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Research Notes

Chemical Composition and Protein Quality of Some High Yielding Varieties of Triticale

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Manuscript Received: 3 *May* 1976

Sixteen improved varieties of Triticale were evaluated for the chemical composition and protein quality from amino acid composition. The varieties differed appreciably in their content of protein but there was not much variation in the contents of calcium, iron and phosphorus. But the two varieties TL-20 and Arm PM-111, contained less moisture and ash as compared to the other varieties. The six varieties of Triticale analysed for amino acid content appeared to be slightly superior to wheat in respect of lysine. Triticales were found to be deficient in tryptophan.

Triticale is a man-made cereal that possesses potentialities that could be suitably exploited for the development of a new field crop, particularly in the rain-fed areas of our country. It is an artificial genus created in an attempt to produce a new cereal with a new combination of characters which will perform better than the present day cereals at least under certain ecological conditions. Triticale was actually produced by doubling the diploid chromosomal complement of the sterile hybrid in the progeny of a cross between *Triticum aestivum* L. or *Triticum turgidum* L. and *Secale cereals* L. and is thus an amphidiploid. Interest in Triticale has been promoted especially because of the possibilities of combining grain quality, productivity and disease resistance of wheat with vigour and hardiness of rye. Studies were, therefore, undertaken to study the chemical composition and protein quality of some of the varieties of Triticale.

Materials and Methods

Fourteen different varieties of Triticale viz. *TL*-101, *TL*-102, *TL*-103, *TL*-104, *TL*-105, *TL*-106, *TL*-107, *TL*-108, *TL*-109, *TL*-110, *TL*-19, *TL*-21, *TL*-22, *TL*-38 grown in Ludhiana were obtained from the Dept. of Plant Breeding, Punjab Agricultural University, Ludhiana and two varieties viz. *Arm PM*-111 and *TL*-20 grown at Hissar were obtained from the Dept. of Plant Breeding, Haryana Agricultural University, Hissar.

Crude protein, ash, moisture, calcium, iron, ether extract and crude fibre were determined, according to methods in AOAC[1] and phosphorus was determined by the method of Fiske and Subba Row[2]. Amino acids were determined by the method of Chatterjee and Abrol[3] with the help of Technicon Sequential Multisample Amino Acid Auto-Analyzer.

Results and Discussion

Moisture, ash, calcium, iron and phosphorous: The contents of different constituents of the various varieties are shown in Table 1. Moisture and ash ranged from 8.6 to 12.9, and 1.8 to 2.6 per cent respectively, while calcium, iron and phosphorus contents ranged from 43.7 to 49.9, 2.6 to 3.0 and 420.9 to 496.7 mg/100 g respectively. There were no marked differences in the contents of phosphorus, calcium and iron in the varieties. Moisture and ash content of the two varieties grown in Hissar were low as compared to those grown in Ludhiana suggestive of differences due to place.

Ether extract and crude fibre: There were no marked differences in the case of crude fibre content and ether extract which ranged from 1.3 to 1.5 and 1.3 to 2.0 per cent respectively. The results are presented in Table 1.

Crude protein: Marked differences were found in the case of crude protein in the different varieties which ranged from 13.2 to 18.5 per cent as shown in Table 1. The Triticales have higher protein content as compared to that of Triticum (9.80-15.80 per cent) as reported by Prasad.[4]

The observations on the chemical composition and nutrient contents indicate that Triticale varieties while in general are comparable to wheat in their chemical composition, are slightly superior to this grain in respect of the protein content.

Amino acid: The amino acid contents of the Triticale varieties viz. *TL*-20, *TL*-21, *TL*-22, *TL*-106, *TL*-110 and *Arm PM*-111 are shown in Table 2. However, the data show that the lysine content of Triticale varieties is higher than that of Triticum varieties. The Triticum varieties viz. *Kalyan Sona* and *Sonalika* contain 2.65 and 2.70 g lysine per 100 g protein respectively. Triticale

TABLE 1. CHEMICAL COMPOSITION OF TRITICALE ON MOISTURE FREE BASIS
(Average of duplicates)

