JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

ASSOCIATION OF FOOD SCIENTISTS & TECHNOLOGISTS, INDIA

VOL. 18 NO. 6 NOV.–DEC. 1981

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(INDIA)

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Manuscripts for publication and books for reviewing in the Journal should be addressed to the Editor, Journal of Food Science and Technology, AFST, Central Food Technological Research Institute, Mysore-570013. The Editor reserves the privilege of editing the manuscript to make it suitable for publication in the Journal.

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JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

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ANNOUNCEMENT

JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

In view of the high increases in production costs, we have been forced to increase the subscription rates from Volume 19, 1982.

The new rates are given on inside front cover and are operative from January 1982. Membership rates are not increased. Subscribers are requested to renew their subscriptions for 1982 at the new rates and cooperate.

Hony. Secretary

RESEARCH PAPERS

Semi-pilot Scale Studies on Wheat Disinfestation by Gamma Radiation

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Manuscript received 3 *April* 1981; *revised* 7 *August* 1981

A semi-pilot scale study on disinfestation of wheat by gamma-irradiation was undertaken to ascertain the feasibility of the process. Wheat was deliberately infested with a common grain pest and stored. In another experiment, infested wheat grains were gamma-irradiated at 30 krad (0.3 kGy) dose levels in throughflow irradiator or conventionally fumigated prior to storage and their storage parameters were compared with that of the control. In irradiated wheat, the insects were destroyed instantly and there was a complete inhibition of development of all the metamorphic stages of the insects at later stage. Whereas, in unirradiated and fumigated wheat they rapidly proliferated. This was also evident from very low percentage of weeviled grain in the irradiated lot. The results further indicated that a single radiation treatment was as effective as three fumigations. The data on economic feasibility of irradiation process showed that it is comparable with fumigation process.

Cereals are generally stored for long periods between harvests, often under conditions that result in extensive losses. Food grain losses during handling, transportation and storage are estimated at about 15 per cent of the total production[1]. A considerable portion of losses during the storage is due to insects and rodents[1]. Chemicals and fumigants have been employed to control the storage pests of food grains. But their use brings problems of toxic residues in the grains or health hazards to the operator. Besides, they are not effective against all the metamorphic stages of insects[2]. Runner[3] had demonstrated for the first time that high doses of X-rays could destroy eggs, larvae and adults of cigarette beetle, *Lasioderma serricorne*. Further, it is shown that the complete and immediate kill of insects can be achieved by gamma-irradiation at 0.3 to 0.5 kGy, whereas, complete reproductive sterilization followed by death is possible within two weeks at 0.1 to 0.2 kGy.[4] Hence, irradiation has a merit as an alternative method of insect control, which is effective against all the metamorphic stages of insects, automatic in operation and free from human error of under or overdosing. Besides, the radiation treatment eliminates the pesticide residue problems in the grains[5].

Realizing the vast potentialities of ionizing radiation for grain disinfestation in India, studies on various aspects of radiation disinfestation of wheat were carried out in comparison with the currently practised fumigation methods for evaluating the feasibility of this technology*.

Materials and Methods

'Punjab Dara' wheat (moisture content 8-9 per cent) filled in jute bags was supplied by Food Corporation of India (FCI). Phostoxin (aluminium phosphide) tablets were purchased from Bayer, India.

Storage bins: Six bins (about 1,600 kg capacity each) were fabricated from corrugated galvanised mild steel, based on some modifications of the design prepared by Punjab Agricultural University (Fig. 1). Neoprene rubber gaskets for inlet and outlet ports were provided. Wingnut-bolts were used for tightening the lid on the manhole with least clearance, for precluding the insect entry and maintaining more or less uniform temperature during storage. A multiscanner recorder, for recording the temperature inside the bin at different heights and points was designed, fabricated and installed. The bins were fumigated before use with phostoxin (one tablet is equal to 3 g) to render them insect-free.

Irradiation: Throughflow irradiator used (Fig. 2) is a self contained irradiation facility, specially designed and fabricated by Atomic Energy of Canada Limited. It is transportable and self-shielded and could process granular, free flowing materials. The gravity fed grain into the hopper on the inlet side of the irradiator is

*These studies were carried out in collaboration with the Food Corporation of India (FCI).

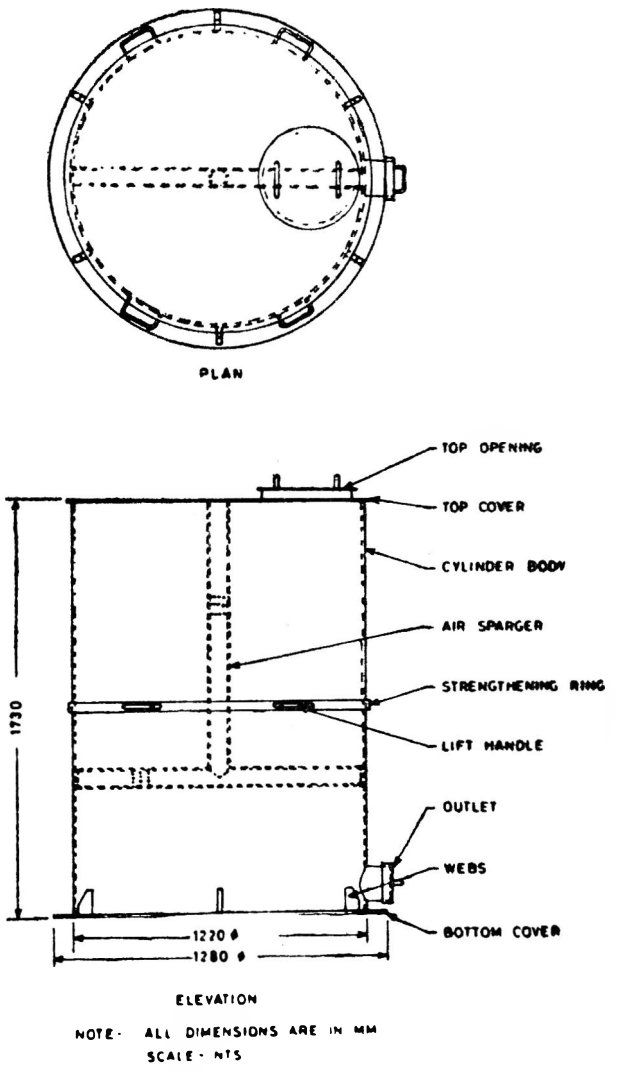

Fig. 1. Grain bin with sparger assembly

picked up by the main screw conveyor; it then gets traversed in a horizontal direction though a tunnel in the centre of the irradiator's main shield. The centre portion of the screw conveyor is surrounded annularly by ^{60}Co source so that the grain gets irradiated as it is conveyed. Irradiator's throughput is controlled by a metering screw conveyor, located on the outlet side of the irradiator. This runs at a slower rate than the main screw conveyor, causing the grain to 'back up' inside the irradiator tunnel. As a result, the main conveyor tunnel operates at approximately 80 per cent capacity and the grain gets throughly mixed during the process of irradiation, an evenflow being maintained at the discharge port.

The metering screw conveyor is directly coupled to a variable speed electric motor. A speed control permits a regulated flow of grain through the irradiator within a range of 5.5 to 34 cft/hr. The source strength of the irradiator is 28,000 curies, having a throughput capacity of 372 kg/hr at 0.15 kGy, minimum dose and bulk desnsity of 0.75 g/cc. The speed of conveyor at 0.15 kGy dose level was calculated and adjusted to 1380 r.p.m.

Dosimetry: To determine the dose delivered to the bulk grain, a dosimetry system was developed. Since the dosimeter has to be mixed with the grain, it should have the same flow characteristics as the grain and it has to resist breakage. Red acrylic pellet dosimeters provide an accurate record of the absorbed dose in a mass of wheat treated by the throughflow irradiator.

Rotor lift mechanism: A rotor lift mechanism is designed for lifting the grain to the inlet and for taking it out from the outlet of the irradiator to the metal bins (Fig. 2). A horizontal and vertical screw conveyor assembly are joined together in such a way that the movement of grain at a desired point is easily obtained. When coupled to the irradiator, the rotor lift mechanism removes the possibility of grain reinfestation. Layout of storage bins and irradiator are shown in Fig. 3.

Infestation of wheat: Six bins were filled up to 5/6th of their capacity; about 3 kg of wheat were infested artifically with eggs, larvae and pupae of rice weevil *Calandra oryzae* and spread over the surface along with about 4,000 newly emerged adults in each bin. The bins were then filled upto the brim and closed tightly and stored. Two bins (3,200 kg) served as control.

Irradiation of wheat: Further, artificially infested (3,200 kg) wheat was passed through ^{60}Co throughflow irradiator assembly (Fig. 1) twice at the interval of 8 hr with a minimum dose of 0.3 kGy. The operational capacity and the source strength of the irradiator necessiated delivery of the dose in two instalments, though care was taken to minimise the interval between the two treatments. Wheat was stored in two bins.

Fumigation of wheat: For comparative studies, 3,200 kg of wheat was fumigated with recommended dose of phostoxin (9 g/ton) for 120 hr. The tablets were placed at the bottom, middle and upper layers of wheat to ensure uniform fumigation. The treatment was given at least three times as and when required, during the course of experiment. All the 6 bins, treated as described above, were stored indoor at ambient temperature ($25\pm5°C$).

Determination of wheat quality: Samples (300 g) were drawn every month for 12 months from top, middle and bottom layers of each bin by a grain sampler for measuring physical properties, as well as for entomological studies.

Measurement of temperature and moisture: Temperature of the grain was measured directly during sampling by the thermometer provided in the grain sampler. Moisture content of wheat (30-40 mesh) was determined using infrared moisture balance and also by air oven

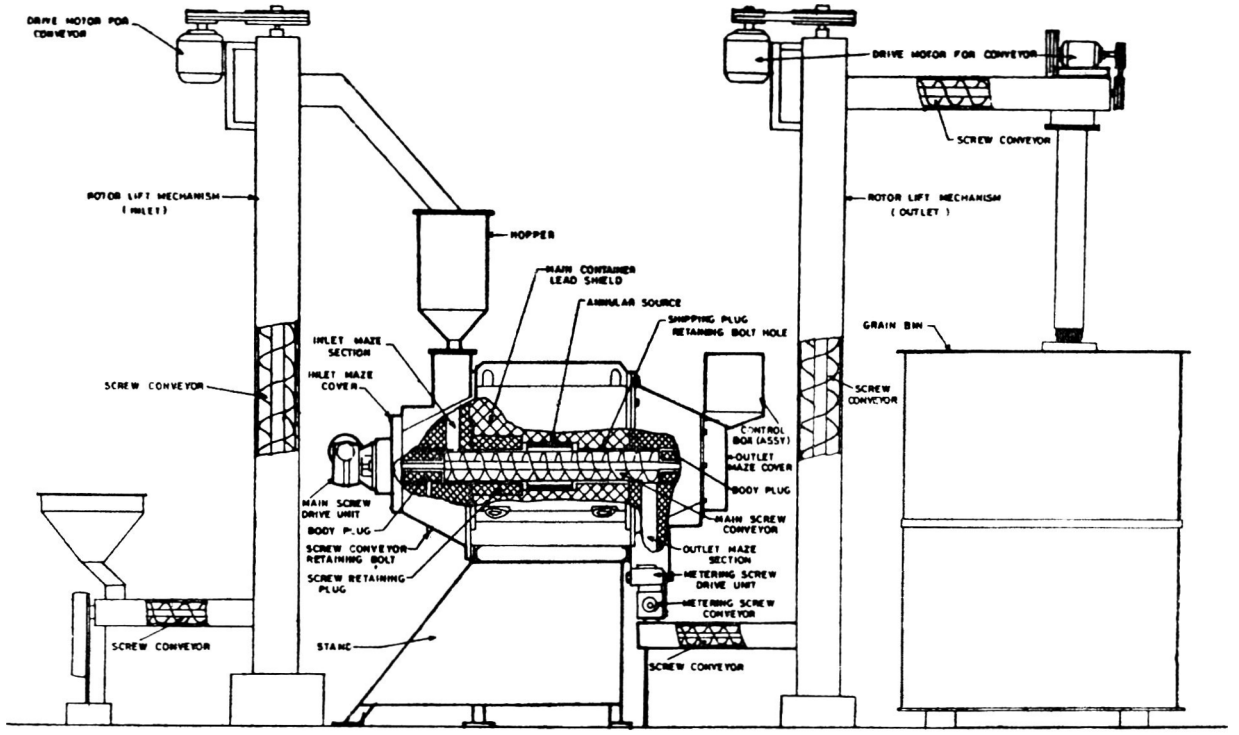

Fig. 2. Throughflow irradiator and rotor lift mechanism

method[6], till constant reading was obtained (approx. 15 min) with $\pm$0.2 per cent variation.

Radiation survival of insects: The insects were sieved out from wheat samples (100 g), collected from three layers in bins and number of living and dead insects were recorded separately. Similarly, per cent weeviled grains in 100 g samples were also counted.

Results and Discussion

Temperature and moisture profiles during storage: Average moisture content and temperature changes in control (untreated), fumigated and irradiated wheat samples, stored for 12 months are shown in Fig. 4. Moisture content of wheat was observed to be in keeping with the seasonal variations in temperature during the

TABLE 1. NUMBER OF INSECTS OF *RHIZOPERTHA DOMINICA* PRESENT UNDER DIFFERENT TREATMENTS DURING STORAGE OF WHEAT

Storage period (months)	Irradiated		Control		Fumigated	
	Repl. 1	Repl. 2	Repl. 1	Repl. 2	Repl. 1	Repl. 2
1	0.0 (0)	0 (0)	2 (0)	0.3 (0)	3.0 (0)	0.0 (0)
2	0.0 (0)	0 (0)	3 (0)	1.0 (0)	3.0 (0)	0.3 (0.3)
3	0.0 (0)	0 (0)	40 (0)	8.0 (0)	12.0 (0.3)	4.0 (0)
4	0.0 (0)	0 (0.3)	0 (93)	0.0 (37)	0.0 (21)	0.0 (20)
5	0.0 (0)	0 (0)	0 (173)	0.0 (24)	0.0 (17)	0.0 (3)
6	0.0 (0)	0 (2)	2 (173)	0.3 (18)	0.0 (19)	0.0 (11)
7	0.6 (0.6)	0 (2)	12 (125)	2.0 (6)	0.3 (20)	0.0 (5)
8	0.0 (0)	0 (0)	8 (103)	7.0 (18)	5.0 (28)	1.0 (3)
9	0.6 (1)	0 (1)	21 (155)	2.0 (269)	0.0 (32)	0.0 (8)
10	0.3 (2)	0 (0.3)	211 (154)	268.0 (304)	0.0 (23)	0.3 (8)

Figures in parentheses represent dead insects in 100 g. wheat.
Values are average of top, middle and bottom layers.

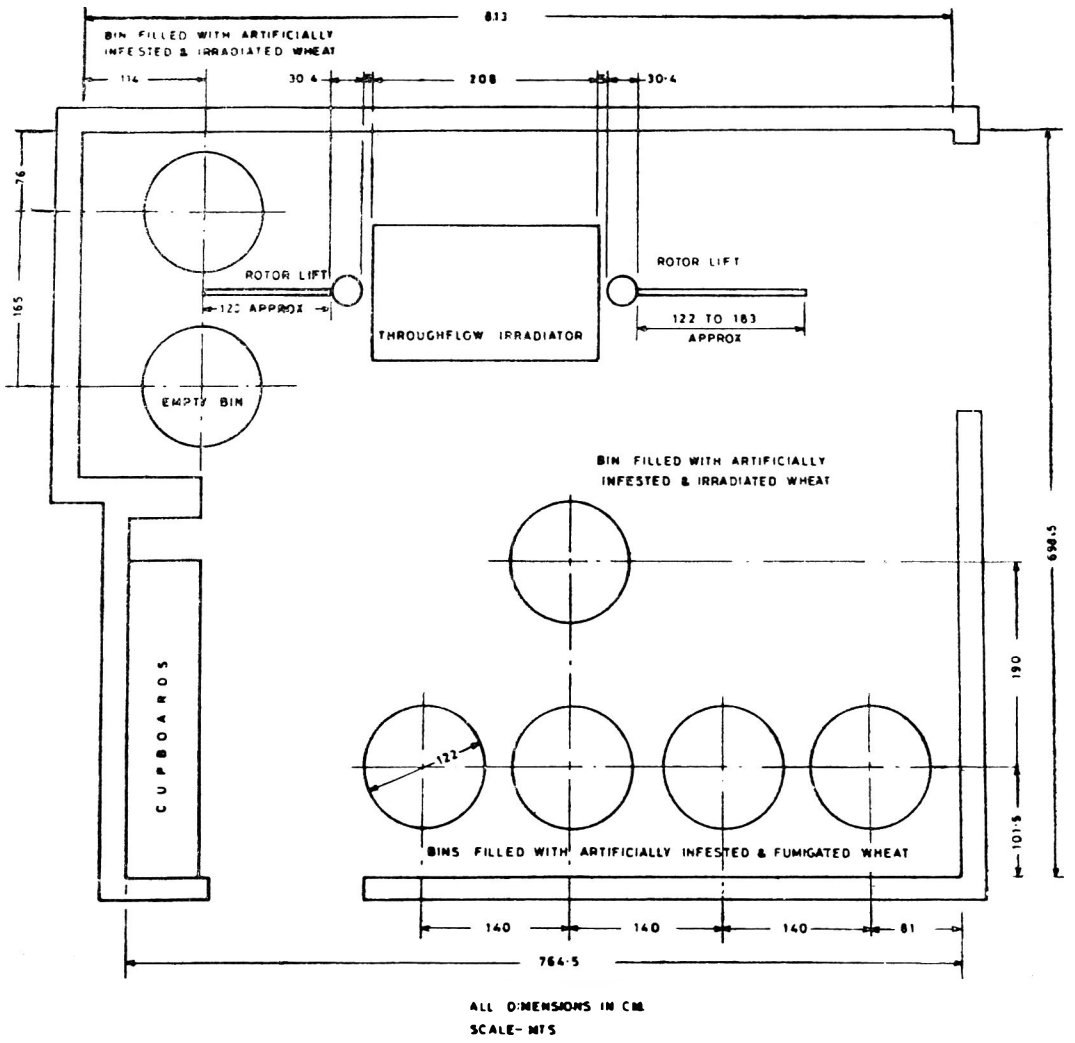

Fig. 3. Layout of storage bins and irradiator

storage period, being higher during the months of May and October, as expected. Higher temperature in control bins may be attributed to the presence of much greater insect population[7,8]. Results describing the effects of irradiation and fumigation on the microbial population and rheological properties of artificially-infested wheat samples, have been reported earlier[9].

