including the Scientific Advisory Committee of CFTRI), and an active participant in ISI activity. He will be missed alike for his wisdom and affability.

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ERRATA

Following corrections are indicated for the paper entitled "Studies on Biochemistry of Higher Fungi II. Submerged Growth of a Few Mushrooms in Synthetic Media" by A. K. Ghosh and S. Sengupta published in **this Journal** 1978, **15** (6), 237-242.

In page 237, column 2, para 2, the sentence starting from: "The complex media used contain (w/v per 100 ml) ..... agar, 2.5., should be read as "The complex media used contain (w/v per 100 ml)..... $MgSO_4$, $7H_2O$, 0.05; $FeSO_4$, $7H_2O$, 0.025; boric acid, 0.057; $CuSO_4$, $5H_2O$, 0.0039, $MnCl_2$, $4H_2O$, 0.0036.....agar, 2.5.

In page 239, column 2, para 2, last line should be read as Zn^{++} Mo^{++} Ca^{++} Mn^{++} boric acid Fe^{++}.

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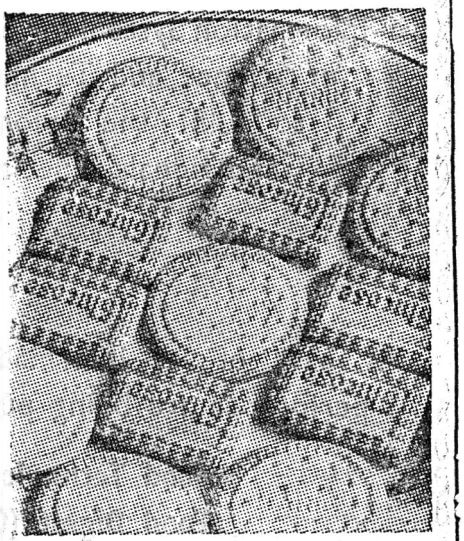

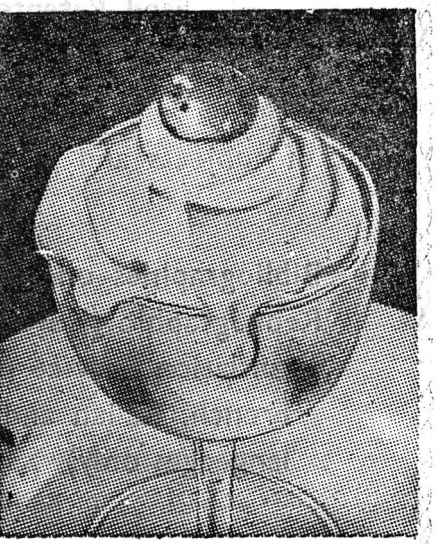

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1. Manuscripts of papers should be typewritten in double space on one side of the paper only. They should be submitted in **triplicate.** The manuscripts should be complete and in final form, since no alterations or additions are allowed at the proof stage. The paper submitted should not have been published or communicated anywhere.

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

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

4. **Abstract:** The abstract should indicate the scope of the work and the principal findings of the paper. It should not normally exceed 200 words. It should be in such a form that abstracting periodicals can readily use it.

5. **Tables:** Graphs as well as tables, both representing the same set of data, should be avoided. Tables and figures should be numbered consecutively in Arabic numerals and should have brief titles. Nil results should be indicated and distinguished clearly from absence of data.

6. **Illustrations:** Line drawings should be made with *Indian ink* on white drawing paper preferably art paper. The lettering should be in pencil. For satisfactory reproduction, graphs and line drawings should be at least twice the printed size. Photographs must be on glossy paper and contrasty; *two copies* should be sent.

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

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

   In the text, the references should be included at the end of the article in serial order.

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

   (*a*) *Research Paper:* Menon, G. and Das, R. P., J. sci. industr. Res., 1958, **18,** 561.

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

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

   (*d*) *Proceedings, Conferences and Symposia:* As in (*c*).

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

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

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Research Papers

Research Notes

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