Variety	Moisture %	Ash %	Calcium mg/100 g	Iron mg/100 g	Phosphorus mg/100 g	Crude fibre %	Ether extract %	Crude protein %
TL-101	12.7	2.2	48.4	2.8	459.1	1.4	1.6	18.5
TL-102	12.7	2.1	49.3	3.0	420.9	1.4	1.5	16.4
TL-103	12.9	2.2	48.9	2.9	441.4	1.3	1.7	16 1
TL-104	12.9	2.1	48.5	3.0	496.7	1.3	1.9	17.2
TL-105	12.5	2.1	47.4	3.0	492.6	1.4	1.9	15.6
TL-106	12.3	2.2	46.3	2.6	482.1	1.4	2.0	14.1
TL-107	12.3	2.4	44.6	3.0	496.5	1.4	1.8	15.2
TL-108	12.1	2.4	45.5	2.8	486.9	1.3	2.0	15.6
TL-109	12.9	2.6	48.7	2.6	496.7	1.4	1.3	16 7
TL-110	12.8	2.3	49.1	2.9	494.7	1.4	1.5	16.0
TL-19	12.3	2.3	46.8	3.0	467.8	1.4	1.5	15.7
TL-20	9.0	1.9	49.9	2.7	485.7	1.5	1.6	13.2
TL-21	12.5	2.2	43.7	2.6	496.7	1.5	1.5	16.1
TL-22	12.4	2.2	49.3	2.8	491.7	1.4	1.6	15.1
TL-28	12.2	2.1	48.4	2 9	482.1	1.4	1.7	17.2
Arm PM-111	8.6	1.8	48.6	2.6	487.6	1.5	1.6	13.5
Average	12.0	2.2	47.7	2.8	479.9	1.4	1.7	15.8

TABLE 2. AMINO ACID CONTENT OF SOME VARIETIES OF TRITICALES

Amino acid	TL 20	TL 21	TL 22	TL 106	TL 110	Arm PM-111
	g/100 g protein					
Lysine	3.6	2.8	2.7	3.5	3.1	3.2
Histidine	2.4	2.4	2.1	2.9	2.9	2.5
Arginine	5.8	5.4	4.0	5.3	3.8	6.5
Aspartic acid	6.3	4.8	4.3	6.1	5.0	6.5
Threonine	2.7	2.1	2.5	3.2	2.0	2.9
Serine	4.5	3.5	3.6	4.3	3.5	4.4
Glutamic acid	27.9	19.4	18.0	26.6	24.6	24.0
Proline	8.5	6.8	6.0	10.4	8.0	10.4
Glycine	4.4	3.8	3.2	4.8	4.2	4.5
Alanine	4.1	3.5	3.7	4.5	4.2	4.9
Valine	5.1	4.8	4.6	5.1	4.7	6.5
Methionine	1.7	1.6	1.5	1.9	1.7	2.8
Isoleucine	4.0	2.7	3.7	4.0	3.4	4.0
Leucine	7.5	5.5	5.7	6.6	5.6	6.7
Tyrosine	2.4	2.6	3.2	3.0	4.0	3.3
Phenylalanine	4.9	4.8	3.8	0.6	4.2	4.6
Tryptophan	0.5	0.7	0.6	0.7	0.7	0.2

thus offers itself as a nutritionally better cereal, being a slightly better source of lysine than wheat.

In general, it was observed that as the protein content in Triticales increased, the percentage of lysine in the protein decreased which suggests that with the increase in the protein content the lysine quantity either remains same or does not increase at the stipulated rate. It was not the case however with other amino acids like threonine and methionine. Comparing the data obtained on Triticale during this investigation and that of Ahuja and Austin[5] on Triticum there was not much difference in the amino acids between the two, however, threonine was much lower in the Triticale varieties. Similarly the contents of glutamic acid and tryptophan were found to be much lower in Triticale as compared to Triticum.

A report from CIMMYT[6] indicated a wider range of variation in the protein and amino acid contents of Triticale than in wheat and rye; and in 100 lines tested protein content ranged from 12 to 21 per cent and lysine content from 0.36 to 0.72 per cent. Protein content, in general increased with the decrease in lysine content, but two lines were found which were rich in protein as in lysine. From this it can be concluded that it is possible to develop lines with high protein and high lysine contents.

It has been observed that the protein content of wheat to a great extent depends upon the environment, and climate soil being the principal denominator. The chief climatic factors are rainfall, humidity, temperature, day length and the length of the ripening period. Applications of nitrogenous and phosphatic fertilizers with or without combination of some of the micro-nutrients viz. zinc, copper and manganese in different doses and at different times of maturity of crops have considerably influenced the protein composition and other qualitative aspects of wheat and wheat products.

As regards protein content, the present study is in agreement with those of many previous workers who reported high protein content in the grains of Triticale. Gill *et al*,[7] in their preliminary trial with twelve Triticales reported protein content which ranged from 12.47 to 16.33 per cent. Similar data have also been reported by CIMMYT.[8]

A report[9] from CIMMYT indicated that in lysine content, Triticale is significantly better than wheat. Among 2700 lines of Triticale tested for protein at CIMMYT in 1973, the lysine level, as a per cent of total protein ranged from 2.7 to 4.4 per cent and the average was 3.7 per cent. The lysine level in commercial wheat is about 2.3 per cent and lysine in Opaque-2 corn is about 4.3 per cent. Thus the lysine level in Triticale can be considered better than wheat, better than normal corn and approaching the level of Opaque-2 corn.