Entomological studies: Wheat, artifically infested with *C. oryzae* had a natural infestation of *Rhizopertha dominica* and *Tribolium* spp. In unirradiated wheat, these species multiplied rapidly during the storage period of one year (Tables 1, 2 and 3). However, insect population could be controlled effectively by using moderately low gamma-radiation dose (0.3 kGy). This can perhaps be explained on the basis of inhibition of insect development or induced sexual sterility in adults[10,11].

Comparative economics of grain disinfestation by fumigation and irradiation: A comparison of the relative economics of irradiation versus fumigation should be reckoned with their relative costs and the difference in losses during storage between irradiated and fumigated grains. In the absence of commercial irradiators for grain disinfestation, the irradiation cost estimates are only tentative. The results are based on our small scale bin storage experiments and on observation made by FCI.

Fumigation is carried out for 4-5 times in a year for wheat stored in gunny bags in godowns compared to only twice for wheat stored in insect-proof silos, to keep the infestation under permissible level. The total cost of each fumigation treatment for silo storage is estimated as Rs. 1.75/ton of wheat/year[12] including the cost of phostoxin tablets (Re. 0.72) and labour charges (Re. 1.00). Since, wheat is stored in silos for a period of 1 to 4 years, the fumigation cost is estimated as Rs. 6.55/ton (Table 4) for the entire period of storage.

However, the actual costing of radiation disinfestation

TABLE 2. NUMBER OF INSECTS OF *TRIBOLIUM* SPP PRESENT UNDER DIFFERENT TREATMENTS DURING STORAGE OF WHEAT

Storage period (months)	Irradiated		Control		Fumigated	
	Repl. 1	Repl. 2	Repl. 1	Repl. 2	Repl. 1	Repl. 2
1	0.0 (0)	1.0 (0)	4 (0)	1.0 (0)	2.0 (1)	0.3 (1)
2	1.0 (0)	4.0 (0.3)	5 (1)	6.0 (1)	2.0 (1)	2.0 (1)
3	1.0 (1)	1.0 (21)	11 (3)	4.0 (3)	3.0 (5)	15.0 (4)
4	0.0 (1)	0.0 (3)	0 (39)	0.0 (7)	0.0 (4)	0.0 (8)
5	0.0 (3)	0.0 (21)	0 (17)	0.0 (3)	0.0 (7)	0.0 (15)
6	0.0 (3)	0.3 (5)	1 (9)	1.0 (3)	0.0 (4)	0.0 (10)
7	3.0 (3)	8.0 (7)	18 (22)	4.0 (7)	0.3 (5)	3.0 (15)
8	1.0 (0.3)	2.0 (3)	5 (5)	3.0 (5)	1.0 (3)	6.0 (11)
9	2.0 (2)	0.3 (3)	14 (14)	0.3 (12)	0.0 (5)	0.0 (26)
10	0.3 (5)	1.0 (2)	45 (152)	0.3 (2)	1.0 (4)	0.3 (16)

Figures in parenthesis represent dead insects in 100 g wheat.
Values are average of top, middle and bottom layers.

TABLE 3. NUMBER OF INSECTS OF *CALANDRA ORYZAE* PRESENT UNDER DIFFERENT TREATMENTS DURING STORAGE OF WHEAT

Storage period (months)	Irradiated		Control		Fumigated	
	Repl. 1	Repl. 2	Repl. 1	Repl. 2	Repl. 1	Repl. 2
1	1.0 (2)	0.0 (0.3)	125 (3)	74 (0)	0.5 (8)	129 (3)
2	1.0 (0.5)	0.0 (0.3)	14 (0)	9 (7)	1.0 (5)	7 (5)
3	19.0 (4)	1.0 (5)	26 (19)	90 (15)	0.3 (5)	49 (5)
4	0.0 (4)	0.0 (1)	0 (21)	0 (45)	0.0 (9)	0 (9)
5	0.0 (5)	0.0 (2)	0 (25)	0 (83)	0.0 (3)	0 (15)
6	1.0 (19)	0.0 (1)	1 (14)	0 (30)	0.6 (4)	0 (17)
7	2.0 (17)	0.3 (1)	4 (18)	28 (94)	0.6 (9)	0 (30)
8	19.0 (2)	0.0 (0)	13 (11)	154 (41)	3.0 (5)	5 (27)
9	12.0 (36)	0.0 (1)	25 (55)	1 (65)	0.0 (7)	0 (29)
10	0.5 (28)	0.0 (1)	16 (43)	1 (206)	0.0 (3)	0 (23)

Figures in parentheses represent dead insects in 100 g. wheat.
Values are average of top, middle and bottom layers.

process is difficult since it depends upon several variations, such as, fluctuation of wheat price, radiation dose as well as efficiency and capacity of irradiator. Besides, there are no large scale commercial grain disinfestation plants in existance anywhere in the world. However, based on the data from U.K. for the bulk grain irradiator with maximum capacity of 96 t/hr, an optimum cost price is estimated for one ton of wheat irradiated at 0.25 kGy. This is plotted against the plant capacity (Fig. 5). It is estimated that irradiation cost may be roughly Rs. 4.75/t (Table 4) when plant capacity and storage capacity at Borivli silos (100,00 tons) are taken into consideration. However, the investment and operating costs may vary to some extent for a plant designed and built in India. Besides, these data were published 15 years back in U.K.[13]. and taking into consideration three-fold increase in price, the cost of irradiation for plant capacity of 32 t/hr, would be estimated as Rs. 7.50/t.

Relative loss in irradiated and fumigated grains: Pilot scale storage studies indicated that the average weeviled grains in fumigated samples were 2 per cent, whereas, weeviled grains were completely absent in irradiated wheat. The average recommended loss for weeviled grain should be taken as 1 per cent. However, in commercial storage facility at Borivli about 0.5 per cent of

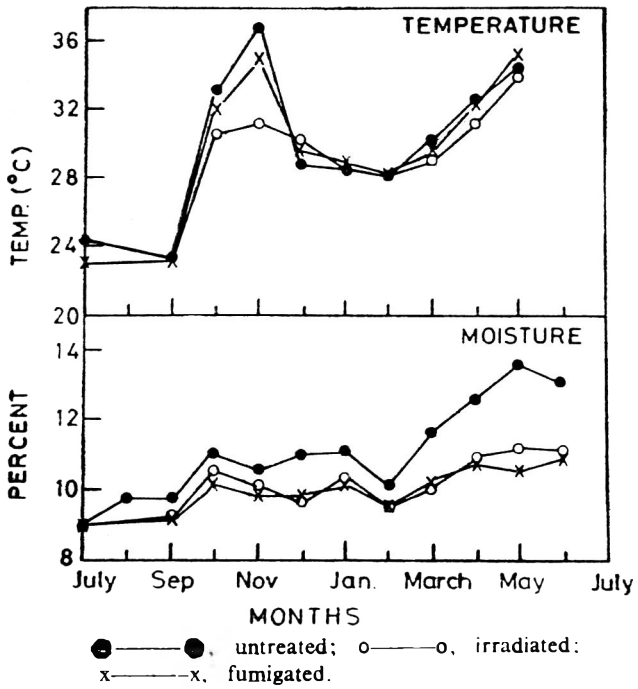

Fig. 4. Variations in temperature and moisture content in wheat during storage at ambient temperature

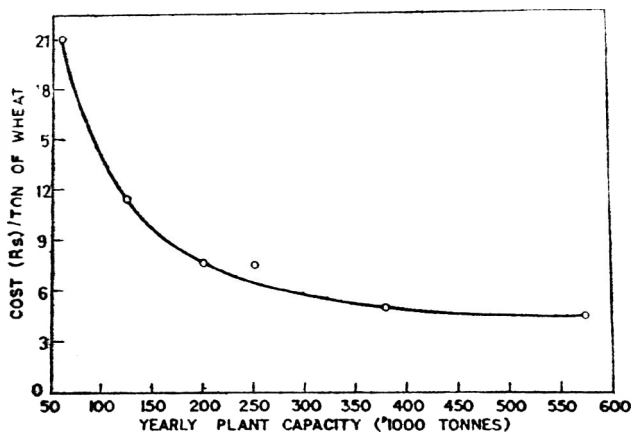

Fig. 5. Relationship between cost of radiation disinfestation treatment of wheat with plant capacity.

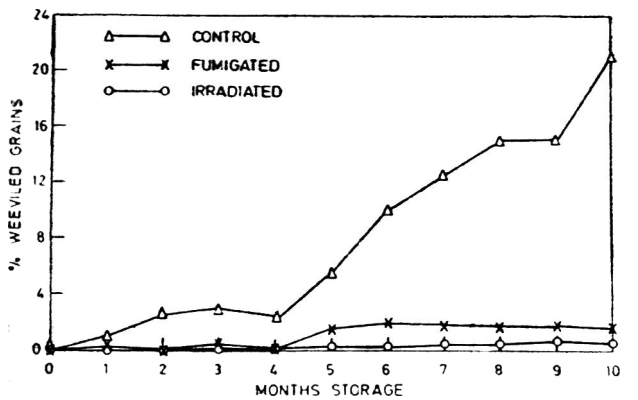

Fig. 6. Progressive increase in weeviled grain during storage, average of two replicates.

TABLE 4. COMPARATIVE ECONOMICS OF FUMIGATION AND IRRADIATION

Capacity (t/yr)	Irradiation Cost (Rs/t)	Value of grain lost	Total (Rs/t)
100,000	13.25	0	13.25
200,000	7.50	0	7.50
300,000	6.00	0	6.00
600,000	4.50	0	4.50

By fumigation, the cost is Rs. 6.55/- t, the value of grain lost is Rs. 3.50/- t, irrespective of the quantity fumigated.

the total grains are infested with weevils[14]. Assuming the price of wheat as Rs. 1.4/kg, the losses in fumigated wheat due to weevils may be about Re. 0.14 and Rs. 3.5 per ton on the basis of Trombay experiments and FCI sources, respectively. Thus, the total cost of the fumigation operation works out Rs. 10.00/t (Table 4). Progressive increase in weeviled grains in bins during storage is shown in Fig. 6. At least 3 fumigations are necessary per year to check insect infestation, whereas, only one irradiation treatment followed by storage in insect-proof containers appears to be sufficient for disinfestation and long term storage of wheat.

Radiation disinfestation of wheat envisages the use of radiation energy from isotopic sources (Co^{60}, Cs^{137}). Since the radiation source cannot be switched off, the process would be economically feasible only when large quantities of grains are handled and stored throughout the year. However, it needs to be emphasized that irradiation offers no protection from reinfestation. It is therefore, imperative that the time gap between irradiation and storage should be minimal to reduce the probable insect attack. This could be achieved by integrating the irradiator with grain loading facility at the storage site.

Thus, the comparative economic data clearly indicate that at plant capacity of 200,000 t./year, radiation treatment for wheat disinfestation would be more economical than fumigation.

Acknowledgement

Thanks are due to Shri G. W. Rahalkar, Biology & Agriculture Division, Bhabha Atomic Research Centre for assessment of insect infestations in wheat during experiment. Assistance provided by Dr S. V. Pingale, Shri H. J. Kakodkar and Shri H. S. Kalbere of Food Corporation of India is also greatly appreciated.

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Studies on *Bacillus cereus* Contamination of Rice and Rice Preparations

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Manuscript received 15 *April* 1980; *revised* 7 *January* 1981

Raw rice, cooked rice and rice products collected from homes in Mysore city were analysed for bacterial count and for *B. cereus*. More than 50% of raw rice samples showed *Bacillus cereus* contamination. Cooked rice samples stored for 12 hr also showed varying levels of contamination. Among the preparations like *Pongal*, *Bisibele bhath*, *Coconut rice*, *Tamarind rice*, *Lime rice* and *Curd rice* examined, only coconut rice showed highest count of *B. cereus* and no contamination was observed in curd rice. Kinetic studies carried out using vomitting type strain *B. cereus* (F. 4810) indicated that unpolished rice and long variety parboiled rice (*SR*-26B) showed slightly better growth rate than the polished rice and short variety parboiled rice. Seven log. difference in *B. cereus* count has been found in 26 hr incubated rice preparations having pH in the range of 5.1–7.8. Among rice preparations whose pH is 4.2–4.3, *lime rice* showed steady growth rate whereas curd rice indicated negative trend.

In recent years, there has been increasing number of reports which has established *Bacillus cereus* as a food poisoning organism. Gilbert and Taylor[1] have reported 34 outbreaks of *B. cereus* food poisioning, wherein fried or boiled rice from Chinese restaurants and "take away" shops have been the source of infection. Goepfert *et al.*[2] have reviewed the evidences relating to the role of *B. cereus* as a food poisoning agent, including properties of the organism, methods of isolation, identification and enumeration. Chopra *et al.*[3] have recorded 100 per cent incidence of *B. cereus* in milk, meat and meat products and 75 per cent incidence in milk products.

In our country, rice is the staple diet. Keeping cooked rice for sometime as practiced in homes under ambient conditions. provides ideal environment for bacterial growth.

The present communication deals with studies carried out on *B. cereus* contamination of raw rice, cooked, rice, and some common rice preparations along with rate of growth of vomitting type of *B. cereus* in the above food preparations.

Materials and Methods

One hundred and seventeen samples of raw rice, 6 samples each of long variety (SR-26B) parboiled rice

and short variety parboiled rice, 7 samples of cooked rice and 4 samples of each rice preparations were collected from different homes, commercial establishments and departmental canteens in Mysore city in sterile containers and examined within 3-4 hr after collection.

The various ingredients in different rice preparations selected for the study are:

Tamarind rice contains cooked rice, tamarind concentrate, groundnut oil/sesame oil, spices, fried groundnut seasoned with curry leaves, dehydrated coconut gratings and Bengalgram.

Curd rice (*Dahi bhath*) consists of cooked rice, milk curds and coconut gratings seasoned with green chillies, coriander, ginger, mustard and Bengalgram. Addition of chopped onion is optional.

Bisibele bhath consists of cooked rice and tur dhal, ghee (clarified butter) and spices with or without vegetables.

Coconut rice contains cooked rice, coconut gratings, cashew or groundnut kernels, refined groundnut oil, seasoned with mustard and dried chillies.

Pongal is prepared from cooked rice, fried and cooked greengram dhal, coconut gratings, pepper, cashewnuts and ghee.

Vegetable bhath contains cooked rice, vegetables like carrot, potato, beans, green peas, onion, tomatoes, coconut gratings seasoned with spices and condiments.

Isolation and identification of Bacillus cereus: All media and reagents were prepared as mentioned in Bacteriological Analytical Manual,[4] and Compendium of Methods for Microbiological Examination of Foods[5].

Raw rice: The enrichment procedure described by Gilbert *et al.*[6] was followed for the isolation of *B. cereus* from raw rice.

Cooked rice and rice preparation: Fifty gram samples of cooked rice/rice preparation were transferred aseptically to 450 ml of 0.1 per cent peptone water to obtain 1/10 dilution. Serial dilutions were made upto 10^{-8}. 0.1 ml of appropriate dilutions were surface spread on mannitol egg yolk polymixin agar (MYP) and Kendall B.C. medium and incubated for 24 hr at 35-37°C. Characteristic colonies of *B. cereus* on MYP medium shows a halo of dense precipitate (indicative of positive lecithinase activity) with a distinct pinkish background (indicative of mannitol negative reaction) were considered presumptive positive reaction for *B. cereus*. Additional confirmation was obtained from growth on Kendal's B.C. medium where colonies surrounded by opaque zone with purple colouration constituted positive reaction of *B. cereus*. Further confirmation of the isolates was made by employing biochemical and sugar fermentation tests.[7]

For kinetic studies, 100 g samples of different rice preparations prepared from "Bangar sanna" variety were aseptically transferred into 250 ml sterilized beakers. The beakers were covered with aluminium foil and sterilized at 15 lb for 15 min. They were then inoculated with 18 hr old suspension of vomiting type strain of *B. cereus* procured from Central Public Health Laboratory, Collindale, London. The samples were incubated at 35-37°C upto 26 hr. Samples drawn at intervals were analysed for *B. cereus*, total bacterial count and changes in pH using pH meter (Systronics, model No. 324).

Results and Discussion

Raw rice could get contaminated with *B. cereus* during harvesting and other agricultural operations. Samples of different varieties of raw rice analysed for *B. cereus* by the conventional surface spread technique using mannitol egg yolk polymixin agar did not show positive indications in any of the sample. The same samples were also analysed by the enrichment procedure described by Taylor *et al*[8]. when many of them proved to be positive for *B. cereus*. Taking all the areas together on an average 66.6 per cent was found to be contaminated with *B. cereus*. The moisture content of commercial raw rice ranged from 12 to 14 per cent. At this moisture level, it is rather not possible for the vegetative forms of *B. cereus* to survive and proliferate. Hence the contamination of raw rice could have been by spores of *B. cereus*.

Samples of cooked rice collected from homes were analysed for the survival of spores of *B. cereus*. The results indicated that 12 hr old samples showed *B. cereus* count in the range of 1.7×10^{2} to 8.1×10^{3}/g, the moisture content of cooked rice ranged from 68 to

TABLE 1. *BACILLUS CEREUS* CONTAMINATION OF RICE PREPARATIONS

Rice preparations	Total bacterial count/g	B. *cereus* count/g	pH
Bisibele bhath	6.2×10^{3} – 4.42×10^{6}	1.31×10^{2} – 4.1×10^{3}	5.7
Coconut rice	2.58×10^{4} – 1.04×10^{8}	1.0×10^{2} – 9.0×10^{5}	6.5
Tamarind rice	5.50×10^{4} – 4.60×10^{6}	2.4×10^{2} – 4.0×10^{3}	4.1
Lime rice	4.6×10^{3} – 8.0×10^{4}	2.1×10^{1} – 4.4×10^{3}	4.3
Mango rice	1.39×10^{4} – 1.2×10^{6}	1.4×10^{2} – 3.47×10^{3}	4.2
Curd rice (Dahi bhath)	1.80×10^{3} – 2.13×10^{7}	Nil – 2.4×10^{1}	4.0

The samples were processed within 3-4 hr of their preparation.

The range of counts indicated is from four batches of preparations prepared on different days.

TABLE 2. GROWTH OF VOMITING TYPE OF *BACILLUS CEREUS* (F. 4810) IN COOKED RICE AND RICE PREPARATIONS

Sample	*B. cereus* (log count/g) after indicated times of incubation at 37°C		
	0 hr	10 hr	26 hr
Plain cooked rice			
Polished rice	2.0(6.7)	6.9(6.5)	8.0(8.0)
Unpolished rice	2.6(6.7)	7.2(6.5)	8.4(7.1)
Parboiled rice (long var.)	2.3(6.5)	7.4(6.3)	8.9(8.3)
Parboiled rice (short var.)	2.3(6.8)	7.6(6.7)	8.8(8.0)
Rice preparations			
Pongal	2.0(6.2)	8.1(6.5)	9.1(6.2)
Coconut rice	3.1(6.2)	8.5(6.0)	9.6(7.8)
Vegetable bhath	2.8(5.9)	8.0(5.7)	9.0(5.5)
Bisibele bhath	2.8(5.1)	6.6(5.1)	7.4(5.1)
Lime rice	2.6(4.3)	4.5(4.3)	7.1(4.3)
Tamarind rice	3.5(4.2)	3.2(4.2)	3.8(4.2)
Curd rice	3.9(4.2)	3.5(4.3)	3.4(4.3)

(Figures in parentheses indicate pH)

70 per cent. This wide variation in the *B. cereus* count indicated may be due to variations in methods of handling, cooking and storage practiced in different homes. Four log increase in *B. cereus* count has been recorded (1.7×10^6-3.1×10^7/g), when the cooked rice was incubated up to 24 hr. At the end of 36 hr this increased to five log (1.6×10^7-3.7×10^7/g.). These were confirmed when 36 hr old samples procured from homes were analysed (3.1×10^7-3.5×10^7). An earlier report[9] indicated *B. cereus* count in the same range in boiled and fried rice incubated for the same period and collected during food poisoning outbreaks in chinese restaurants and 'take away' shops in Great Britan. The cooked rice showed slight discolouration when the counts were 10^6/g. Further increase in count by one log resulted in the development of off-flavour. As *B. cereus* is proteolytic, off-flavour may be due to proteolysis.

Studies carried out on the effect of types of cooking on the survival of *B. cereus* showed that keeping after 24 hr at room temperature, pressure cooked rice did not show *B. cereus* contamination, whereas rice cooked at atmospheric pressure showed signs of spoilage after 12 hr (6.5×10^2-8.0×10^3/g).

Various types of rice preparations contain cooked rice as the base, which is seasoned and flavoured with spices and condiments. Some of the preparations are acidified with lime juice, tamarind extract, etc. It is possible that these added ingredients may exert influence on microflora with particular reference to *B. cereus*. As seen from Table 1, the highest count for *B. cereus* (9.0×10^5) is seen in coconut rice and lowest (2.4×10^1) in curd rice. In coconut rice, coconut gratings are added in the end and the cooked rice is allowed to air cook before other ingredients are added. No further heat processing is carried out after fresh coconut gratings are added. This may be the reason for higher *B. cereus* count in coconut rice. This point is further corroborated when results of kinetic studies are examined (Table 2). In 26 hr of incubation, 6.5 log difference is seen when pure culture of *B. cereus* is used. Perhaps the very nature of coconut rice preparation with some of its ingredients can support survival and growth of *B. cereus*.

The curd rice has shown the lowest count for *B. cereus*. This may be due to the presence of lactic acid or antagonistic effect of lactic acid bacteria. This is reflected in kinetic studies also where downward trend in growth rate is observed.

With regard to total bacterial count highest count of (1.04×10^8 is observed in coconut rice and lowest count (1.80×10^3) in curd rice. As pointed out earlier the same reasons may hold good for higher total bacterial count in coconut rice and lowest count in curd rice.

The range of pH for the growth of *B. cereus* in laboratory media has been reported[2] to be 4.9-9.3. But the present studies show the survival of *B. cereus* in rice preparations below pH 4.9 also. It is not known whether other ingredients in the preparations had any influence on the survival of *B. cereus*.

It was of interest to study the rate of growth of *B. cereus* in different types of rice preparations. In these studies, vomiting type strain of *B. cereus* has been used. Melling *et al.*[10] has reported a strain of *B. cereus* from food poisoning outbreak having the property of inducing vomiting in Rhesus monkeys only, when they are grown on rice but not on other media. In contrast, a strain isolated from diarrhoeal outbreak produced diarrhoea only, when grown on various media.

In Table 2, the rate of growth of vomiting type *B. cereus* in different types of rice and rice preparations has been indicated. The growth rate was slightly better in unpolished rice than in polished rice. Similar trend was noticed in long variety parboiled rice. With regard to rice preparations they can be divided into two groups based on their initial pH. Some rice preparations have pH above 4.5 and few others have pH below 4.5. Better rate of growth of *B. cereus* has been observed in rice preparations having pH above 4.5. In *Pongal*, coconut rice and vegetable *bhath*, six log difference was noticed after 10 hr incubation and seven log difference at the end

of 26 hr. But *Bisibele bath* showed slightly decreased rate of growth. Among the samples whose pH was below 4.5, lime rice induced steady rate of growth. Net increase between 10 and 26 hr was more than initial and 10 hr samples. Similar trend is not observed in tamarind rice whose pH is also below 4.5. In curd rice a lowering of counts for *B. cereus* is noticed. This may be due to different types of organic acids involved in their preparation. The tamarind rice has tartaric acid derived from tamarind extract, whereas Lime rice has citric acid derived from lime juice. The data obtained show more inhibitory influence of tartaric acid in growth of *B. cereus* compared to citric acid. Lower pH itself does not seem to completely control the growth of *B. cereus*.

Acknowledgement

The authors are thankful to Dr R. J. Gilbert, Central Public Health Laboratory, Collindale, London, for providing the type strain of *B. cereus* used in this study.

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Sorption Affinity of Various Stored Products Towards Acrylonitrile

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Manuscript received 31 *December* 1980; *revised* 23 *June* 1981

Sorption quality of stored food products like cereals and their products, pulses and their products, oilseeds, and their cakes, spices, dry fruits, nuts, instant foods, and infant foods, towards acrylonitrile was studied. Analysis was done by GLC. In general, most of the products were sorptive towards acrylonitrile. The sorption values were very high for paddy, wheat flour, cowpea, some split pulses, sesame, sunflower seeds, coriander, cumin and papad. Sorption was moderate for raisin, dry dates, figs, instant food beverages like 'Nestum', 'Nutramul', 'Horlicks', 'Viva', 'Maltova', maize, some of the pulses, tamarind pulp and brown sugar. Sorption was least for refined sugar, 'Glaxose-D', 'Boost' and 'Bournvita'.

Successful disinfestation of stored products can be achieved by maintaining a lethal concentration of fumigant in the atmosphere for a definite period. Despite satisfactory gas-tight conditions in fumigation systems, the initially applied fumigant concentration drops rapidly or gradually depending on the nature of the fumigant, moisture content and chemical constituents of the stored produce fumigated, the treatment temperature and (reduced or atmospheric) pressure. Besides the basic insect bioassay studies, an understanding of sorption affinity of stored products towards a fumigant at a specified temperature is necessary while fixing the effective dose. The minimum effective dose necessary to control the pest is often considerably less than those applied in practice[1]. Acrylonitrile (AN), one of the highly toxic fumigants, has been used in combination with nonflammable adjuvants for fumigation of cereals, tobacco, walnut, etc., and as a spot fumigant in pro-

cessing machinery[2]. In the present investigation, various stored products were fumigated with AN and their sorption affinities studied.

Materials and Methods

All the foods and food products tested were purchased from the local market except the Energy food, which was a ready-to-eat low-cost processed food containing wheat (54 per cent), brown sugar (27 per cent), chickpea (9 per cent), peanut cake (9 per cent), vitamin mix (0.9 per cent) and calcium carbonate (0.9 per cent), prepared at CFTRI, Mysore. The moisture contents were estimated as described earlier[3]. Round-bottomed-flasks of 305±5 ml volume fitted with stop-cock and a side tube closed by rubber septum (for administering the fumigant liquid) were used throughout the study. Prior to fumigation, volume of empty flask was determined by complete evacuation and subsequent air-filling with a 100 ml syringe (V_1). Hundred grams of stored product was weighed into each flask and again the volume of the flask with the content was determined as above (V_2). The 'effective volume' was then calculated for each flask after deducting the void in the flask with load V_2, from the volume of the flask without load V_1.

An effective dose of 100 mg/l of AN was applied through the septum cap with a microsyringe. There were three replicates for each stored product. On each day, 4-6 commodities were fumigated along with three control replicates of empty flasks fumigated at the same dose. All the fumigations were carried out at the room temperature of 25-30°C. At the end of 24 hr exposure period, AN concentrations in flasks with and without the stored products were estimated by GLC with thermal conductivity detector (Willy Giede GCHF 18). The operating parameters were as follows: column: 1m × 0.63 cm i.d. stainless steel; packing: 5 per cent OV-17 on plain chromosorb W 60-80 mesh; column, injector and detector temperature 100°C; carrier gas: N_2 37ml/min; AN retention time: 6 min 35 sec.

AN sorbed by the treated stored product in 24 hr was calculated by deducting the quantity of AN obtained with load from that of the control empty flask.

Results and Discussion

Based on sorption values, the products tested may be grouped into four categories viz., very highly sorptive (>400 ppm), highly sorptive (300-400 p.p.m.), moderately sorptive (200-300 p.p.m.) and less sorptive (<200 ppm). The products which showed very high sorption affinity are given in Table 1. These include sorghum, rice (raw/para-boiled / steamed / brown or polished), rice bran, wheat, semolina, pearl barley, white oats, corn flour,

TABLE 1. SORPTION AFFINITIES OF STORED PRODUCTS TOWARDS ACRYLONITRILE

Stored Product	Moisture content (%)	Acrylonitrile sorbed in 24 hr: (ppm) Mean±S.D.
Very highly sorptive		
Paddy (with husk)	10.0	413± 4.12
Refined wheat flour	9.5	412± 0.71
Whole wheat flour	13.0	408± 2.12
Cowpea	11.0	407± 2.12
Split cowpeas	7.5	410± 0.71
Split peas	13.5	410± 0.00
Split black gram	9.5	439± 1.73
Sunflower seeds	4.8	421± 0.00
Sesame (black)	3.4	403±11.36
Coriander	....	436± 1.75
Cumin	....	423± 3.61
Papad	15.6	400± 0.00
Moderately sorptive		
Maize	11.0	295± 1.00
Pigeon pea	12.5	277± 2.55
Horsegram	9.5	250±11.31
Tamarind pulp	....	289± 2.12
Raisin	18.2	290±12.04
Dates	9.2	263± 4.08
Figs	14.2	256± 6.52
'Lactodex'	....	257± 1.73
'Lactogen'	3.0*	249±19.00
'Nestum'	5.0*	250±11.02
Horlicks	....	274±20.26
Milk chocolates	....	295± 1.00
Brown sugar	....	268± 8.06
Less sorptive		
'Viva'	....	165± 6.08
'Nutramul'	....	153±25.33
'Maltova'	....	101±11.07
'Boost'	....	58± 6.00
'Glaxose-D'	....	10± 0.00
'Bournvita'	....	4± 0.00
Refined sugar	....	18± 0.00

*as given by the manufacturer

corn starch, field bean, pea (dehydrated or dried), green gram, soybean, chickpea flour, split pulses (lentils, field bean, green gram, pigeonpea, soybean) shelled or unshelled peanuts, cotton seed, linseed, and oilseed meals of peanut and sesame. Many of the spices including cinnamon bark, nutmeg, dry ginger, turmeric fingers/powder, cloves, aniseed, black pepper, cardamom, curry powder, chillies, instant mix powders (*jamun*, *idli*, *vadai* and *dosai*), infant foods ('Cerelac', 'Farex' and 'Nespray)', Energy food, 'Complan', custard powder, apricot, almond, cashewnut, spaghetti, macaroni, poultry feed, fish meal, dried sardines, dry prawn, sal seed, coffee seed and tobacco flakes also showed higher sorption affinity. Moderate sorption was observed (Table 1) with dried foods like milk powders ('Lactogen' and 'Lactodex'), maize, pigeon pea, horse gram, tamarind, raisin, dates, figs and brown sugar. Sorption was less or negligible with refined sugar, instant beverage mixes like, 'Viva', 'Maltova', 'Boost', 'Nutramul', 'Bournvita' and 'Glaxose-D' (Glucose).

Moisture and oil/fat contents are important factors influencing the sorption of fumigants[4,5]. Since AN is soluble in water (7.5 g/100 ml at 25°C) majority of the tested products showed greater sorption affinity towards AN. Some of the infant milk foods (Lactodex, Nestum, Lactogen) and instant food beverages having less than 5 per cent moisture showed moderate or less sorption. However, products with low moisture, like sunflower seeds (4.8 per cent), shelled peanuts (5 per cent) and sesame (3.4 per cent) also showed higher sorption of AN, probably, because of their high fat content. Sesame (fat content 43.3 per cent) and sunflower seeds (fat content 52.1 per cent) were the most sorptive among oilseeds.[6]

Paddy (with husk) and maize, have been reported to show greater sorption affinity towards fumigants[7,8]. In the present study, paddy was the most sorptive (413 ppm) commodity and maize the least sorptive (295 ppm). The greater sorption affinity towards AN observed with milled pulses or cereals may be attributed to the greater surface area available for fumigant sorption[9,10] and the chemisorption by protein in pulses[4,11].

Dry fruits including raisin, dates and figs even at higher moisture levels (upto 18.2 per cent) showed only moderate sorption. 'Glaxose-D', brown or refined sugar were found less sorptive. Kroger and Schuler[12] also observed that sorption of AN was not much with sugars.

The above results indicate that AN can be used for fumigation of processed dry foods, instant food beverages and dry fruits, where the sorption is low. However, doubts are being expressed about the safety of foods containing AN[13]. Thorough aeration of fumigated food materials and residue determination should be carried out to assure that AN is not present.

Acknowledgement

The authors gratefully acknowledge the support and encouragement received from Mr. S. K. Majumder, Deputy Director and Mr. C. P. Natarajan, Director of this Institute.

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Malathion Poisoning in Non-target Species: An Acute Oral Study in Poultry *(Gallus domesticus)*

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Manuscript received 18 *March* 1981, *revised* 15 *June* 1981

Technical Malathion and its 50% EC formulation exclusively, used as ectoparasiticides in poultry were evaluated for their acute oral toxicity in chickens (*Gallus domesticus*). The computed LD_{50} values were 948.1 and 1195 mg/kg body weight for the technical grade and formulation, respectively. Liver and kidney of treated birds showed marked hypertrophy. Histological observations revealed cellular infiltration and necrotic changes in liver and moderate cellular infiltration in kidney. Malathion in emulsifiable concentrate proved more toxic to birds than the technical grade.

Malathion, S-(1,2-bis (ethoxy-carbonyl ethyl) O, O dimethyl phosphorodithioate, a non-systemic insecticide and an acaricide[1], is being extensively employed for controlling ectoparasites in poultry, cattle and pigs[2-4]. As malathion is employed in various public health and domestic pest control programmes, the possibility of its poisoning of non-target species cannot be ruled out. Reports on the acute oral toxicity of malathion in poultry are scarce. Hence this study describes the single dose oral toxicity of malathion (technical and EC formulation) to domestic fowl and its gross effects on liver and kidney.

Materials and Methods

Animals and diet: 8-10 weeks old male birds (*Gallus domesticus*) (White Leghorns) of the weight range 1100-1300 g were randomly grouped and individually caged. They were maintained on commercial grower mash and tap water *ad libitum*.

Pesticide: Malathion technical (96.9 per cent) and malathion 50 per cent EC (a.i.-50 per cent) obtained from Volrho Ltd, India were used.

Experimental design and treatments: The birds were conditioned for a period of one week. Prior to intubation of the pesticide, they were starved overnight. The dosages employed for technical malathion were 700, 800, 1000, 1100 and 1200 mg/kg body weight and for the formulation were 400, 600, 1200, 1600 and 2000 mg/kg body weight. An untreated control group was maintained; and six birds were included for each dosage. The compounds were intragastrically administered through a rubber catheter. The treated birds were not fed on the day of intubation. Observations for the onset of symptoms were made at regular intervals and mortality was recorded. The survivors were maintained for three weeks, during which the feed consumption and weekly body weights were recorded. The birds were autopsied at the end of third week. Fresh weights of liver, kidney, heart, lungs, spleen, adrenals, thyroid, testes and brain were recorded and a portion of each organ was fixed in Bouin's fluid and processed for histological examination.

The LD_{50} values with 95 per cent confidence limits and LD_{90} values were computed individually for both the samples, by probit analysis[5].

Results and Discussion

Morbidity and mortality: Both technical malathion and its formulation evoked similar symptoms. The intoxicated birds manifested typical signs of organophosphate poisoning. Symptoms appeared about 6 hr after intubation and no correlation could be observed between the dosage and the intensity of symptoms. The predominant symptom was lethargy, accompanied by foamy salivation and ataxia. Cyanosis of the combs were observed in dying birds. Few birds developed mild diarrhoea. Paralysis was invariably observed and convulsions preceded death. Symptoms in case of non-fatal intoxication were of short duration ranging for about 6-8 hr. In general, all deaths in both technical malathion and EC formulation occurred within a period of 6-20 hr. The computed LD_{50} and LD_{90} values are presented in Table 1.

Food intake and body weights: Slight anorexia was observed among the survivors in both technical malathion and EC formulation and feed consumption was

TABLE 1. THE ACUTE ORAL TOXICITY OF MALATHION TO POULTRY

Pesticide	Regression equation	LD_{50} (mg/kg b.w.)	Confidence limits 95% level		LD_{90} (mg/kg b.w.)
			Lower	Upper	
Malathion technical	Y = 13.0125X - 33.7360	948.1	842.0	1060	1189.0
Malathion 50% E.C.	Y = 7.5156X - 18.1277	1195.0 (597.5)*	930.9	1687	1769.0 (884.5)*

*mg of active ingredient (Malathion)

reduced only during the first 24 hr. The treated birds in general lost weight at the end of first week, but gained weight *on par* with controls during the subsequent weeks.

Autopsy results: At autospy, no gross pathology of any of the vital organs could be observed. Both technical malathion and EC formulation induced a dose-dependent increase in the fresh relative weights of liver and kidney. This significant increase calculated as per cent hypertrophy is presented for technical malathion treatment (Fig. 1). Fresh relative weights of other vital organs were comparable to those of controls.

Microscopic examination of liver sections of birds treated with technical malathion and EC formulation showed mild to moderate hypertrophy, focal necrosis (Fig. 2A) and cellular infiltration in the centrilobular areas (Fig. 2B). Kidney showed marked cellular infiltration and disruption of renal tubules in some areas. No noticeable histological changes were observed in other organs.

Chickens administered malathion exhibited typical symptoms of organosphosphate poisoning such as lethargy, salivation, ataxia, paralysis and convulsions. Similar observations were reported by earlier workers[3,6]. Malathion has been demonstrated by labelled studies to be absorbed within 30 min and distributed to various

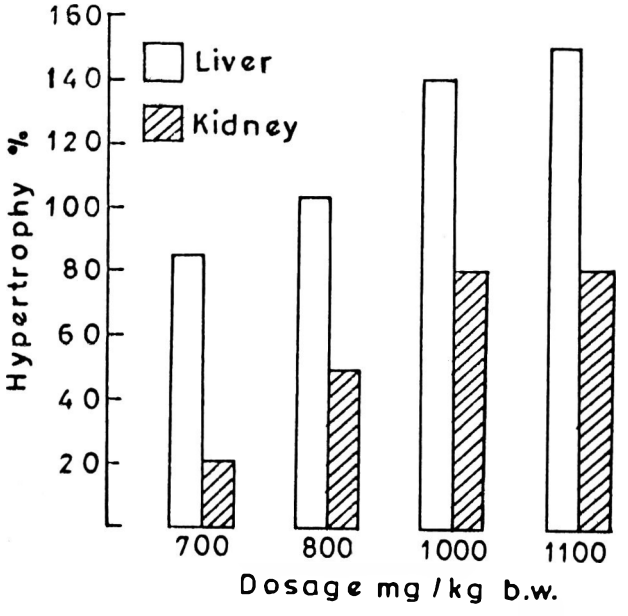

Fig. 1. Hypertrophy of liver and kidney in male birds induced by malathion (technical).