Biological assay of Triticale, that is, feeding to laboratory animals to test the animals growth response, has demonstrated that Triticale has superior feeding values/unit of protein compared with normal corn. Triticale further contains more units of protein by crop weight than corn[10].

Acknowledgement

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Detection of Tobacco Seed Oil in Sesame and Other Oils by Thin Layer Chromatography

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Thin layer chromatographic separation of unsaponifiable fraction on silica gel G with petroleum ether (40°-60°C)-solvent ether-acetic acid (60:40:2) as developer and 1:1 ortho-phosphoric acid as spray enables the detection of adulteration of sesame oil with tobacco seed oil as low as 5 per cent level. Two discrete blue spots of Rf 0.25 and 0.15 are characteristic of tobacco seed oil, which are absent in sesame oil. Tobacco seed oil in other oils like groundnut, sunflower seed also can be detected similarly.

Tobacco (*Nicotiana tobacum* Linn) seed oil, had received attention for its possible use as an edible oil when refined. The yield of seed varies from 80 to 300 lb per acre and contains 33 to 41 per cent oil. The cold process of extracting oil in country wooden mill yields a pleasant smelling drying oil of an agreeable taste which has properties similar to good quality sesame oil[1]. It can usually be refined without any difficulty by the ordinary methods and deodourised satisfactorily. Tobacco seed oil is used as an adulterant in various edible oils, since the composition of sesame oil and tobacco seed oil are very similar[2,3] and it is difficult to distinguish them on the basis of physical and chemical constants. Suitable methods are not available for the detection of tobacco seed oil in sesame oil when present in small proportions. A rapid thin layer chromatographic method is described here, which can be successfully employed for the detection of small amounts of tobacco seed oil in sesame and other oils.

Materials and Methods

(*a*) *Thin layer plate:* Glass plates 10×20 cm were coated singly by spreading silica gel G and water (1:2) with a stationary type spreader to give a layer thickness of 300 mμ. After brief air drying, the plates were activated by heating in an air oven at 105°C for one hour and cooled in a desiccator.

(*b*) *Solvent system*[4]*:* Petroleum ether (40°–60°C): ether:acetic acid (60:40:2).

(*c*) *Chromogenic reagent:* 1:1 ortho-phosphoric acid in water.

Preparation of sample: The seeds of pure sesame, sunflower, groundnut and tobacco were crushed in a stainless steel pestle and mortar and extracted with petroleum ether-solvent ether mixture. The solvent was then distilled off and the residue left was taken as unrefined oil. This unrefined oil was treated with alkali and charcoal to get refined oil.

Preparation of unsaponifiable matter[5]*:* To about 5 g of oil or refined oil in a conical flask, 30 ml of distilled 95 per cent ethanol and 2.5 ml of 50 per cent (w/w) KOH solution were added and heated on a water bath until saponification was complete. The solution was cooled, diluted with water and extracted with solvent ether (3 to 4 times). The combined ether extract was washed with water to make it alkali free, and dried over anhydrous sodium sulphate. The unsaponifiable fraction was dissolved in chloroform (5 ml).

Chromatography: 10 μl of the chloroform solution of the unsaponifiable matter was spotted in a thin layer plate and developed until the solvent front was 10 cm from the origin. A single run was made for 20 min. After the development, the plates were dried at room temperature for 15 min to remove the residual solvent. They were then sprayed with the chromogenic reagent and heated at 85 to 90°C for 5 to 10 min.

Results and Discussion

It was found that while sesame, sunflower seed and groundnut oil gave two discrete spots at the upper half (above Rf 0.5), the number of spots increased to four in case of tobacco seed oil. Of these, the two blue spots at the lower half (Rf 0.25 and 0.15) were very prominent and characteristic of tobacco seed oil, which are totally absent in sesame and other oils. It is evident from Fig. 1 that individual chromatographic pattern of the unsaponifiable matter of tobacco seed oil (both refined and unrefined) is distinctly different from that of sesame and other oils (both refined and unrefined).