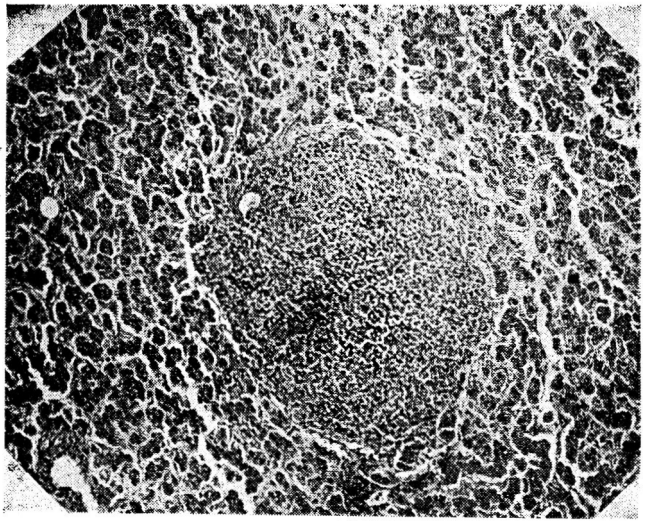

Fig. 2A.: Liver section of technical malathion treated bird (1000 mg/kg.b.w.) Note focal necrosis. H & E; × 80.

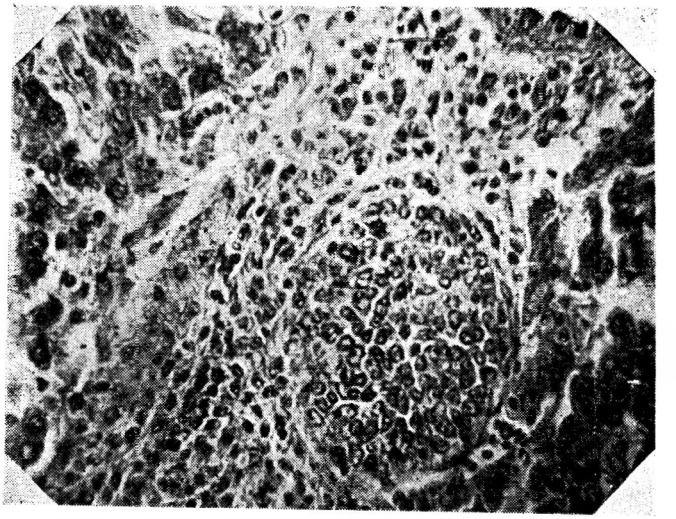

Fig. 2B.: Liver section of technical malathion treated bird (1000 mg/kg.b.w.). Note cellular infiltration. H & E; × 320.

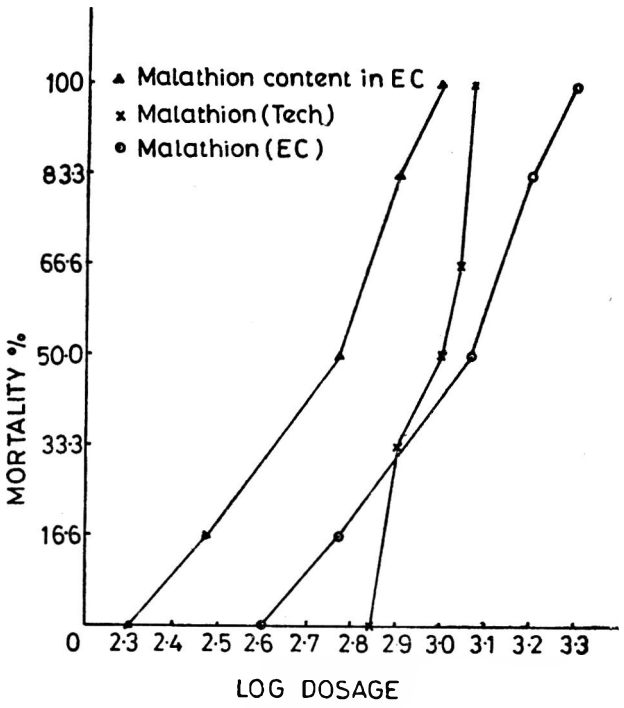

Fig. 3. Mortality responses of poultry to malathion (Technical & E. C. formulation).

vital organs within 6 hr after administration[4]. The probable reason for mortality to begin after 6 hr in the present study is that the malathion was present in deleterious concentrations in various vital organs within 6 hr so as to cause derangement of various metabolic activities resulting in deaths. Further, malathion administered intraperitoneally is shown to be eliminated within 24 hr[3]. This quicker elimination could be the reason for short duration of symptoms in non-fatal intoxication.

In toxicity studies, the fresh relative organ weights of treated animals often serve as a useful index of intoxication[7,8]. Liver hypertrophy is common among animals treated with toxic compounds, as a result of increased physiological demand[9]. The marked dose-dependent increase in relative weights of liver and kidney and moderate histopathological lesions (Fig. 2A & 2B) observed in these organs of the treated birds in the present study, could be attributed to acute malathion poisioning.

In the present study, the oral LD_{50} value of 948.1 mg/kg body weight computed for technical malathion is lower compared to that of the EC formulation (1195 mg/kg body weight). However, the LD_{50} value in terms of active ingredient present in the formulation is lower, revealing that the EC formulation is more toxic than the technical grade malathion (Fig. 3). Similar results have been reported in acute oral toxicity study of malathion with rats[10]. The oral toxicity of carriers such as Aromex and xylene conventionally employed in the emulsifiable concentrate formulations has been well demonstrated in rats[11]. Our results with chicken also confirm that the carriers significantly contribute to the toxicity of a pesticide in such formulations.

From these studies, it is evident that chickens are susceptible to malathion poisoning and hence warrants careful application of the pesticide for food protection and ectoparasite control.

Acknowledgement

The authors thank Sri B.S. Ramesh for statistical assistance, Sri H. P. Ramesh for processing tissues and Sri K. Nanjundiah for photomicrographs. They also thank Sri S. K. Majumder and Sri C. P. Natarajan for their keen interest in this study.

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Intake of Lead Through Food in India

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Manuscript received 5 *September* 1977; *revised* 10 *June* 1981

Lead content of commonly used foods in Indian diet has been analysed by using Atomic Absorption Spectroscopy. Daily intake of lead was estimated by collecting hospital diet (24 hr collection) of uniform caloriffic value. Cereals and pulses were found to contain higher amounts of lead than other food groups. Average daily intake of lead was 550 μg by actual food analysis and per capita daily intake was 478 μg based on lead content of foods. The lower intake of calcium and proteins in Indian population may result in an increased lead absorption. This was reflected in high content of lead in rib bone samples of Indians as compared to those of Western countries. The residence time of lead was found to be 3765 days.

The element lead is being introduced continually to the bio-sphere and enters the body through various chains. The intake through food is a major factor for determining chronic exposure to stable lead. The study of Lehnert *et al*[1] on German foods showed that practically no lead free food exists. Harley[2] reported that in American population, the diet contributed the major portion of lead intake. Not much data are available on the lead content of various foods consumed in India.

The present study is intended to estimate the lead content of various components of an average Indian diet. The total intake of lead has been estimated from these data and compared with the values obtained using 'actual food collection' procedure[3]. The information is useful in arriving at an estimate of the residence time of stable lead using a single exponential model of ICRP[4].

Materials and Methods

Sample collection: The collection of different food materials was carried out at random in triplicate from different sources. These included cereals, pulses, vegetables, fruits, meat products, milk and spices for estimating the total average intake. A set of total diet was obtained from the foods served in various local hospitals. The contents of the menu were pooled for 24 hr and assumed to represent a fairly well balanced normal diet of approximately 1800 calories.

Estimation of lead: Five grams of homogeneous, raw vegetarian and non-vegetarians food samples were weighed and dehydrated at 110°C for 48 hr and were ashed in a muffle furnace at 400°C. The uniform mixture of 24 hr collected diet was dehydrated and from this, five grams were ashed. The ash was dissolved in 2 ml of double distilled nitric acid and made upto 10 ml with deionised distilled water. Atomic absorption spectrophotometry was used for lead estimation. A Perkin-Elmer 303 atomic absorption instrument was employed at the following operating conditions:

Flame: air acetylene; wave length: 217 nm; lamp current: 20 mA; slit width: 4.

The solutions were aspirated directly into the flame since no inter-element effect was observed on the estimation of this element in an air-acetylene flame[5-7]. The accuracy and precision of the results were determined by carrying out recovery experiments on a set of samples.

Results and Discussion

The percentage recovery of lead added to the sample solutions was ± 5 per cent. Triplicate analysis of various samples showed that the reproducibility of the results was within ± 5 per cent.

Data on the contents of lead in different food materials are given in Table 1. The mean consumption of various food items has been estimated earlier[8]. The mean lead

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TABLE 1. LEAD CONTENT OF VARIOUS FOOD MATERIALS

	Food material	Lead content (mg/kg)		Fooc Material	Lead content (mg/kg)
1.	**Vegetables**[a]	0.36		Turmeric (*Curcuma longa*)	0.20
	Spinach (*Spinacea oleracae*)	0.23		Nutmeg (*Myristica fragrans*)	0.78
	Amaranthus (*Amaranthus spinosus*)	0.28		Black pepper (*Piper nigrum*)	0.50
	Coriander leaves (*Coriandrum sativum*)	0.42			
	Radish leaves (*Raphanus sativus*)	0.58	4.	**Cereals**[b]	0.73
	Kadhineem (*Murrya Koenigi*)	1.82		Pearl millet (bajra) (*Pennisetum typhoideum*)	0.30
	Cowpea leaves (*Vigna catjang Walp*)	0.42		Wheat (*Triticum aestivum*)	0.50
	Potatoes (*Solanum tuberosum*)	0.58		Rice (*Oryza sativa*)	1.00
	Tomatoes (*Lycopersicum esculentum*)	0.09		Jowar (*Sorghum vulgare*)	1.10
	Cabbage (*Brassica oleracea* var. *capitata*)	0.09			
	Cauliflower (*Brassica oleracea* var. *tortrytis*)	0.14	5.	**Pulses**[b]	0.92
	Brinjal (*Solanum melongana*)	0.11		Green gram (*Phaseolus aureus*)	1.10
	Bitter gourd (*Momordica charantia*)	0.31		Pigeon pea (*Cajanus cajan*)	0.90
	Lady's finger (*Hibiscus esculentus*)	0.16		Black gram (*Phaseolus radiatus*)	1.35
	Radish (*Raphanus sativus*)	0.08		Gram (*Cicer arietinum*)	0.14
	Onion (*Allium cepa*)	0.15		Cowpea seeds (*Vigna sinensis*)	0.90
	Tamarind (*Tamarindus indica*)	2.65*		Dried peas (*Pisum sativum*)	1.10
	Green chilly (*Capsicum annum*)	0.36			
	Garlic (*Allium sativum*)	0.39	6.	**Fruits**[a]	0.40
				Orange (*Citrus reticulata*)	0.53
2.	**Meat and meat products**[a]	0.55		Mango (*Mangifera indica*)	0.43
	Shark muscle	0.75		Mosambi (*Citrus sinensis*)	0.24
	Chicken muscle	0.53			
	Fish	0.83	7.	**Milk**	0.15
	Egg	0.44		Aaray milk[a]	0.15
	Sausage	0.41		Dairy milk[a]	0.16
	Ham	0.36		Baby food[b]	0.50*
	Pork	0.24		Tinned milk-I[b]	1.00*
	Bacon	0.50		Tinned milk II[b]	1.40*
	Mutton	0.90			
			8.	**Drinking water**	
3.	**Spices**[b]	0.66		Tap water (Bombay)	0.005
	Cumin (*Cuminum cyminum*)	1.00		Well (Dombivali)	0.005
	Coriander seeds (*Coriandrum sativum*)	0.80		River (Ganges)	0.005
				Reservoir (Mahabaleshwar)	0.005

a - wet basis; *b* - dry wt. basis; *Not used for estimating mean

value obtained for each food item and their mean consumption by average Indian population[8] has been used to obtain the daily mean lead intake.

Vegetables like kadhineem, potatoes and radish leaves showed high lead content. Green and leafy vegetables generally contained high amounts of lead. The high lead content of potatoes has been noted by Los and Pyatnitskays[9]. The lead content of tamarind was very high.

Harley has stated that approximately 30 per cent of average lead intake of Americans is provided through the meat part of the diet. In India, cereals and pulses are consumed more by majority of population and the average lead content of these is high compared to other food groups. Therefore, the total intake of lead by Indians has been found to be high as compared to European and American countries[2].

The calculated per capita daily intake of 478 μg lead agreed fairly well with the mean amount of 550 μg of lead estimated by actual food analysis as shown in Table 2. The present mean value compares well with the daily intake of 518 μg obtained by Lehnert *et al*[1]. in Germany, but is higher than that obtained by Kehoe *et al*[10]. They stated that an intake of 550 μg of lead,

TABLE 2. TOTAL LEAD CONTENT IN HOSPITAL FOOD SERVED IN A DAY (24 HR)

Type of food	Sample No.	Water content (%)	Total wt. (g)	Total lead (μg)
Non-vegetarian	1	80.6	1750.5	420
	2	68.4	1275.5	680
	3	74.2	1530.5	670
Mean		—	—	590
Vegetarian	1	68.8	1318.7	360
	2	68.2	1490.0	400
	3	69.0	1470.0	780
Mean		—	—	510

Mean lead content for all samples: 550 μg

would not cause a potentially degenerative condition, however, Indian population may be susceptible to lead induced metabolic abnormalities since increased lead absorption may occur because of the lower intake of calcium and proteins[3,8].

The lead content of a few human bone rib samples collected from accidental death cases in Bombay was found to be 8.32 μg/g which is higher than the average values of 6.90, 2.32. 6.20 and 6.70 μg/g reported from U.S.A., Poland, Japan and Germany respectively[11]. However, the present value agrees with that reported by Bagchi *et al*[12]. as 8.40 μg/g. Similar conclusions were reached by Khandekar and Anand[13] who observed higher contents of lead-210 in bone samples of Indians. The biological half life of lead calculated according to Holtzmann's equation[14] is estimated to be 3765 days which is slightly high as compared to 3650 days calculated by ICRP[4].

In conclusion, it is time for consideration to control lead pollution in India to keep away the population from induced metabolic abnormalities due to lead.

Acknowledgement

The authors are grateful to Shri S. D. Soman, of Health Physics Division, Bhabha Atomic Research Centre, for his constant encouragement.

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Relative Evaluation of Yield and Quality Attributes of Nigerian and Exotic Strains of Chicken

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Manuscript received 2 *March* 1981; *revised* 9 *July* 1981

Extensive consumer prejudice against the consumption of broiler meat in Nigeria arose because of its poor palatability and extreme tenderness when compared with meat from spent hens. In the present study, an attempt has been made to evaluate the light indigenous strain of chicken in relation to two dual purpose imported strains that have been raised under the same feeding and management regimes. Parameters studied included carcass grade, a composite of conformation, fleshing and finish, liver colour score, water pick-up, per cent cut-out values as well as objectives and subjective scores for various traits. Data on these parameters suggest that intensive feeding and management tend to reduce the toughness and variable organoleptic attributes generally associated with rearing the indigenous chicken under free range.

The present high cost of broilers and their comparatively poor cooking characteristics have greatly limited the use of this class of poultry in the Nigerian diet. Most of the poultry requirement has therefore, been met by using spent exotic layers and the indigenous strain of chicken. The meat from these sources are said to be tough, desirably chewy and palatable.

About 92 per cent of the 14 million chicken population of Nigeria are of indigenous strain[1]. Despite this, no systematic investigation has been carried out into many of its characteristic attributes vis-a-vis the exotic strains. The present investigation attempts to obtain comparative data on carcass traits and organoleptic attributes of the indigenous and two exotic strains of spent chicken.

Materials and Methods

Fifty four spent hens were randomly selected in three equal numbers from three larger flocks of the indigenous, 'Amocathman' and 'Hyline' strains of birds. These birds had been raised at the University of Ibadan Teaching and Research Farm under the same feeding and management regimes and were 18 months old at the time of slaughter.

The birds were handpicked after scalding at 60°C, dressed and eviscerated. Each liver was visually scored on the basis of its colour. Livers with yellow, light mahogany or mahogany colours were graded 1, 2 or 3 respectively indicating the order of lipid accumulation from high to low. The weighed warm carcasses were chilled in a 1:1 cold water-ice slush mixture for 20 hr with a change of the chilling medium every four hours in a 2°C cold room. They were drained for 20 min prior to determining the chilled weight and amount of water pickup.

The carcasses were graded following the provisions of the Canada Agricultural Products Act[2] into A, B, C or D grades. The graded carcasses were disjointed and the weights of the breast, foreback, hindback, wings, thighs, drumsticks and neck were determined. Sarcomere length and fibre diameters were determined on small muscle strips from the belly of the pectoral muscle[3].

The breasts were individually wrapped in aluminium foil and broiled to an internal temperature of 70°C. The measured heating time averaged 35 min for the indigenous strain and 40 min for the two exotic strains. Weights were taken prior to and after cooking to determine cooking losses.

Shear force values were determined on two strips from the left pectoral muscle. Each strip has a cross section of 0.7×1.65 cm across the fibre direction and was sheared in three places perpendicular to the fibre. Hardness and adhesion values of meat from the right pectoral muscle were determined on an Instron Universal Testing Machine[4] except that the muscle strips for hardness determination were 1 cm in depth with the fibre running along the long axis of the strips. A plunger with a diameter of 0.5 cm was also used. The fibres in the strips for adhesion measurements were 1 cm long and run across the short axis of the strips perpendicular to the line of strain. The remaining meat from the left and right pectoral muscles were cut into pieces of $1.5 \times 1 \times 1$ cm and presented to a six member taste panel for evalu-

TABLE 1. RELATIVE WEIGHTS AND PROPORTION OF CUT-UP PARTS OF THE CARCASSES OF THE THREE STRAINS OF CHICKEN

Cut-up parts	Indigenous (kg)	Indigenous (%)	Amocathman (kg)	Amocathman (%)	Hyline (kg)	Hyline (%)
Breast	0.174[a]	23.88	0.292[b]	23.01	0.308[b]	23.42
Wings	0.076[a]	10.66	0.131[b]	10.35	0.141[b]	10.75
Thighs	0.100[a]	13.74	0.145[b]	11.70	0.150[b]	12.59
Drumsticks	0.084[a]	11.60	0.133[b]	10.50	0.161[b]	12.20
Hindback	0.110[a]	14.91	0.211[b]	16.59	0.187[b]	14.22
Foreback	0.083[a]	11.50	0.141[b]	10.93	0.130[b]	9.88
Neck	0.049[a]	6.70	0.103[b]	8.26	0.091[b]	6.95

Means in each row followed by the same superscripts are not significantly ($P<0.05$) different.

ation of tenderness and overall acceptability rating on a nine-point hedonic scale.

The data obtained were subjected to analysis of variance[5] while Duncan's multiple range test[6] was used to determine significant differences among means.

Results and Discussion

The 'Amocathman' and the 'Hyline' strains were not different in the absolute weights of the various cut-up parts (Table 1), but were all significantly heavier, ($P<0.05$) than those of the indigenous strain. On percentage basis however, no significant differences were observed for the various cuts in all strains. Oluyemi *et al*[7]. similarly observed that the indigenous fowl did not differ significantly from the exotic breeds in plucked and carcass weight when expressed as percentages of the live weight. The breast, thigh and drumstick account for 49.22 per cent of the whole carcass in the indigenous strain compared to 45.21 and 48.21 per cent in the 'Amocathman' and 'Hyline' strains respectively. These values were generally lower than those reported for broilers.[8,9]

Mean values for moisture pick-up, liver colour score, carcass grade, sacrcomere length and fibre diameter are presented in Table 2. No significant differences were obtained in the amount of water pick-up although the 'Hyline' strain had the least. Previous values reported for broilers varied from 4.6 per cent[10] to 6 per cent[11,12] thus indicating that spent hens exhibit higher water uptake than those of broilers probably due to their larger surface area.

Distinct differences in conformation fleshing and finish were observed in the three strains. The 'Amocathman' had the best carcass traits while the indigenous strain showed the poorest conformation and fleshing and hardly any finish. The indigenous and 'Amocathman' strains showing distinctly different grading scores had similar water pick-up values. The indigenous strain with its small size had a larger internal surface and external skin area per unit weight of carcass while the 'Amocathman' with its superior finish had a greater amount of loose connective tissue and better development of ovaries. These traits contribute for good moisture absorption.

Significant ($P<0.05$) differences were observed in the liver colour score for the three strains. The livers of the indigenous strain tended to be mahogany coloured while those of the 'Amocathman' were yellow with the 'Hyline falling in between. As observed by Wolford and Polin[13] visually scoring the liver for lipid content was positively and significantly ($P<0.01$) correlated with chemically extracted lipid content. Cunningham and Morrison[14] also noted that dietary energy levels had no influence on liver colour score suggesting that such differences in colour between the strains may in fact be of genetic origin.

The sarcomere length and fibre diameter did not differ among the three strains. The sarcomere length of 1.70μm in the indigenous strain falls within the type II fibres as definied by Locker[15]. The fibre diameter varied from 57.68 μm in the 'Amocathman' strain to 63.29 μm in the 'Hyline' strain. These values agree with a previous

TABLE 2. COMPARISON OF SOME PHYSICAL PARAMETERS IN THE THREE STRAINS OF CHICKENS

Strains	Moisture pickup (%) Mean±S.D.	Liver colour score Mean±S.D.	Carcass grade	Sarcomere length (μm) Mean±S.D.	Fibre diam (μm) Mean±S.D.
Indigenous	8.04±1.32[a]	2.50 ±0.55[a]	D	1.70±0.31[a]	58.90±6.86[a]
Amocathman	8.01±3.66[a]	1.50 ±0.84[c]	B	1.96±0.31[a]	57.68±2.13[a]
Hyline	6.82±2.10[a]	2.00[b]±0.89[b]	C	1.86±0.07[a]	63.29±3.77[a]

Figures are from the mean of 18 birds

Means in columns followed by the same superscripts are not significantly ($P<0.05$) different.

TABLE 3. OBJECTIVE AND SUBJECTIVE DATA ON BREAST MUSCLE FROM THE THREE STRAINS OF CHICKEN

Strains	Breast cooking loss (%) Mean±S.D.	Shear force value (kg) Mean±S.D.	Instron hardness (kg) Mean±S.D.	Instron adhesion (kg/cm²) Mean±S.D.	Tenderness score Mean±S.D.	Overall acceptability Mean±S.D.
Indigenous	26.82 ±6.96^{a}	4.02±1.12^{a}	3.20±1.26^{a}	2.27±1.26^{a}	6.31±1.02^{a}	6.40±1.09^{a}
Amocathman	33.24 ±2.64^{b}	4.44±1.19^{a}	3.52±0.53^{a}	2.24±0.53^{a}	6.14±0.53^{a}	6.78±0.44^{a}
Hyline	28.30^{ab}±3.83^{ab}	4.95±2.13^{a}	3.61±0.80^{a}	2.34±0.41^{a}	6.11±0.84^{a}	6.95±0.65^{a}

Means in columns followed by the same superscripts are not signifantly ($P<0.05$) different.

report[16] of 64 μm for fowl pectoralis muscle. Aberle *et al*[17]. however, reported extremely low values of 14.0 to 14.2 μm and 16.8 to 17.2 μm for layers and broilers respectively. The non significant difference in the sacromere length and fibre diameter of the three strains vis-a-vis the relative sizes of their breast muscle suggests that the number of fibres and perhaps their relative lengths in the breast muscle of the exotic strains greatly exceed those in the indigenous strain in line with similar observation by Smith[18].

Results of objective and subjective estimation of meat from the breast meat are shown in Table 3. The 'Amocathman' strain, having the best finish, had significantly ($P<0.05$) higher cooking loss than the indigenous strain while the 'Hyline' strain did not differ from the other two strains. However, the indigenous strain with the least mean percentage cooking loss had the highest variation of 6.96.

There was no significant difference in the shear force values, taste panel tenderness and overall acceptability scores for breast meat from the three strains. Peterson and Lilyblade[19] however, reported shear force values which indicated that breast muscle from atrophic (light-weight) mutant strain of chicken were significantly more tender while those from hypertrophic (heavy weight) mutant strain were significantly tougher than normal muscles. Data for instron hardness and instron adhesion indicated no significant difference among the three strains although values for instron hardness followed the same trend as the shear force values. Subjective scores for overall acceptability confirm the previous findings of Oluyemi *et al.*[7] that the indigenous strain was less palatable than the two exotic strains.

The lack of significance in differences among the three strains for most of the parameters measured indicated that proper management and nutritional regime may to a reasonable extent upgrade the quality of meat from the indigenous strain.

Acknowledgement

The fund for this project was provided through the Senate Research, Grant (6:302:26) University of Ibadan. The author gratefully acknowledges the technical assistance of J. Kolade, O. Bolarinwa and J. Famoriyo.

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Protease Inhibitors and *In vitro* Protein Digestibility of Pigeonpea (*Cajanus cajan* (L.) Millsp.) and Its Wild Relatives

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Manuscript received 30 *September* 1980

Seven wild relatives and cultivated species of *Cajanus* were studied for the trypsin and chymotrypsin inhibitor activities and for the *in vitro* digestibility of seed proteins. The trypsin inhibitor activity was generally higher in the wild species. A clear difference in the chymotrypsin inhibitor activity was observed between the wild and the cultivated species. The highest trypsin and chymotrypsin inhibitor activities were observed in *Rhynchosia rothi;* only exception was *A. cajanifolia* in which the inhibitor activities were similar to those in the cultivated species. *Rhynchosia rothi* showed the lowest value for *in vitro* protein digestibility. Otherwise no large differences were observed in protein digestibility for the wild and cultivated species.

The nutritive value and protein digestibility of legumes are improved by processing or cooking leading to the destruction of heat labile antinutritional factors[1]. Among these factors, trypsin and chymotrypsin inhibitors have been studied in detail[2].

The variability for protein content within the pigeonpea cultivars is small. At our Institute, some of the species of *Atylosia*, a related genus of *Cajanus* were found to have higher protein content and have been used in intergeneric crosses for selecting high protein derivatives having desirable agronomical characters[3]. However, the use of wild relatives as high protein source in a breeding programme will be limited, if they are known to contain considerable amount of certain antinutritional factors. Therefore, a study of other quality attributes such as the digestibility, and antinutritional factors of wild species of pigeonpea was carried out. This report, presents the results of such an investigation.

Materials and Methods

Seed samples of three pigeonpea cultivars—'Baigani' 'Pant A-2' and 'UPAS-120' and seven wild relatives as listed in Table 1 were obtained from the Genetic Resources Unit of our Institute. All analyses were carried out on decorticated split seed (dhal) samples. For decortication, seeds were soaked in water and stored at 5°C overnight. Excess water was decanted and the seed coats were removed with forceps. The decorticated material was dried and ground in a Udy cyclone mill to pass through a 60 mesh sieve and defatted in a Soxhlet apparatus using hexane.

Total nitrogen was determined by the micro Kjeldahl procedure[4] and crude protein was calculated using the factor 6.25. The trypsin inhibitor activity (TIA) and the chymotrypsin inhibitor activity (CIA) were assayed according to Kakade *et al*[5,6]. Trypsin and chymotrypsin inhibitors in samples (200 mg) were extracted with 10 ml of 0.1 M phosphate buffer (pH 7.6) and 0.1 M borate buffer (pH 7.6) respectively at room temperature for 1 hr. After diluting the extracts fourfold, aliquots of 0.2, 0.4, 0.6, and 0.8 ml were assayed for trypsin and chymotrypsin inhibitor activities. Protein content in the extracts was determined according to the method of Lowry *et al*[7].

Results and Discussion

Table 1 gives the protein content, trypsin and chymotrypsin inhibitor activities and the values for *in vitro* protein digestibility of pigeonpea cultivars and the wild relatives. Protein percent ranged from 23.1 to 26.2 for pigeonpea cultivars whereas it was 27.1 to 29.3 for wild relatives. The mean protein content of the wild relatives was about 15 per cent higher than that of the pigeonpea cultivars. The variation in the trypsin and chymotrypsin inhibitor activities within the pigeonpea cultivars was smaller as compared with that of wild relatives. The trypsin inhibitor activity (units inhibited/ mg meal) ranged from 13.3 to 25.8 for the *Atylosia* species and from 12.5 to 15.1 for *Cajanus*. The trypsin units inhibited were the highest (82.4 units/ mg meal) for *Rhynchosia rothi*. The mean chymotrypsin inhibitor activity in the wild species was more than three fold than

Submitted as J.A. No. 157 by the International Crops Research Institute for the Semi-Arid Tropics.

TABLE 1. PROTEIN CONTENTS, TRYPSIN AND CHYMOTRYPSIN INHIBITORS AND PROTEIN DIGESTIBILITIES IN CULTIVARS OF PIGEONPEA AND THE WILD RELATIVES

Cultivars/species	Protein N×6.25 (%)	Trypsin inhibition (Units/mg meal)	Trypsin inhibition (Units/mg protein)	Chymotrypsin inhibition (Units/mg meal)	Chymotrypsin inhibition (Units/mg protein)	*In vitro* protein digestibility (%)
Cajanus cajan cultivars						
Pant A-2	24.4	12.5	69.7	5.0	27.8	57.9
UPAS-120	23.1	12.9	71.3	4.2	23.1	59.5
Baigani	26.2	15.1	67.1	3.5	15.3	64.1
Mean	24.6	13.5	69.4	4.2	22.1	60.5
Wild species						
Atylosia scarabaeoides (*L.*) Benth.	27.8	14.2	60.4	14.2	60.9	67.8
A. sericea Benth Ex. Bak.	28.4	17.9	76.4	20.1	85.3	68.1
A. albicans W. & A.	28.5	19.4	81.9	22.0	92.4	62.6
A. volubilis (Blanco) Gamb.	27.1	25.8	121.4	11.5	60.9	52.6
A. platycarpa Benth	29.3	13.3	54.5	11.5	47.1	59.3
A. cajanifolia Haines	29.1	14.9	61.3	5.9	24.2	56.0
Rhynchosia rothii Benth. Ex. Aitch	27.6	82.4	445.7	20.9	113.2	40.9
Mean	28.3	26.6	127.6	15.2	69.1	58.2
SE[a]	±0.3	±0.5	±2.0	±0.2	±1.3	±1.6

[a]Standard error of estimation

the mean of the cultivated species. However, in the case of *A. cajanifolia* the level was similar to that of pigeonpea. CIA was the highest (20.9 units inhibited/mg meal) for *Rhynchosia rothi* and thus was similar to TIA.

A similar variation was observed between the cultivated and wild species when the values of TIA and CIA were expressed as units inhibited per mg of extracted protein. TIA was several times higher in *Rhynchosia rothi* while *A. volubilis* exhibited the highest level among the *Atylosia* species. TIA of *Rhynchosia rothi* was comparable with the reported values for soybeans[8]. Large differences were observed between the cultivated and wild species, in the CIA except in *Atylosia cajanifolia* which had values similar to those of the pigeonpea cultivars.

There were only small differences in the *in vitro* digestibilities except for *Rhynchosia rothi* which had a substantially lower value (40.9 per cent). The low protein digestibility of this species might be due to the presence of high levels of protease inhibitors.

The protein quality of pigeonpea is affected by the presence of protease inhibitors asin other grain legumes[2]. The high levels of protein inhibitors in some of the wild species are evident from the present investigation. Therefore, it is suggested that intergeneric lines obtained from crosses of *Cajanus* with wild species should be tested for the levels of protease inhibitors. However, the antimetabolic nature of such compounds could provide chemical resistance against some insect pests. Elevated levels of TIA in cowpea (*Vigna unguiculata*) have been reported to confer resistance against the attack of the bruchid beetle, *Callosobruchus maculatus* (F.)[9]. Clear differences in the levels of CIA between the wild species and pigenopea have been observed in the present investigation. It would be worthwhile to find out if these compounds are associated with insect resistance mechanisms in pigeonpea.

Acknowledgement

Authors thank Dr. A. N. Murthi for the supply of seed material and Mr. N. Subrahmanyam for technical assistance.

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Large Scale Production of Pectolytic Enzyme by Solid State Fermentation

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Manuscript received 24 *May* 1980; *revised* 12 *November* 1980

The studies on large scale production of pectolytic enzyme by solid state fermentation revealed that the production of enzyme by *Aspergillus carbanerius* CFTRI 1048 on wheat bran medium was highest at 21 hr of fermentation. Control of temperature of the solid state fermentor at 30°C is necessary. Steaming of wheat bran medium at 15 Pa for 45 min is needed to get good results. The extraction of the enzyme from moldy bran by water at ambient temperature (25-28°C) as well as the use of plate and frame filter press for clarification of the enzyme extract gives satisfactory results.

Pectoytic enzymes find extensive application in industry. The main uses are in fruit processing industries for the clarification of fruit juices and wines, in the expression of fruit juices from fruits like banana, mango, guava, papaya and apple, in the manufacture of hydrolysed products of pectin, in the retting of textile fibres, in the manufacture of pectin-free starch, in the refinement of vegetable fibres, in the curing of coffee, cocoa and tobacco and finally as an analytical tool for the estimation of plant products[1-3]. Though pectolytic enzymes are used in smaller quantities as compared to other hydrolytic enzymes[4], the requirement of this enzyme was met by import till 1976. A process for the production of fungal pectinases has been developed[5,6]. This communication describes some of the scale up studies such as relation between enzyme activity and fermentation time, fermentation temperature, steaming of wheat bran, precipitation and keeping quality of the enzyme.

Materials and Methods

Fermentation time and enzyme activity: Wheat bran and acidic mineral salts solution were mixed[5,7] and spread in perforated aluminium trays (2 kg moist bran in each tray) of 16 in. × 33 in. × 1½ in. size to a depth of 1-1.25 in. and sterilized in autoclave at 121°C for 60 min. After cooling, the bran was inoculated with spores of *Aspergillus carbanerius*[5] CFTRI strain 1048, raised in flasks on bran medium, with inoculum size of 2.5 per cent based on moist bran. The trays were charged in the chamber of a surface fermentor of commercial size accommodating 96 trays (Fig. 1), equipped with humidity, temperature and air circulation controls. The organism was allowed to grow for 20-21 hr at 30-35°C under 90 per cent R.H. The air inlet and outlet ports were controlled to maintain heat in the cabinet. A composite sample from 10 trays (10 g from each tray) was collected at intervals for the determination of enzyme activity.

Fermentation temperature and enzyme activity: Wheat bran trays prepared and inoculated under similar conditions were divided into two sets; one set was charged in a fermentor cabin where optimum temperature of 30°C was maintained while another set was incubated in a chamber where the temperature was not controlled.

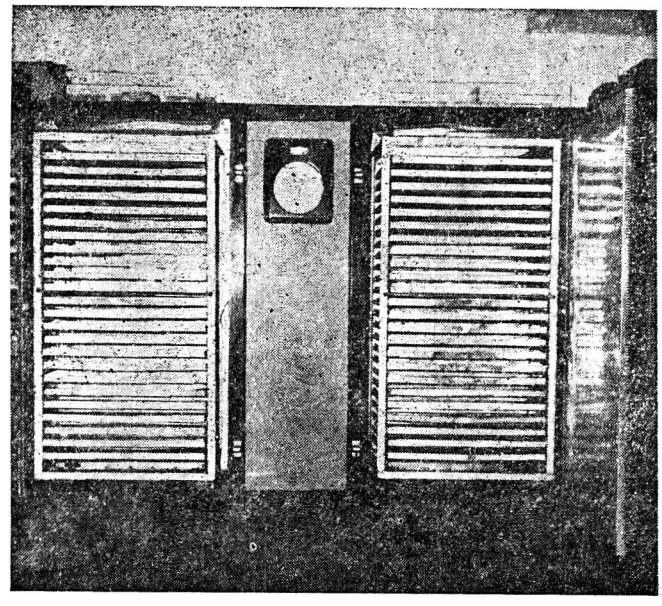

Fig. 1. Commercial size surface fermentor

Samples were removed at intervals and analysed for enzyme activity.

The temperature of the solid medium in the tray was measured by inserting a probe inside the bed and the temperature was noted from Aplab temperature indicator-cum-controller. The temperature and humidity in the chamber were recorded on Bestobel recorder.

Effect of steaming of bran on enzyme production: The trays containing wheat bran and mineral salts were steamed for varying periods before inoculation and were fermented under optimum conditions for growth. The enzyme was extracted from the moldy bran to study its characteristics and activity.

Extraction of enzyme: To a 10 g portion of composite sample, 100 ml water was added and allowed for 60 min with intermittant stirring. The contents were filtered through a filter paper and volume of the filtrate was measured. Fresh water equal in volume to that retained in the bran, was added to the bran and the process of mixing and filtration was repeated. The two aliquots after mixing (100 ml) was used in the estimation of enzyme activity.