In spite of the lack of specific information about the components of the unsaponifiable fraction of the oils, these patterns yielded a method for unequivocal and

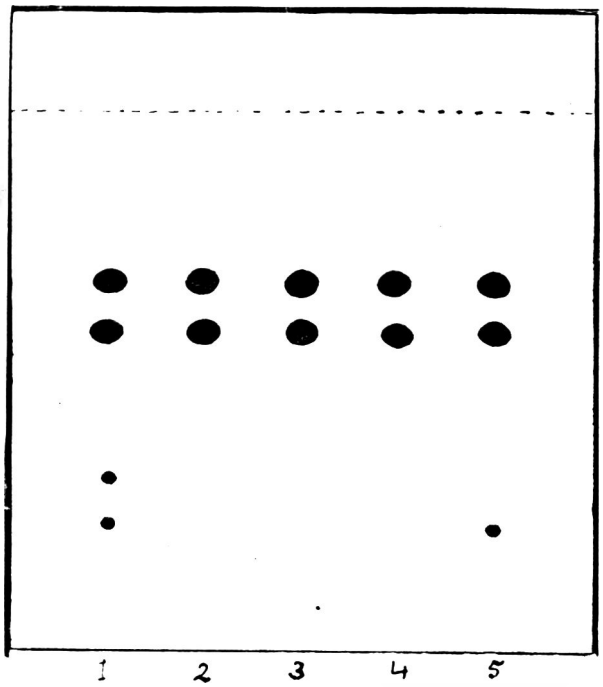

Fig. 1. Chromatogram of unsaponifiable matter of refined oils 1, Tobacco seed; 2, sesame; 3, groundnut; 4, sunflower seed; 5, tobacco seed in sesame.

reproducible separation. Using this method, it has been observed that tobacco seed oil can successfully be detected in sesame and other oils at levels as low as 5 per cent, as it gives an additional blue spot at Rf 0.15. The other blue spot at Rf 0.25 though present at higher concentrations is usually insignificant for detection purposes at 5 per cent level.

Acknowledgement

Our thanks are due to Dr. B. R. Roy, Director, Central Food Laboratory for his keen interest in the present investigation.

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State of Aflatoxin in Groundnut Oil

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Aflatoxin in commercial groundnut oil could be sedimented by centrifugation to the extent of 60 to 65% which is present in the state of suspended particles. Studies with membrane filtration, preparative ultracentrifugation and adsorption on the state of aflatoxin have revealed that the remaining portion of the toxin may perhaps be in the solubilized state.

In earlier communications from this laboratory[1,2] partition of the toxin among various constituents of the groundnut kernel during their separation was reported. The results showed that 10-15 per cent of the toxin separated with the oil while the remaining was with the cake. The observed uneven distribution between cake and the oil reported in these papers may perhaps be due to limited solubility of the toxin in oil. Feuell[3] and Parker and Melnick[4] have suggested that aflatoxin in oil appears to be present in suspension associated with cell debris carried over mechanically. There is however not adequate experimental data on the physical state of the toxin in oil. To avoid or eliminate the toxin contamination of the oil, these data are needed. This communication presents results obtained on the state of the toxin in the oil medium.

Groundnut oil, available commercially, invariably carried a considerable amount of suspended matter. Preliminary trials were therefore confined to study the effect of centrifugation on the toxin content of the supernatant. Further studies were carried out on membrane filtration, adsorption and ultracentrifugation in order to find out the state of aflatoxin in commercial groundnut oil.

Materials and Methods

Centrifugation: Thirty-gram samples of well mixed commercial groundnut oil were centrifuged at diffe-

rent forces (in Janetzki K. 24 centrifuge and Spinco Model L-40 preparative centrifuge) for 30 min at 5°C and the toxin contents of the supernatants as well as the residues were determined by the method of Pons *et al.*[5]. A few experiments were also done by adding known amounts of the pure aflatoxin to refined as well as commercial oil. Toxin in these cases was added to the oil through a chloroform solution of the pure sample and heating it subsequently on water bath (90-95°C) for 10 min to remove the solvent. High toxin oil was obtained by crushing aflatoxin contaminated groundnut seeds in Carver Laboratory Hydraulic Press and mixed with commercial oil for the above experiments.

Preparative ultracentrifugation: Beckman L_2-65B preparative ultracentrifuge was used at 218,000 × g for 30 min at 5°C. Both supernatant and residue were analysed for aflatoxin as before.

Membrane filtration: The commercial groundnut oil containing 0.08 ppm of aflatoxin B_1 was filtered through a membrane filter (pore size: 0.45μ; size: 50 mm; Sartorius membrane filter, GMBH) under mild vacuum at the rate of 10 ml of filtered oil per hour. Aflatoxin was analysed in the filtrate. Other filtrations through Whatman No.1 filter paper and sintered glass funnel (Jena Glass Type 11 G3) were also performed for purposes of comparison.

Dissolution of aflatoxin in refined oil: In order to determine the degree of solubility of aflatoxin in refined oil, 3.0 mg of pure aflatoxin B_1 was uniformly dispersed in 35 g of pure refined oil in a 100 ml conical flask. It was heated on a water bath for 10 min, cooled and centrifuged in Beckman L_2-65B preparative ultracentrifuge at 218,000 × g for 60 min at 5°C. Aflatoxin was determined both in the supernatant as well as in the residue.