Clarification of enzyme extract: Clarification by Sharples centrifugation, filtration using plate and frame filter press and decantation were employed to study their effect on the enzyme activity and to determine their relative efficiency. Sodium benzoate at 0.1 per cent level was added to the clarified extract and was stored at 6-10°C.

Precipitation of the enzyme: This was carried out to study the effect of the contact time of 66 per cent ethanol with enzyme extract on the recovery of the precipitated enzyme. The contact time studied ranged from 5 to 360 min. Ethanol concentration required for optimum precipitation of the enzyme was also determined with a contact time of 5 min.

Enzyme assay: Reduction in viscosity of one per cent pectin solution has been used as the basis for the assay of enzyme activity[5]. Two 50 ml Erlenmeyer flasks containing 24 ml of one per cent pectin (supplied by M/s Manackchand & Co., Uttran) dissolved in citrate buffer (pH 4.0) were equilibrated at 40°C for 20 min. To one flask, 1 ml distilled water was added while to the other, 1 ml of diluted enzyme (1 ml made to 10 ml) was added and the reaction was allowed to proceed at 40°C for 30 min. The viscosity was measured immediately (within 60 sec) by rotational viscometer. Fifty per cent reduction in viscosity under the above conditions is defined as one unit of enzyme. The total protein in the filtrate was estimated by the Biuret method[8].

Results and Discussion

Enzyme activity at different stages of fermentation: It is observed (Table 1) that the activity of the enzyme increased upto 21 hr and remained constant upto 25 hr of fermentation at which the experiment was terminated due to sporulation.

Effect of fermentation temperature on the activity of enzyme: Data given in Table 2 indicate that the bed temperature increased from 25 to 45.2°C due to heat generated during fermentation. The heat generation was 1.6 Btu/hr per tray resulting in about 12 per cent decrease in the enzyme yield. This clearly shows the need for temperature control in large fermentors.

TABLE 1. ENZYME ACTIVITY AT DIFFERENT STAGES OF GROWTH

Time (hr)	% reduction in viscosity at 1/10 dil.	Enzyme units/ ml	Enzyme units/ g of moldy bran
15	18.1	3.62	36.20
17	36.3	7.26	72.60
19	50.0	10.00	100.00
21	54.5	10.90	109.00
23	54.5	10.90	109.00
25	54.5	10.90	109.00

TABLE 2. EFFECT OF FERMENTATION TEMPERATURE ON THE ENZYME ACTIVITY

Growth period (hr)	Temp. controlled fermentation				Temp. uncontrolled fermentation			
	Chamber temp. (°C)	Tray temp. (°C)	Enzyme activity (Unit/ml)	Enzyme activity (Unit/g of moldy bran)	Chamber temp. (°C)	Tray temp. (°C)	Enzyme activity (Unit/ml)	Enzyme activity (Unit/g of moldy bran)
16	26.0	30.0	4.26	42.6	36.2	39.6	3.48	34.8
18	26.5	30.0	7.42	74.2	37.5	41.9	6.54	65.4
22	26.5	30.0	11.36	113.6	39.0	45.2	10.12	101.2
24	26.5	30.0	11.43	114.3	38.3	44.0	10.22	102.2

TABLE 3. EFFECT OF STEAMING AND STERILIZATION OF BRAN MEDIUM ON THE ACTIVITY AND QUALITY OF THE ENZYME

Fermentation time (hr)	Control* (units/g moldy bran)	Enzyme (unit/g of moldy bran) at indicated periods of steaming		
		1 hr	1.5 hr	2 hr
20	100.0	36.2	61.0	68.2
25	118.2	45.0	68.4	92.6
30	118.2	59.0	79.2	102.4
36	—	71.2	97.0	100.8

*Sterilized at 15 Pa for 45 min.

Effect of steaming of wheat bran before inoculation on the activity and the quality of the enzyme: The wheat bran moistened with mineral media was steamed (at slightly above atmospheric pressure) for 1, 1.5 and 2 hr. A control sample was also sterilized at 15 Pa for 45 min. All the trays were inoculated and incubated in the chamber where humidity and temperature were maintained at 95 per cent and 30°C respectively. The data on enzyme activity at various periods of fermentation are given in Table 3. In all the steamed samples the enzyme activity was low as compared to the control, probably due to low degree of hydrolysis of wheat bran constituents on steaming. It was difficult to extract moldy bran from those steamed for 1 and 1.5 hr due to the leaching of the gummy material in the extractant. The extracts from the steamed samples gave grainy odour.

Effect of extraction temperature and different extractants: The moldy bran, dried at 28-30°C, was extracted with water, distilled water and 0.1 M phosphate buffer at 25-28°C and at 4°C. The results are shown in Table 4. The enzyme activity when extracted in water, distilled water or phosphate buffer at ambient temperature (25-28°C) was almost the same. Extraction in distilled water or in phosphate buffer at 4°C showed higher recovery of the enzyme however the difference is not significant, considering the additional costs involved.

TABLE 4. EFFECT OF TEMPERATURE OF EXTRACTANTS ON THE RECOVERY OF ENZYME FROM MOLDY BRAN

Extractant	Extraction temp. (°C)	Enzyme activity (units/ml of extract)	Protein (mg/ml of extract)	Specific activity of enzyme/mg of protein
Water	25–28	10.00	0.187	53.48
Distilled water	25–28	9.52	0.175	54.40
Distilled water	4	10.96	0.137	80.00
Phosphate buffer (0.1 M) (pH. 7.1)	25–28	9.20	0.175	52.57
Phosphate buffer (0.1 M) (pH. 7.1)	4	11.20	0.175	64.00

TABLE 5. EFFECT OF CONTACT TIME OF ETHANOL WITH ENZYME SOLUTION ON THE ACTIVITY OF ENZYME IN THE PRECIPITATE

Ethanol contact time (min)	Viscosity reduction at 1/10 dil (%)	Enzyme activity (units/ml extract)
5	58.1	11.62
30	43.5	8.70
60	26.0	5.20
90	23.0	4.60
360	13.0	2.60

The total precipitate was dissolved in and made to the original volume of enzyme extract. The activity in the enzyme extract taken for precipitation was 11.64 units/ml.

Clarification of the extract: The enzyme extract was clarified by centrifugation in Sharples centrifuge, filtration in plate and frame filter press and by settling and decantation at 6-10°C for 24 hr. Not much difference was observed in the enzyme activity of the extract before and after clarification. However, there was 8 and 10 per cent loss in activity of the enzyme during centrifugation and filtration respectively, probably due to foaming of the extract. Difficulties were observed in the separation of the extract from the residue in decantation as about only 79 per cent extract

TABLE 6. EFFECT OF ETHANOL CONCENTRATION ON THE ENZYME ACTIVITY IN THE PRECIPITATE

Ethanol in enzyme mix. (%)	Enzyme activity (units/ml extract)*	% enzyme pptd.
30	7.54	68.55
35	9.51	86.45
40	9.52	86.55
50	10.82	98.36
60	9.84	89.45
70	8.20	74.55

*The total precipitate was dissolved in a volume of water equivalent to the original volume of enzyme extract.

The initial activity of enzyme taken for precipitation was 11.00 units/ml

TABLE 7. KEEPING QUALITY OF ENZYME EXTRACT, ENZYME POWDER AND ENZYME PRECIPITATE SUSPENDED IN BUFFER SOLUTION

Storage period (days)	Enzyme extract at 25–28°C (units/ml extract)	Enzyme powder at 25–28°C units/ml extract)	Ppt suspended in buffer (pH 7) at temp. of	
			25–28°C (units/ml extract)	4°C (units/ml extract)
0	13.08	12.04	9.64	9.64
6	12.86	—	8.10	9.64
13	12.60	12.08	5.78	8.82
20	11.98	—	4.68	—
27	11.80	—	4.46	8.76
34	12.02	11.74	4.02	7.92
37	12.06	11.26	*	7.06

*Contaminated.

could be recovered. The clarified extracts obtained by centrifugation and filtration remained clear even after storage for 30 days in cold room while heavy fungal growth was observed in decanted extract.

Precipitation: The clarified enzyme extract was precipitated using various concentrations of ethanol for different time intervals for getting maximum recovery of the enzyme. As the time of contact increased from 5 min to 6 hr, the enzyme activity decreased sharply (Table 5). It is therefore, necessary to complete the entire precipitation process as quickly as possible. Data in Table 6 indicate that the optimum concentration of alcohol in enzyme-alcohol mixture needed for maximum recovery is 50 per cent. When precipitation was carried out at 4°C instead of ambient temperature (25-28°C), about 25 per cent more recovery of the enzyme was observed.

Keeping quality of enzyme: The keeping quality of the enzyme extract, precipitate and buffer suspended precipitate was studied and the results are given in Table 7. It is observed that the enzyme precipitate, when suspended in buffer of pH 7, lost its activity rapidly both at 25-28°C and at 4°C. The dried powder lost about 6.5 per cent activity in 37 days whereas the original extract lost about 8 per cent activity at ambient temperature during this period.

Acknowledgement

The authors are grateful to Sri C. P. Natarajan, Director of the Institute for encouragement given in the course of this work.

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RESEARCH NOTES

IMPROVEMENT IN THE CONVENTIONAL PARBOILING OF RICE THROUGH PREVENTION OF HUSK OPENING

Husk opening and exposure of kernel during steam treatment in parboiling was prevented by application of 0.5 to 1.0% of common salt. This ensured husk sealed grains in parboiling which facilitated quick drying besides improving the appearance of milled rice. Closed grains remained free from fungal growth when drying is slow. The prevention of husk opening was common to 'IR 20', 'ADT 8', 'ADT 31', 'Co 25' and 'Co 33' paddy varieties.

In parboiling, during steam treatment the husks open (lemma and palea separate or split) exposing the kernel to the external mileu. This opening leads to a higher moisture absorption by the kernel which prolongs the drying. In open yard drying, the exposed kernels are liable for mechanical damage while turning and spreading for uniform drying. Splitting of rice kernel also leaves an unsealed fissure in milled rice. The exposure of kernels and the leachate therein impregnate the paddy with nutrients which harbour fungal growth during cloudy and humid weather and in monsoon rains. The mechanical damage and mould attack leave a scar on the milled product. Hence, husk opening was considered as a disadvantage in parboiling and the results of studies undertaken to prevent husk opening are reported here.

Paddy was soaked for 3 days in cold water and parboiled in a vessel with water at the bottom. Dipping of soaked paddy for 2 min in 15-16 per cent solution of common salt (commercial sample with 85 per cent NaCl content) before steaming was used as the standard for determining prevention of husk opening while untreated paddy served as control Coarsely powdered salt was mixed with 5 kg of 'IR 20' soaked paddy at 0.5, 1.0, 2.0 and 5.0 per cent levels on the weight of paddy. Another set of 5 kg-lot of paddy was treated with 16 per cent salt solution @ 20 ml/kg (v/w) of paddy which gave 0.32 per cent (w/w) salt concentration on paddy and @ 125 ml/kg of paddy which gave 2 per cent (w/w) salt concentration on paddy. The prevention of husk opening by application of 0.5, 0.8 and 1.0 per cent w/w) of salt was determined in 'ADT 8', 'ADT 31' and 'Co 33' paddy varieties each with 1 kg lot.

The moisture drop in sun-drying of treated and untreated 'IR 20' paddy was determined in 2 t lot paddy parboiled in a conventional mill adopting double steaming. The moisture content was determined by drying a known quantity (5g) of whole grains in an oven at 105°C to constant weight.

Extent of fungal infection in salt treated parboiled paddy was studied in 10 kg lots of 'IR 20' paddy by treating separately with 0.5 and 1.0 per cent salt, followed by parboiling and heaping to pick up natural infection. After 6 days of such inadequate drying the grains in each lot were thoroughly mixed and dried separately under fan. The chalkiness of the brown rice was assessed to determine the degree of fungal infection. The milling yield and breakage were determined in the treated and untreated lots by milling in a McGill Miller No. 1 and 3. The salt content in paddy was estimated by titrating against silver nitrate and expressed as sodium chloride equivalents.

Mixing of coarsely powdered salt at 0.5, 1.0, 2.0 and 5.0 per cent level with soaked paddy prior to steaming prevented husk opening (Table 1). The prevention of husk opening at these salt concentrations were equivalent to that achieved in 16 per cent brine dip for 2 min. Sprinkling 16 per cent salt solution at the rate of 2 per cent (v/w) over paddy or mixing with salt at 2 per cent level (w/w) also prevented husk opening. The exposure of kernel by "husk opening" (separation of lemma and palea) and by "husk splitting" (splitting of lemma and palea themselves) were prevented by salt application. Prevention of husk opening in parboiling by dipping in brine for 2 min was reported earlier[1]. A concentration of 0.5 to 1.0 per cent was found to be adequate to prevent the husk opening. However, when large quantities of paddy parboiled after treating with 0.5 per cent salt were heaped in hot condition in the yard prior to spreading, a few grains opened. This was not observed in concentrations nearing 1.0 per cent salt. Hence, for commercial scale operation, application of 500 g of

TABLE 1. EFFECT OF APPLICATION OF SALT AS SOLUTION AND AS DRY MIX ON PREVENTION OF HUSK OPENING IN IR 20 PADDY

Brine/salt	Mode of application	Husk opened grains (%)
16% brine	20 ml/kg paddy	0.6
16% ,,	125 ml/kg paddy	0.9
16% ,,	Paddy dipped 2 min	1.9
0.5% salt	Dry salt mix	1.5
1.0% ,,	,,	0.5
2.0% ,,	,,	0.1
5.0% ,,	,,	—
Control		26.6

TABLE 2. EFFECT OF SALT APPLICATION IN PER CENT HUSK OPENED GRAINS IN DIFFERENT VARIETIES

Varieties	Control	0.5% salt	0.8% salt	1.0% salt
Co 33	47.9	2.9	1.4	1.1
Co 25	89.3	18.7	6.7	5.2
ADT 8	52.5	6.1	2.3	1.0
ADT 31	90.7	22.1	4.2	2.8
IR 20	44.3	3.5	1.9	0.5

commercial common salt per bag (57 kg) of raw paddy offered a level of around 0.8 per cent which was found to be optimum. The prevention of husk opening achieved by this level of salt was consistent with 'ADT 8', 'ADT 31', 'Co 33' and 'Co 25' paddy varieties (Table 2).

The moisture absorption by the closed grains, during steaming was limited. In a conventionally parboiled sample with 8 per cent husk opening, the opened grains had 36.8 per cent moisture as against 28.0 per cent in closed grains; the moisture of the bulk paddy was 30.5 per cent. The moisture content of paddy with 66 per cent husk opening was 32.7 per cent. The low initial moisture content of sparsely opened lot always facilitated quick drying. After 5 hr of drying in open yard the moisture content of the sparsely opened lot came down to 24.1 per cent from 32.7 per cent. The exosmotic action of salt also withdrew a portion of water absorbed by the grains. When 4 bags (57 kg) of 'IR 20' paddy was steamed in a kettle, 7.1. of water drained out in untreated paddy during steaming, while 21.1. of water drained out in 0.8 per cent salt treated lot. In the conventional parboiling, only the adhering water and the steam condensate water drained out, while in salt treated paddy the water withdrawn by the exosmotic action of salt increased the amount of water drained out. The salt treated paddy dried faster than conventionally parboiled grains in sun-drying (Table 3). The low initial moisture content and closed nature of the grains facilitated rapid drying of salt treated paddy.

TABLE 3. MOISTURE DROP IN OPEN YARD DRYING OF SALT TREATED AND CONVENTIONALLY PARBOILED 'IR 20' PADDY

Treatments	Moisture content after parboiling (%)	Moisture (%) in paddy* after drying for			
		0 hr	2 hr	4 hr	6 hr
Control	32.9	32.1	30.3	25.2	16.3
0.8% salt	30.1	29.8	26.4	19.5	13.5

*Wet basis

The checking of husk opening prevented inside kernel being protruded outside and secured compactness of the grain after drying while the conventionally parboiled grains were plumpy. The plumpy opened grains lead to uneven polishing during milling. Moreover, the burst opening and splitting of rice kernel, resulted in mechanical damage and fungal infection due to which a scar was left on the milled product thereby reducing its market appeal. On the other hand, the salt treated grains got evenly milled and presented a "pearly" appearance. The colour of the treated rice was relatively whiter than untreated rice as determined by an Elico Reflectance meter model Cl 28.

An increase of 0.1 to 0.4 per cent out turn in the milled rice was observed due to prevention of grain opening at 0.8 per cent salt treatment (Table 4). This increase might be due to the lesser degree of brokens besides a slight entry of salt. The addition of salt at this concentration increased the head rice recovery. However, the head rice recovery in commercial milling is largely influenced by the evenness of drying. The salt content of treated paddy was 0.5 per cent and this might not pose any problem of corrosion to the milling machinery, as the paddy is milled dry and the raw paddy itself contained about 0.1 per cent sodium chloride.

During slow drying of parboiled paddy in rainy humid weather the moulds initiate infection on the expos-

TABLE 4. EFFECT OF SALT TREATMENT TO IR 20 PADDY ON THE POTENTIAL YIELD OF MILLED RICE

Type of milling	Salt concn (%)	Paddy taken (g)	Milled rice* (g)	Brokens (g)	Out turn (%)
Milling in McGill	0.0	750	514.0	12.5	70.2
Miller No. 3 (as paddy)	0.8	750	517.0	10.5	70.3
Milling in McGill	0.0	130	87.1	4.5	70.4
Miller No. 1 (as paddy)	0.8	130	88.0	4.1	70.8

*Only head rice

TABLE 5. INFLUENCE OF SALT TREATMENT ON MOULD INFECTION AND MILLING YIELD IN INADEQUATELY DRIED IR 20 PARBOILED PADDY

Treatments	Brown rice infection (%)	Milled rice (g)	Huller bran (g)	Brokens (g)	Head rice (g)	Out turn (%)
Control	58.7	81.7	60.8	6.5	65.0	54.4
0.5% salt	21.1	94.7	50.1	3.9	78.0	63.1
1.0% salt	11.6	99.5	44.5	4.5	87.0	66.3
Normal drying	—	106.4	41.8	1.3	95.0	70.8

ed kernel surface and through the micropylar opening. The husk opening exposed a larger area for fungal invasion from which they grew and ramified the whole lot. *Aspergilli* incite localised infection on the the exposed kernel. These organisms can gain entry only through micropylar opening provided the grains remain closed after parboiling. The fungi like *Mucor* and *Rhizopus* ramify the grains only superficially and do not penetrate deep into the kernel. The salt treatment prevented husk opening and thus prevented fungal infection resulting in higher out turn. Inadequate or slow drying for 6 days resulted in more fungal growth and lowering the milling yield of rice. In the control, the infection was severe as they gained deep entry into the kernel turning a number of grains chalky which broke on milling (Table 5). The salt treatment aided in quick drying rendering the grains to escape from mould contamination, even if the showers interfere with drying after a few hours of parboiling. If unexpected showers come just after parboiling of paddy and interfere with drying, then the paddy could be preserved for a week by the application of 5 per cent salt and 40 per cent husk powder[2] or 0.5 per cent acetic acid[3].

Thanks are due to Mr. A. N. Dyaneswaran, Project Head, Paddy Processing Research Centre, Tiruvarur for encouragement.

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Received 19 *Sept.* 1980
Revised 11 *March* 1981
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QUANTITATIVE CHANGES IN THE AMINO ACIDS OF COPRA DUE TO FUNGAL INFECTION

The amino acid pattern of copra (dried coconut kernel) was found to be altered due to infection by fungi. Copra infected by *Botryodiptodia theobromae* did not show such variation compared to the control, while samples infected by *Mucor hiemalis* and *Curvularia senegalensis* indicated lower and *Rhizopus oryzae* infected samples showed higher amino acid contents.

The traditional method of processing coconut kernel into copra makes them susceptible to infection. The present paper aims at studying the changes in amino acid content and pattern as influenced by the infection of different fungi which have been involved in the deterioration and spoilage of copra (dried coconut kernel) under the conditions, existing in Kerala (India).

Samples of copra were prepared, sterilized and inoculated with *Aspergillus niger*, *A. flavus*, *Rhizopus oryzae*, *Mucor hiemalis*, *Penicillium citrinum*, *Botryodiplodia theobromae* and *Curvularia senegalensis* and incubated for one month under natural conditions. The fungal species used in the study had earlier been consistently isolated from naturally deteriorated copra collected from various centres and at different seasons in Kerala. Defatted samples of copra (100 mg) were hydrolyzed with 6 N HCl at 110°C in sealed evacuated tubes. After hydrolysis, it was evaporated in a rotary vacuum evaporator and taken in 0.05 N acetate buffer. The amino acids were determined in an automatic amino acid analyser (Perkin Elmer Model KLA 33).

Results are given in the Table 1. As the data are not amenable for statistical comparision, only variations of 20 per cent on either side of the control value, are calculated.

It was observed that *B. theobromae* infected sample did not show variation with control to the tune of 20 per cent in respect of any of the amino acids. Copra samples infected by *M. hiemalis* and *C. senegalensis* showed similar amino acid pattern and recorded lower amino acid content than control. Samples infected by

TABLE 1. EFFECT OF INFESTATION BY FUNGI ON THE AMINO ACID CONTENT OF COPRA*

Amino acids	*A. niger*	*A. flavus*	*R. oryzac*	*M. hiemalis*	*P. citrinum*	*B. theobromae*	*B. senegalensis*	Control
Aspartic acid	8.13	5.57	9.21	3.24		7.18	4.01	7.18
Glutamic acid	21.25	18.08	17.71	8.23	23.18	18.13	7.90	17.76
Hydroxy proline Threonine	3.57	2.38	3.47	1.12	3.25	2.53	1.53	2.15
Serine Proline	3.38	2.70	3.68	1.40	4.27	3.27	1.79	2.83
Alanine	4.19	3.54	5.20	1.76	4.46	3.64	2.27	3.95
Glycine	10.20	8.94	6.14	4.16	12.47	4.65	2.42	4.41
Valine			5.96			4.44	1.22	3.91
Isoleucine	3.44	2.09	4.88	1.72	4.16	3.23	1.70	2.71
Leucine	6.37	6.95	8.02	2.61	7.44	6.07	3.24	5.48
Tyrosine	2.27	1.76	2.89	1.52	1.85	2.34	1.73	2.84
Phenylalanine	5.04	3.89	5.67	2.29	4.95	4.80	2.31	5.88
Lysine	4.09	3.04	3.90	1.62	3.60	3.83	1.46	4.60
Histidine	—	0.82	1.07	—	0.44	1.66	0.39	1.91
Ammonia	9.12	5.32	8.89	8.42	6.90	5.59	3.91	6.75
Arginine	14.28	13.57	18.28	6.57	14.54	17.50	3.80	20.76

Ammonia is expressed as ammonium chloride. *g amino acid per 100 g protein

R. oryzae in general showed higher quantities while samples of *A. niger* infection was more or less comparable with control, but showed greater quantities of hydroxy proline, threonine, glycine, valine and isoleucine, but lower quantities of arginine. Arginine content was reduced in all cases.

Due to infection, the amino acid pattern of copra was found to be altered. Similar quantitative changes in the amino acid pattern have been observed[1-4]. In the copra material with a uniform pattern of distribution of amino acids, infection by different fungi thus causes variations in the content of certain amino acids. The decrease itself may cause an increase in the content of the unutilised amino acids, or it might have been due to the pathogen metabolism. Plant pathogens cause remarkable changes in the nitrogen metabolism of host plants. Compounds like free and bound amino acids have been reported to disturb the degree of virulence of pathogens. Consequently the disease inciting agents change the quantitative and qualitative content of amino acids and other compounds[5-7].

The senior author is thankful to the Kerala Agricultural University for deputing her to undergo the programme and to the ICAR for providing a senior fellowship. Thanks are also due to Dr. R. S. Iyer, Professor of Agricultural Chemistry and to the Biochemistry Department of the Tamilnadu Agricultural University for the amino acid analysis.

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Received 25 *July* 1980
Revised 1 *April* 1981

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YEASTS OF COCONUT AND PALMYRAH PALM WINES OF SRI LANKA

Twenty three strains of yeast, isolated from samples of fermented coconut and palmyrah palm wines (toddy) by direct plating, were characterized and identified by biochemical and morphological characteristics. The yeasts belonged to three genera: *Saccharomyces* (twenty one strains), *Schizosaccharomyces* (one strain) and *Candida* (one strain). The predominant species were *Saccharomyces marxianus* (eleven strains) and *Saccharomyces exiguus* (nine strains).

Coconut and palmyrah palm wines (*toddy*), the traditional beverages of Sri Lanka, are the saps collected from the young inflorescences of the coconut palm (*Cocos nucifera*) and the palmyrah palm (*Borassus flabellifer*) and fermented. The unfermented sap (sweet *toddy*) contains about 18-20 per cent (w/v) sugars (mainly sucrose) which are fermented to ethanol and minor components by a mixture of wild yeast and bacteria. Fermentation of the palm sap involves three main conversions viz. alcoholic fermentation by a variety of wild yeasts, formation of various flavour components by a mixture of wild yeast and bacteria and finally, spoilage due to the production of volatile acids and gases mainly due to bacterial activity. The fermented palm wines which contain about 7 per cent (by volume) alcohol are normally drunk fresh. However, coconut palm wines are also distilled to produce palm brandy (*arrack*) or bottled (pasteurized) or used to prepare vinegar by acetic acid fermentation.

In a previous study[1] 17 yeasts (8 species) isolated from coconut palm wine were characterised and identified.

In this study 23 yeasts isolated from coconut palm wines (twenty strains) and palmyrah palm wines (three strains) obtained from different parts of Sri Lanka were characterized and identified using both biochemical and morphological characteristics.

Representative samples of palm wines from different parts of Sri Lanka, were collected in sterile containers from the collection pots 24 hr after tapping. Yeasts were isolated by direct plating on a medium which had the following composition in g/l. of distilled water; glucose, 20.0; yeast extract, 3.0; peptone, 5.0; and agar agar, 20.0. Single colonies of different morphological types were isolated, purified and maintained on slopes of the same medium at 4°C.

Methods used to study the biochemical and morphological characteristics are those described by Jayatissa *et al*[1]. Identifications were made mainly on the basis of the identification codes assigned to the yeasts as described by Beech *et al*[2].

Table 1 shows the sugar fermentation, assimilation and morphological characters of the isolated yeasts.

TABLE 1. IDENTIFICATION OF YEASTS OF COCONUT AND PALMYRAH WINE

Fermentation[a]	Assimilation[a]				Morphology[a]						Completed yeast code	No. of strain in C/P
Code[b]	KNO_3	Ethanol	Code[b]	Sugars (Code[b])	Cell shape	Ascospore shape	Ps	My	Pe	C[b]		
MB	—	—	C	ISM	Elongated	Oval elipsoidal	+	+	+	FF	MB/01SM/FF	*Schizosaccharomyces versatilis* (C1)
GA	—	+	0	3	Subglobose or ellipsoidal	Crescentiform oblong	+	—	—	2B	GA/03/2B	[c]*Saccharomyces marxianus* (C9)+(P2)
GA	—	+	0	3	Ellipsoidal	Spherical	—	—	—	1	GA/03/1	*Saccharomyces exiguus* (C9)
GA	—	+	0	3	Ovoid	—	+	—	—	2B	GA/03/2B	[c]*Candida macedoniensis* (C1)
GMA	—	+	0	4	Spheroidal, subglobose	Spheroidal	—	—	—	1	GMA/04/1	*Saccharomyces cerevisiae* (P1)

[a]Criteria used —Ps, pseudomycelium; My, mycelium; Pe, pellicle; C, code; Gl, glucose; Ga, galactose; Su, sucrose; Ma, maltose; Me, mellibiose; Ra, raffinose; Eth, ethanol; KNO_3

[b]Code— based on method by Beech *et al*.[2]
MB—Gl, Su, Ma, Me, Ra; GA—Gl, Ga, Su; GMA—Gl, Ga, Su, Ma; ISM—Gl, Su, Ma, Ra
3—Gl, Ga, Su, Ra; 4—Gl, Ga, Su, Ma, Ra.

[c]*Saccharomyces marxianus* (perfect state)/*Candida macedoniensis* (imperfect state)

[d]'C'—Coconut palm wine, 'P'—Palmyrah palm wine.

In addition to criteria used by Beech *et al*[2]. the ascospore formation and the utilization of ethanol were also studied. The inclusion of melibiose in the fermentation experiments was merely to study the extent of raffinose fermentation.

Fermentation of palm wine is not done under controlled conditions and the yeast inoculum comes mainly from the unsterilized pots, previously used for collecting the sap. Therefore, the complexity of the yeast flora[1] is more or less in accordance with the expectation. It is seen from this and the previous study[1], that the yeast flora of coconut palm wine is more complex than that of palmyrah palm wine. This may be a result of the type of climate found in the two different areas in which these palms are cultivated and the composition of the saps. Coconut palms are cultivated in areas of high humidity and rainfall, and moderate temperature, whereas the areas in which palmyrah palms are cultivated are very dry and hot. A detailed study of the composition of the unfermented saps have not been carried out. However, a comparative study of the composition of the two palm saps may be able to throw some light on the nature of the yeast flora of the two palm wines.

An interesting feature of the present study was the isolation of a fission type yeast *Schizosaccharomyces versatilis* in coconut palm wine. No fission type yeasts were encountered in our previous studies. It is very significant that the bulk of the yeasts found in palm wines belong to genus *Saccharomyces*. This is mainly due to their numerical predominance and superior fermentative ability.

Natural fermentation of palm wines by wild yeasts has been found to produce ethanol contents much lower than the theoretical yield[3]. The main contributory factor is the low fermentation efficiencies of the fermentative yeasts present in the palm wines. It is also seen from this and a previous study, that majority of the yeasts isolated from palm wines are able to utilize ethanol when it is used as the only carbon source available. However, yeasts utilize sugar in preference to ethanol, which may be used only in the absence of sugars or when the concentration of the sugar is very low. Therefore, it may be possible, that part of the ethanol produced is utilized by these yeasts towards the end of the fermentation when the sugar concentration is low.

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Received 29 *January* 1981
Revised 14 *July* 1981

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FREE FATTY ACID CHANGES DURING STORAGE OF *BAJRA (PENNISETUM TYPHOIDEUM)* FLOUR

Storage of *bajra* flour for eight weeks resulted in progressive increase in the free fatty acids. The change was much less when the flour was heated to 100°C for 2 hr before storage.

During the storage of cereal grains and their flours, titratable acidity of their lipid extracts increases and these grains undergo a deterioration in quality. These changes have been studied extensively in wheat and rice by various investigators[1-5], but not much attention has been paid to these changes in milled millets. The present study was, therefore, undertaken to find out the changes taking place in milled *Bajra* during storage. This millet is a staple food in many parts of India especially among the poorer sections of the population.

About 2 kg of *Bajra* was ground into flour and divided into two equal parts. One part was stored at room temperature in the dark, while the other was heated in an oven at 100°C for 2 hr after spreading in a thin layer and then stored. About 50-g lots of both types of *Bajra* flour were slurried with 200 ml of petroleum ether (BDH, 40-60°C b.p.). The contents were filtered under suction through a sintered glass funnel. The residual flour was extracted two times with 100 ml of petroleum ether each time. The filtrates were mixed and evaporated to dryness under vacuum. The residue was dissolved in 50 ml of 2:1 by vol. chloroform-methanol mixture, extracts transferred to a separating funnel and freed of the non lipid contaminants as per the procedure described by Folch *et al*[6]. The extraction of the lipid from both types of flour was carried out at weekly intervals and the extracts stored in the refrigerator. Free fatty acids were determined by the procedure described in IS:3506-1966[7] and were expressed as percent oleic acid. Unheated *Bajra* flour had a moisture content of 12.0 per cent, whereas heated *Bajra* flour had 1.5 per cent moisture. Table 1 shows the free fatty acid contents in the heated and unheated *Bajra* flour at different periods of storage.

TABLE 1. CHANGES OF FREE FATTY ACIDS IN HEATED AND UNHEATED *BAJRA* FLOUR DURING STORAGE

Storage period (weeks)	% free fatty acids (as oleic acid) Heated	Unheated
1	6.8	13.7
2	7.6	22.8
3	8.2	33.4
4	9.7	41.8
5	14.0	47.4
6	18.5	53.1
7	23.1	57.2
8	25.2	59.2

It is evident from the table that development of free fatty acids is considerably slower in the heated *Bajra* flour which is 25.2 per cent as against 59.2 per cent in the unheated flour after eight weeks of storage. This increase in the free fatty acid contents on storage is in agreement with the findings of Carnovale and Quaglia[8], that hydrolytic and autoxidation changes in the lipid fractions become apparent after storage for one month and increase with storage. These findings suggest that *Bajra* contains an active lipase enzyme which is responsible for the breakdown of glycerides and consequent increase in the free fatty acids and thus can cause deterioration in the quality of the grain/flour during storage. Its action can be inhibited and the spoilage prevented by preheating the grain/flour before storage.

Thanks are due to Lt. Col. S. N. Datt and Lt. Col. J. L. Koul for their keen interest in the investigation.

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Received 28 *August* 1980
Revised 23 *March* 1981

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OCCURRENCE OF *CLOSTRIDIA* IN MILK AND MILK PRODUCTS

A total of 547 samples of milk and milk products were examined for *Clostridia* by standard procedures. Amongst 310 positive samples, the incidence of *Clostridia* was highest in processed cheese (66.4%), followed by infant foods (65.5%), pasteurised milk (59%), raw milk (49.5%) and dried milk (44.4%). The average *Clostridial* spore count in milk and milk products was as follows: raw milk 1500/100 ml; pasteurised milk, 300/100 ml; processed cheese (bulged) cans), 1000/g; processed cheese (normal cans), 500/g; dried milks, 100/g and infant foods, 150/g.

Contamination of milk by *Clostridial* spores is widely recognised by several workers,[1,2]. While the incidence of these organisms in milk has been reported from 16 to 80 per cent[2,3], their incidence in processed cheese varied from 50 to 70 per cent[4,5] and in dried milk from 15 to 48 per cent[6]. Reports of occurrence of *Clostridia* in infant foods, ice cream and yoghurt are also available[7,8]. Lack of sufficient information in India on the occurrence of *Clostridia* in milk and milk products prompted the present investigation.

Samples of milk and milk products examined were procured from various markets and from organised dairies in Karnal, Delhi, Bangalore, Madras, Bombay, Ludhiana and Chandigarh. The distribution of samples analysed was as follows: raw milk, 210; pasteurised milk, 132; processed cheese, 140; dried milk, 36 and infant foods, 29.

Standard methods and procedures[4,9,10] employed comprised of pour plate method with sulphite-polymyxin-sulphadiazine agar, sulphite-iron-agar and differential reinforced *Clostridial* medium (DRCM) with agar (1.5 per cent) and MPN technique using DRCM alone. The samples were serially diluted in 0.1 per cent peptone water and heat treated at 70°C for 20 min in water bath before inoculating into media. Incubation was carried out for 48 to 72 hr at 37°C under anaerobic conditions using nitrogen gas in McIntosh jar. All the black colonies were counted for enumeration.

Out of a total of 547 samples of milk and milk products analysed (Table 1), 310 contained *Clostridia*. The incidence of *Clostridia* was highest in processed cheese (66.4 per cent), followed by infant foods (65.5 per cent), pasteurised milk (59 per cent), raw milk (49.5 per cent) and dried milk (44.4 per cent).

Enumeration of *Clostridial* spores involving all samples of milk and milk products by MPN method (Table 2) revealed that the spore count in raw milk was more (1500/100 ml) than in pasteurised milk (300/100ml). The lower count encountered in pasterurised milk might be partially due to destruction of a few spores which were

TABLE 1. INCIDENCE OF *CLOSTRIDIA* IN MILK AND MILK PRODUCTS

Product name	Samples examined (no.)	Samples positive (no.)	Per cent
Raw milk	210	104	49.5
Pasteurised milk	132	78	59.0
Processed cheese	140	93	66.4
Dried milk	38	16	44.4
Infant foods	29	19	65.5

TABLE 2. ENUMERATION OF *CLOSTRIDIA* IN MILK AND MILK PRODUCTS

Source	Samples analysed (no.)	Anaerobic spore counts*	
		Range	Average
Raw milk	210	$20—3\times10^4$/100ml	1500/100ml
Pasteurised milk	132	$10—1\times10^4$/100ml	300/100ml
Processed cheese			
Normal cans	65	$10—2\times10^5$/g	500/g.
Bulged cans	75	$5\times10—5\times10^5$/g	1000/g.
Dried milk	36	$10—5\times10^3$/g.	100/g.
Infant food	29	$10—6\times10^3$/g.	150/g.

*by MPN Method.

in sporulation stages. The bulged cans of processed cheese had a *Clostridial* count of 1000 spores/g, while in normal cans the numbers were as low as 500/g, which indicates the proliferation of *Clostridia* in processed cheese cans utilising the available nutrition and favourable anaerobic atmosphere.

The number of *Clostridia* present in milk and milk products depends on such factors as seasonal variation, method of enumeration, hygienic conditions on the farm, type of milk, kind of feeds and fedders including silage and concentrates[4,11]. The growth and multiplication of *Clostridia* is also affected by such factors as redox potential, initial bacterial load, pH, salt concentration, moisture, type of heat treatment and presence of inhibitory substances[1,4,10].

The high incidence as well as recovery of large number of isolates of *Clostridia* from milk and milk products in the present study can be attributed to unhygienic management practices on the farm and in processing. Some *Clostridial* species are reported to be pathogenic[12], a few cause spoilage of food products and several types are both pathogenic and cause food spoilage. Hence, the occurrence of these organisms in large numbers in milk and milk products is of great public health significance.