Adsorbents and filter-aid: Adsorbents like aluminium silicate, magnesium silicate, activated carbon and Fullers earth (both activated and unactivated) were used to study the adsorption of aflatoxin. Celite was used as filter-aid. These were added in proportion of 0.5 g adsorbent to 100 g of commercial oil containing 0.08 ppm of aflatoxin B_1 and mixed individually on a magnetic stirrer for a period of 30 min. Higher levels of 1.0 and 2.0 per cent were also used similarly in the case of activated Fullers' earth. The samples were centrifuged at 10,000 × g for 30 min at 25° C and the supernatants were analysed for aflatoxin.

Results and Discussion

In a preliminary experiment to study the state of aflatoxin in commercial groundnut oil, the degree of sedimentation of the toxin was studied by centrifuging upto 218,000 × g for 30 min. Fig.1 presents data on the centrifugal force applied to oil and the toxin content

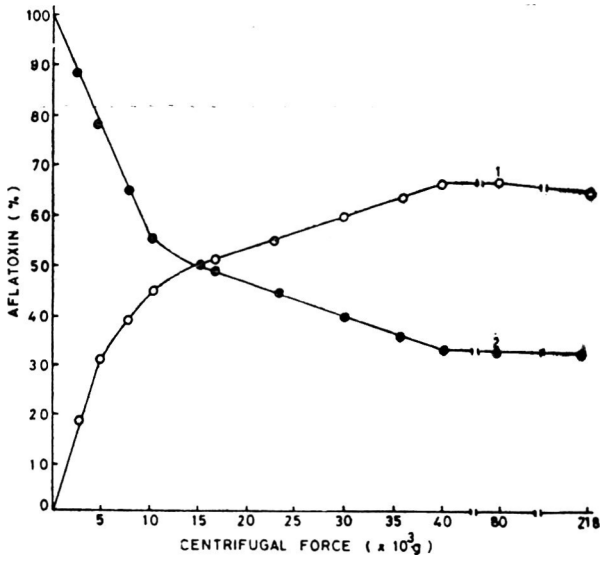

Fig. 1. Distribution of aflatoxin into residue and supernatant on centrifugation of groundnut oil for 30 min

1, Residue; 2, Supernatant.

of the residue as well as the supernatant. With the increase in centrifugal force the toxin content of the residue also increased till it reached 40,000 × g. At this level, 65 per cent of the total toxin sedimented. Beyond this point even at 218,000 × g for 30 min in a preparative ultracentrifuge, no more toxin sedimented. This suggested that nearly 35 per cent of the toxin remained perhaps in the solubilised state in the supernatant.

In this connection, an experiment was conducted to determine the degree of solubility of aflatoxin B_1 in pure refined oil. The data presented in Table 1 show that as high as 871 μg is not sedimentable even at 218,000 × g for 60 min suggesting that this is probably the limit of solubility of the toxin in 35 g of pure refined oil.

In a recovery experiment, commercial oil fortified with high toxin oil and commercial oil as well as refined oil fortified with pure aflatoxin were subjected to centrifugation at 80,000 × g for 30 min. The results in Table 2 show that while the toxin occurring in oil as a natural

TABLE 1. DEGREE OF SOLUBILITY OF AFLATOXIN IN PURE REFINED GROUNDNUT OIL

	Micrograms
Aflatoxin B_1 taken in 35 g of pure refined oil	3,000
Total aflatoxin B_1 solubilized in 35 g of the supernatant of the centrifuged oil (218,000 × g for 60 min at 5°C)	871
Total aflatoxin remained undissolved in residue	2,100

TABLE 2. RECOVERY EXPERIMENT ON SEDIMENTATION OF AFLATOXIN IN OIL AT 80,000×g FOR 30 MINUTES

Initial aflatoxin (μg)	Aflatoxin in supernatant (μg)	Aflatoxin in residue (μg)
80.0[a]	28.4(35.5)	43.0
500.0[b]	300.0(60.0)	170.0
200.0[c]	192.0	0.0

Figures in parentheses indicate percentage of aflatoxin in supernatant oil. Initial aflatoxin content in commercial oil:0.08 ppm.

[a]Commercial oil mixed with high toxin oil obtained by crushing aflatoxin contaminated seeds in Carver Laboratory Hydraulic Press.

[b]Commercial oil fortified with pure aflatoxin.

[c]Refined oil fortified with pure aflatoxin.

TABLE 4. EFFECT OF STIRRING OF THE OIL* WITH DIFFERENT ADSORBENTS ON THE SEDIMENTATION OF AFLATOXIN BY CENTRIFUGATION (10,000×g FOR 30 MINUTES)

Adsorbent	Aflatoxin B_1 in supernatant (ppm)	Per cent removal
Fullers' earth (activated)	0.027	66
Fullers' earth (unactivated)	0.037	54
Carbon (activated)	0.030	64
Magnesium silicate	0.030	64
Aluminium silicate	0.037	54
Celite (filter aid)	0.037	54
Control (centrifuged without adsorbent)	0.054	32

*Initial aflatoxin content: 0.08 ppm

contaminant could be removed (as estimated in the supernatant) by centrifugal force to the extent of 64.5 per cent, pure toxin added to refined oil could not be sedimented. Since the commercial oil has, in addition to the toxin, some suspended particles, sedimentation of the toxin by centrifugation is perhaps aided by these particles. Centrifugation of the refined oil fortified with aflatoxin does not help in the sedimentation of the toxin, perhaps because of such suspended particles. It is further noted that in the commercial oil 52 μg of the toxin could be sedimented along with the suspended particle. On the other hand, when the same oil is fortified with pure toxin 200 μg toxin could be sedimented. This raises the question as to why the suspended particles in the commercial oil could not take more than 52 μg toxin when they can take more of the added toxin. This could be either due to a high concentration gradient built up by the added toxin or due to increased capacity of the particles to adsorb more toxin as a result of heating to remove chloroform added to fortify the oil with pure toxin.

A parallel study was conducted to check whether the sedimentable particles carrying aflatoxin could be filtered off by using various filters like, filter paper, sintered glass and membrane filters. The data presented in Table 3 show that membrane filtration separates maximum amount (50 per cent) of aflatoxin present in the oil as compared to sintered glass filtration (44 per cent) and filtration through filter paper (30 per cent). This difference among different filters is obviously due to varying pore sizes of the filters, the smallest being those of the membrane filter. Even with membrane filters of pore size 0.45 μ only 50 per cent of the toxin could be separated.

To remove the unsedimentable part, the efficiency of different adsorbents was also, studied. The adsorbents examined are Fullers' earth (both activated and unactivated), activated carbon, magnesium silicate, aluminium silicate and a filter-aid like celite. The results in Table 4 show that addition of Fullers' earth (unactivated), aluminium silicate and celite promote sedimentation of aflatoxin to the extent of 54 per cent as compared to 64-66 per cent sedimentation in cases of Fullers' earth (activated), magnesium silicate and activated carbon on centrifugation at 10,000×g for 30 min.

TABLE 3. REMOVAL OF AFLATOXIN IN GROUNDNUT OIL* BY FILTRATION

Filtration	Aflatoxin B_1 in filtrate (ppm)	Per cent removal
Membrane filter	0.040	50
Sintered glass	0.045	44
Filter paper	0.056	30

*Initial aflatoxin content: 0.08 ppm.

TABLE 5. EFFECT OF FULLERS' EARTH ON THE SEDIMENTATION OF AFLATOXIN IN GROUNDNUT OIL* (10,000×g FOR 30 MINUTES)

Fullers' earth in 100 g oil (g)	Aflatoxin B_1 in supernatant (ppm)	% retained in sedimented material
0.0	0.054	32.5
0.5	0.030	62.5
1.0	0.020	75.0
2.0	0.006	92.5

*Initial aflatoxin content: 0.08 ppm.

The control sample of oil with no addition gave a value of 32 per cent sedimentation of aflatoxin under similar conditions.

Since activated Fullers' earth has been found to sediment more aflatoxin than the other adsorbents under the above conditions, the efficiency of higher levels of activated Fullers' earth was examined. Results in Table 5 show that sedimentation of aflatoxin increased as the level of activated Fullers' earth increased in the oil recording 92.5 per cent sedimentation with 2 per cent Fullers' earth even at 10,000 $\times$g. This indicates that activated Fullers' earth when mixed with oil adsorbs even the unsedimentable portion of the toxin and thus nearly all the toxin can be removed by sedimentation at 10,000 $\times$g.

It is clear from the above data obtained by the studies on centrifugation, ultracentrifugation, membrane filtration and adsorption, that aflatoxin in commercial groundnut oil is present to the extent of 60-65 per cent along with the sedimentable cell debris of the oil. The remaining portion of toxin may perhaps be in solubilized state. The results obtained on adsorption of aflatoxin by Fullers' earth have indicated the possible use of this material in industry for decontamination of groundnut oil from aflatoxin.