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Received 7 *October* 1980
Revised 29 *June* 1981.

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UTILIZATION OF DRIED KHOA FOR *PEDA* MAKING

***Peda* (a khoa based sweet meat) was prepared from fresh as well as stored cow and buffalo khoa powder. *Peda* made from cow khoa powder was chalky and sticky. Addition of chocolate improved the taste. *Peda* made from buffalo khoa powder (BKP) had slight khoa like flavour and smooth body. Acceptable quality of *Peda* could be made using BKP stored upto 105 days at room temperature.**

Khoa, a heat coagulated and partially dehydrated whole milk product is used as a base for various types of sweet meat preparations such as *Peda* and *Burfi*. The keeping quality of khoa is low during summer. Therefore, any attempt to increase the keeping quality of khoa at room temperatures will obviate the shortage. Patel and De[1] reported the production of dried khoa having substantial shelf life. This study was carried out to compare the suitability of cow and buffalo milk khoa powder for the preparation of *Peda*.

The khoa powders were prepared, using milk from the Institute's Experimental Dairy after standardization to 4.0 and 5 per cent fat for cow and buffalo milk

TABLE 1. SENSORY CHARACTERISTICS OF KHOA POWDERS

Type	Colour	Body and texture	Flavour
Cow	Creamy yellow (8.0)	Fairly uniform (8.0)	Slight khoa like (8.0)
Buffalo	White with greenish tinge (8.0)	Fairly uniform (8.0)	Slightly cooked (7.8)

Figures in parenthesis are sensory scores

TABLE 2. SUITABILITY OF KHOA POWDERS FOR *PEDA*

Type	*Peda* Colour	Flavour	Body texture	Sensory score	Remarks
Cow	Creamish yellow	Chalky	Soft and sticky	6.5	Gummy
	*Brownish	Chocolate	Sticky	6.8	Gummy
Buffalo	White	Slightly khoa like	Smooth	8.0	Acceptable
Fresh khoa	White	Khoa like	Compact	9.0	Excellent

*Chocolate added.

TABLE 3. SENSORY SCORE OF *PEDA* MADE FROM STORED KHOA POWDER

Type	Storage temp (°C)	Storage period (days) 0	30	45	60	75	90	105	120
Cow	R.T.*	6.5	6.5	6.5	6.5	6.5	6.0	5.5	5.5
	37±1	6.5	6.0	6.0	6.0	5.4	5.0	4.0	—
Buffalo	R.T.	8.0	8.0	8.0	7.5	7.0	6.5	6.0	5.7
	37±1	8.0	7.0	6.0	5.5	4.0	3.0	—	—

R.T.*—Room temperature

respectively, by the method of Patel and De[1] with slight modification (Fig. 1). The control *Peda* was made by the conventional method as delineated by Gill and De[2]. The experimental *Peda* was prepared by mixing 100 g khoa powder in 50 g sugar (ground) dissolved in 70 to 80 ml of water and the contents concentrated in a Karahi over a low fire till the product attained desired consistency. It was then set in a plate and allowed to cool.

The khoa powders and the sweets were subjected to sensory evaluation by a selected panel of judges, using 9-point hedonic scale.

The khoa powders, prepared from both cow and buffalo milk were of acceptable quality (Table 1), and contained 3.0 and 2.9 per cent moisture; 31.8 and 33.9 per cent fat[3]; 24.1 and 27.0 per cent protein[4]; 31.0 and 30.9 per cent lactose and 4.8 and 45.3 per cent ash[5] respectively. Our results for buffalo khoa powder are similar to that obtained by Patel and De[1]. The *Peda*

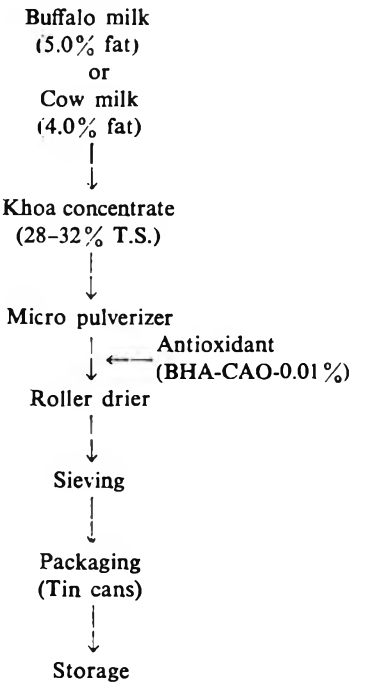

Fig. 1. Flow sheet for manufacture of khoa powder

made from buffalo khoa powder scored highest (8.0 liked very much) (Table 2). *Peda* made from cow khoa powder had chalky flavour and soft body. To improve the flavour of *Peda* made from cow khoa powder, 1 g of Cadboury's chocolate powder was added to the dry mix. Although addition of chocolate improves the flavour, the *Peda* had a tendency to stick to the palate. The sensory score of *Peda* as given in Table 3 indicated that fairly good quality of *Peda* could be prepared from buffalo khoa powder stored at room temperature for a period of upto 105 days. Samples stored at 37±1°C had shelf-life of 60 days and 45 days respectively for cow and buffalo khoa powders. The study showed that khoa can be stored in dried form for preparation of acceptable quality *Peda*.

Division of Dairy Technology
National Dairy Research Institute,
Karnal-132 001.

D. K. THOMPKINSON
SUKUMAR DE

Received 14 *December*, 1980
Revised 27 *January* 1981.

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1. Patel, A. A. and De, S , *Indian J. Dairy Sci.*, 1977, **30**, 325.
2. Gill L. K. and De, S., *Publication* 119, National Dairy Research Institute, Karnal, India 1974.
3. *Laboratory Manual*, 1959, Milk Industry Foundation,, Washington. D. C.
4. McKenzie, H. A., *Milk Protein*: *Chemistry and Molecular Biology*, Vol. 1, Academic Press, New York, 1970.
5. *Specification for milk powder* (*whole and skim*), 1967, IS: 1165, Indian Standards Institution, Manak Bhavan, New Delhi.

BOOK REVIEWS

Economic Aspects of Small Scale Fish Freezing: P. R. Street, I. J. Clucel, A. Jones and R. C. Cole, Tropical Products Institute, London, 1980, pp. 48, Price, £ 1.70

This report concerning with small (202 tonnes/year) to medium scale (3360 tonnes/year) fish freezing plants discusses physical and financial cost models. Quick freezing by plate contact and blast freezing methods is discussed; the design of the cost models pertains mainly to contact freezing. The end product price ranges for prawns and fish have been worked out.

Tnis report which provides details of capital, equipment and operational requirement would be useful in the identification, appraisal, financing and implementation of small scale fish freezing plants.

M. N. MOORJANI
C.F.T.R.I., MYSORE

A Stirrup Operated Coconut Grater: by J. F. Cecil and W. H. Timmins, Rural Technology Guide, Tropical Products Institute, 56/62, Gray's Inn Road, London WC 1 ×8LU, 1980; pp 28; Price: £ 1.70.

The booklet describes how to make a simple pedal actuated tool for grating coconut meat. All the tools and materials required are listed at one place in pages two and three. These tools and materials are generally available in a rural carpentry/smithy shop. The making of the frame, the blades and the shaft, the assembling of all the parts together and the final preparation of mounting the tool on a table are described in full detail with illustrations. The illustrations are so vivid as to enable even an uneducated artisan to fabricate the tool. At the end, instructions to use the tool are given.

From the illustration one may notice that the shaft of the grater is placed perpendicular to the operator so that one can use both his hands to press the halved coconut on to the greater blades without much strain which is a welcome feature. But in this arrangement of the shaft, when the stirrups are operated by the feet the loops of the stirrup on the shaft can over lap and block the movement. Tnis could be avoided by providing two 25 mm diameter holes both in the tool base and the table, instead of the big rectangular slot and passing the strirrup string through the holes to avoid the loops coming together.

This tool can be of much help to the cottage industry to improve productivity and yield in the field.

P. VEERRAJU
C.F.T.R.I., MYSORE

Innovation in Food Science and Technology to Meet the Nutritional Needs: Proceedings of the Workshop held at Bombay on November 10, 1979, under the auspices of Protein Foods and Nutrition Development Association of India, Bombay, India, pp: 94: Price: Rs. 40.

The first session deals with the need for innovation, covering topics such as nutrition of prematures and infants, importance of parenteral nutrition, lipid and amino acid formulations for parenteral alimentation, and special nutritional needs of diabetic and obese subjects. Relation between dietary factor and coronary heart diseases, and advantage of Indian diets for those with chronic renal failure have also been discussed by different experts in the field.

The main emphasis, in the second session is on the role of technology in meeting the challenge in the development of special food products. Topics covered in the session include development of special foods for infants and other age groups. Use of protein isolates in the development of special foods has been discussed. Application of food technology in developing therapeutic diets and problems of marketing special foods in India have also been discussed.

The two sessions were followed by a panel discussion on the topics covered in workshop.

The workshop showed the urgent need for special types of foods. It also brought out the fact that enough scientific know-how and technology to produce such products is availabe and a good market exists if such products are sold at a price which is not beyond the reach of the middle class.

KANTHA S. SHURPALEKAR
C.F.T.R.I., MYSORE

ASSOCIATION NEWS

Trivandrum Chapter

The Annual General Body Meeting of 1980–81 was held on 26 June 1981 at Regional Research Laboratory, Trivandrum. Shri H. Sreemula Nathan, Hony. President of the Chapter presided over the meeting.

Sri K. C. M. Raja, Jt. Secretary, presented the report of the Chapter for the year 1980, highlighting the major activities of the Chapter. These are: the EC met 8 times during the year, three technical seminars were held besides a symposium on *Adulteration of foods*, the proceedings of which has been already published. The Secretary pointed out that the present membership is 70. This was followed by the presentation of the statement of accounts for the year 1980. Both the secretary's report and the statement of accounts were accepted by the General Body.

Shri V. V. Nair, Secretary of the Chapter announced the names of the office bearers for the year 1981. They are:

Vice-President	—Smt. Satyavati Krishnankutty
Secretary	—Shri N Sreedhara
Jt. Secretary	—Dr. V. P. Potty
Treasurer	—Miss M. Omanakutty Amma

Since no nomination has been received for the post of President, Dr. N. Balakrishnan Nair, was nominated by the Executive Committee for the Presidentship which was approved by the General Body.

The following members were nominated by the CE for the coming year for EC membership.

1. Shri R. Sudhir
2. Shri P. Achutha Kurup
3. Dr. L. Prema Aiyer
4. Sri Mahadevan
5. Dr. G. Soman
6. Sri A. Govindan
7. Sri K. C. M. Raja
8. Shri H. Sreemula Nathan —Invitees
9. Shri V. V. Nair — -do-

Two guest lectures were also arranged. The first was by Sri Ashok Koshi, Managing Director, KL & MM Board, Kerala on 'Administering Milk'. The second was on 'Production perspectives of tubers' by Sri P. K. Thomas, Acting Director, Central Tuber Crops Research Institute, Trivandrum.

Ludhiana Chapter

The Annual General Body meeting of the Ludhiana Chapter was held on 20 June 1981. The following were elected as office bearers for the current year:

President	—Dr. K. Kirpal Singh
Vice-President	—Dr. M. S. Kalra
Hony. Secretary	—Dr. K. S. Sekhon
Hony.Jt.-Secretary	—Mr. B. S. Ahluwalia
Hony. Treasurer	—Dr. H. P. S. Nagi
Exec.-Councillor	—Mrs. B. K. Mann
	Dr. S. P. S. Saini

Delhi Chapter

The Annual General Body Meeting of the above Chapter was held on Sunday, May 3, 1981.

The outgoing President welcomed the members. The Secretary presented the report for the year and the Treasurer presented the statement of accounts. The following office bearers were elected unanimously.

President	—Sri P. N. Narang
Vice-President	—Dr. V. B. Mitbunder
Hony.-Secretary	—Dr. Susanta K. Roy
Hony.-Treasurer	—Dr. D. S. Khurdiya
Councillor	—Dr. N. K. Dadlani

The Seventh World Cereal and Bread Congress

"Cereals '82: Bread and Peace for All Nations" is the motto of the 7th World Cereal and Bread Congress which will be held in Prague, Czechoslovakia from June 28 to July 2, 1982.

The official languages of the Congress are English, Russian, French, German and Czech (Slovak). The technical programme will include two plenary sessions (at the opening and at the end of the Congress), technical sessions, poster program, symposia and round table discussions. Ten topics will cover all aspects of research, production, processing, utilization and consumption of cereals and cereal products.

Deadline for submission of abstracts of papers and poster program contributions is September 20, 1981 and for the payment of the registration fee is February 1, 1982.

For more information contact at: 7th World Cereal and Bread Congress Secretariat, Na Pankraci 30, 140 04 Prague, 4, Czechoslovakia.

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SRI B. N. GUPTA

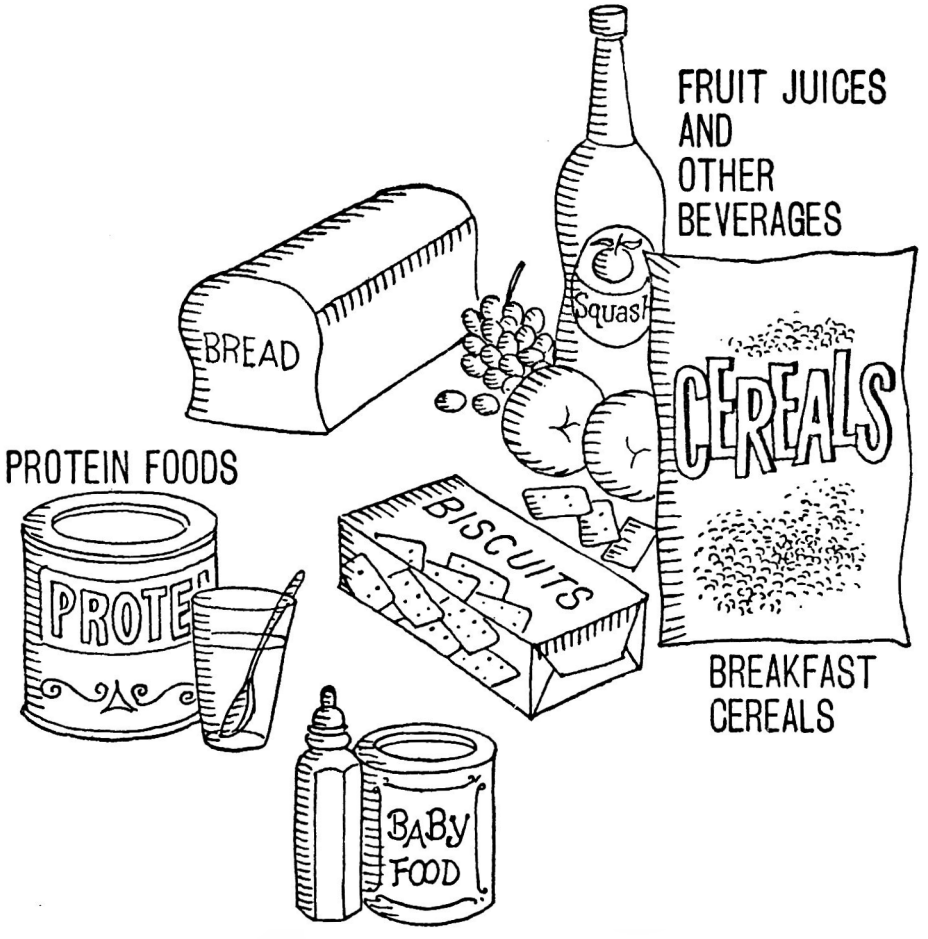
FOOD SCIENCE AND TECHNOLOGY UTILISATION AWARD

This award sponsored by the Bangalore Chapter of AFST(I) will be given on the basis of All India selection and the recepient would be required to base his operation in an appropriate village of his choice in Karnataka. The Awardee will have to stay in the village for 8-12 weeks and work on areas where he can meaningfully interact and contribute his experience to make a lasting impact on their living conditions. He should be a graduate, preferably in Food Science and Technology and allied subjects. A post-graduate qualification and working knowledge of Kannada are desirable. He should have about 5 years experience in teaching and research or extension work in food utilisation and preservation in a recognised institution or organisation. Industrial experience is desirable but not essential.

The Award will be for a period of 8-12 weeks and may be extended by a few weeks, if necessary. He will be paid an honorarium of Rs. 250/- per week and a sum of Rs. 5,000/- as contingency expenses. On completion he should submit a report of the work done for the Association.

Applications enclosing detailed project proposals and bio-data may be sent to Convener, Sri B. N. Gupta Awards Committee, 19, Platform Road, Bangalore-560 023 to reach on or before 31–1–1982. Any further information could also be obtained from the above address.

FORTIFY



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INSTRUCTIONS TO AUTHORS

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

REG. NO. 24918/64

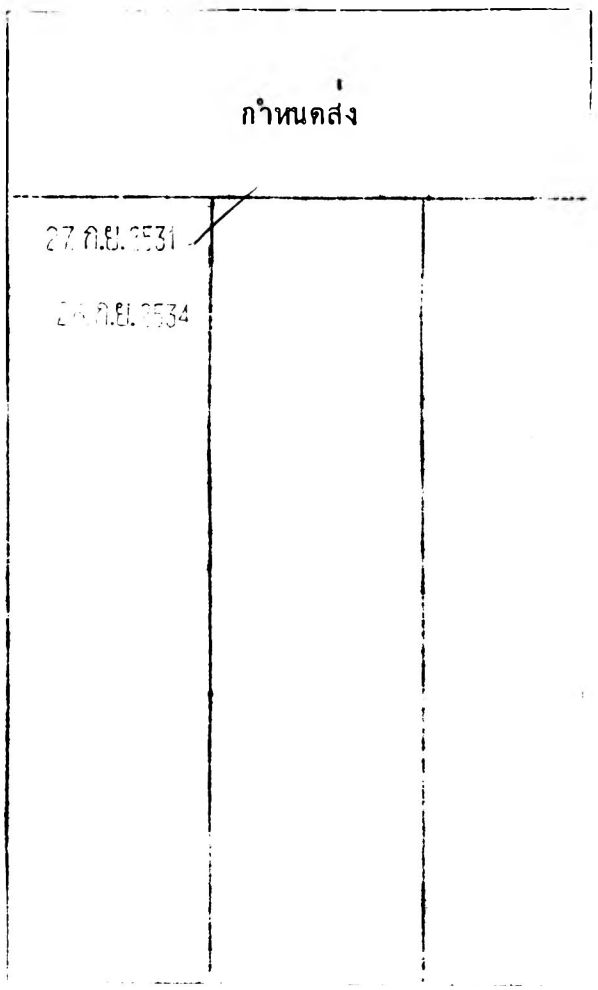
JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

Vol. 19 No. 1 **Contents of forthcoming issue** **Jan.-Feb. 1982**

Research Papers

Research Notes

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