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Thin Layer Chromatography and Identification of Dyes in Indian Country Liquors

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The differentiation of authentic coloured country liquors from spurious ones is possible by comparing dyes present in them by TLC and absorption spectrophotometry. The results show that most of the spurious country liquors, in general contain prohibited food colours.

The addition of coal tar dyes (synthetic permitted food colours) to the country liquors is a common practice followed by all the manufacturers. The low cost and high demand of the country liquors in India have resulted in the manufacture of imitation country liquors by adding different dyes either to the illicit liquors or diluted ordinary denatured spirit keeping ethanol percentage the same as in genuine ones. This poses a great problem for the differentiation of the genuine country liquors from the imitation ones. However, genuineness of a coloured country liquor could be based on the study of dyes in a specific brand of country liquor.

Paper chromatography[1-7], ion exchange chromatography[8], electrophoresis[9,10] and thin layer chromatography[11,12] have been used to detect the coal tar dyes in alcoholic products. In this communication the comparison of dyes from genuine country liquors and fake country liquors by thin layer chromatography and absorption spectrophotometry is reported.

Materials and Methods

Thin layer glass plates (10 $\times$20 cm) coated with Silica Gel G (E. Merck, Germany) to a uniform thickness (250 μm) were dried for 15 min at room temperature and then activated in an oven at 160°C for 1 hr (activa-

TABLE 1. CHROMATOGRAPHIC PROPERTIES AND ABSORPTION MAXIMA OF THE DYES IN COUNTRY LIQUORS MANUFACTURED IN MAHARASHTRA

Name	Place of manufacture	Liquor colour	Absorption max (nm) in Acid	Alkali	Alcohol	Rf value	Colour of spot	Absorption max (nm) in Acid	Alkali	Alcohol	Identified dye
Mosambi	Sholapur	Yellow	440	400	440	0.12	DY	440	400	440	Tartrazine
Ananas	,,	Yellow	450	NSA	450	0.12	DY	440	400	440	Tartrazine
						0.26	DP	525	475	525	Red 10, B
						0.36	DO	490	450	490	Cos Sol Orange G
						0.41	DP	525	475	525	Carmoisine
Narangi	,,	Orange	495	450	495	0.36	DO	490	450	490	Cos Sol Orange G
						0.41	DP	525	475	525	Carmoisine
Angoor	,,	Pink	530	500	530	0.15	DP	535	475	535	Amaranth
						0.34	PP	525	475	525	Not identified
						0.41	DP	525	475	525	Carmoisine
Pineapple	Satara	Yellow green	440	400	440	0.12	DY	440	400	440	Tartrazine
						0.34	DB	625	625	625	Indigo Carmine
Orange	,,	Orange	495	440	495	0.26	DP	525	475	525	Red 10 B
						0.36	DO	490	450	490	Cos Sol Orange G
						0.41	DP	525	475	525	Carmoisine
Mosambi	,,	Yellow	460	430	460	0.12	DY	440	400	440	Tartrazine
						0.26	DP	525	475	525	Red 10 B
						0.36	DO	490	450	490	Cos Sol Orange G
						0.41	DP	525	475	525	Carmoisine
Gulab	Chitli	Pink	520	NSA	530	0.15	DP	535	475	535	Amaranth
						0.41	DP	525	475	525	Carmoisine
Santri	,,	Orange	495	440	495	0.26	DP	525	475	525	Red 10 B
						0.36	DO	490	450	490	Cos Sol Orange G
						0.41	DP	525	475	525	Carmoisine
Pink Country Liquor	Sangli	Pink	530	575	530	0.35	DBR	525	475	525	Not identified
						0.95	DP	535	475	535	Not identified
Ananas	Ahmednagar	Yellow	450	350	450	0.12	DY	440	400	440	Tartrazine
						0.15	DP	535	475	535	Amaranth
						0.41	PP	525	475	525	Carmoisine
Orange	,,	Orange	500	NSA	500	0.15	DP	535	475	535	Amaranth
						0.36	DO	490	450	490	Cos Sol Orange G
						041.	DP	525	475	525	Carmoisine
Angoor	,,	Pink	500	NSA	500	0.15	DP	535	475	535	Amaranth
						0.36	DO	490	450	490	Cos Sol Orange G
						0.41	DP	525	475	525	Carmoisine
						0.48	DP	525	475	525	Not identified
Pineapple	,,	Orange	480	440	480	0.12	DY	440	400	440	Tartrazine
						0.15	DP	535	475	535	Amaranth
						0.36	DO	490	450	490	Cos Sol Orange G

DY: Dark Yellow; DP: Dark Pink; DO: Dark Orange; DB: Dark Blue; DBR: Dark Brick Red; PP: Pale Pink; NSA: No. Sharp Absorption. All the above liquor samples contained 42 to 43% ethanol.

TABLE 2. CHROMATOGRAPHIC PROPERTIES AND ABSORPTION MAXIMA OF DYES IN SPURIOUS COUNTRY LIQUORS

Brand name	Liquor colour	Rf value	Colour of spot	Absorption max (nm) in Acid	Alkali	Alcohol	Identified dye	% ethanol
Angoor	Red	0.65	Red	515	480	515	Acid Red	45
Angoor	Red	0.52	Red	515	445	515	Crocine Scarlet	38
Gulab	Red	0.25	Red	515	445	515	Basic Red	42
Mosambi	Yellow	0.68	Yellow	520	445	430	Metanil Yellow	45
Gulab	Pink	0.69	Pink	565	557	565	Rhodamine B	45
Mohini	Green	—	—	520*	445	430	—	32
				620	—	620		
		0.53	Blue	620	—	430	Malachite green	—
						620		
		0.68	Yellow	520	445	430	Metanil yellow	—

*Absorption maxima for whole extract.

tion of plates at 160°C was found to give good resolution with compact spots). After cooling, the plates were used. The authentic samples of coloured country liquors procured from manufacturers and samples of imitation coloured country liquors claimed to be genuine received in this laboratory were used.

Equipment: (*i*) Chromatographic apparatus—closed glass developing tank; (*ii*) micropipettes; (*iii*) hair drier and (*iv*) Carl Zeiss Specord UV-VIS automatic recording spectrophotometer.

Reagents: (*i*) 95 per cent ethanol; (*ii*) chromatographic solvent: n-butanol glacial-acetic acid-ethanol-water, 10:2:0.5:5 (vol./vol.) and (*iii*) 0.01 per cent solutions of the following coal tar dyes (permitted/non-permitted) in 40 per cent alcohol was prepared and used: (1) Tartrazine, (2) Cos Sol orange G, (3) Carmoisine, (4) Amaranth, (5) Indigo carmine, (6) Red 10 B, (7) Acid Red, (8) Crocine Scarlet, (9) Basic Red, (10) Metanil yellow and (11) Rhodamine B.

Procedure: The liquor sample (10 ml each) was evaporated and the residue was dissolved in 1 ml of 40 per cent ethanol. Ethanol solution (10 µl) of each sample was spotted on the activated silica gel plate and the chromatograms were developed by using the solvent to a distance of 10 cm. Then the plates were removed and dried at room temperature and Rf values were noted. Each spot was scrapped off and extracted with ethanol. After evaporation of ethanol the dry residue was dissolved in 40 per cent ethanol, 0.1 N HCl or 0.1 N NaOH to give approximate concentration of 0.01 per cent of the dye. The visible spectra of these solutions were recorded in the spectrophotometer using 1 cm path cell. Moreover the dry residue obtained directly from 10 ml of liquor sample was dissolved in 40 per cent ethanol, 0.1 N HCl or 0.1 N NaOH and their spectra were also recorded.

The dyes (permitted/non-permitted) were examined by thin layer chromatography and spectrophotometry as described above.

Results and Discussion

The Rf values obtained on TLC and absorption maxima of various dyes in genuine country liquors and imitation country liquors are given in Table 1 and 2 respectively. It can be seen that the dyes found in genuine country liquor vary as the source or type of flavour present. For example "Mosambi" manufactured at two different distilleries contains a mixture of permitted four colours and one colour respectively with different maxima in visible range (Table 1). These data also indicate that these genuine country liquors are prepared by diluting pure alcohol and adding different permitted food colours. However the imitation country liquors contain one or a mixture of two prohibited food colours which have been identified from their chromatographic behaviour and absorption maxima (Table 2).

Although considerable work has been done on the analysis of dyes by paper chromatography[5-7] and by electrophoresis[13]; the present TLC procedure in combination with the spectrophotometric study of the dyes in country liquors is more reliable and corroborative for the differentiation between imitation and genuine coloured country liquors.

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Studies on the Methods for the Preparation of Enriched Flavour Distillate from Lactic Starter Cultures

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A simple and convenient method has been described for maximum production of enriched flavour distillates from broth and milk cultures using selected lactic starters. The effect of some factors such as level of inoculum, agitation, oxidation of fermented medium, etc. on the recovery of flavour distillate, has also been studied.

Although lactic starter cultures have been known to bring about the natural development of flavour and aroma in dairy products, distillates recovered from selected starter cultures have been used in recent times for this purpose[1]. In the present communication a simple and convenient method has been described for the maximum recovery of the enriched flavour distillates from selected lactic starter cultures. The effect of some factors on the production of distillates has also been investigated.

Materials and Methods

Streptococcus B used in the present study was a variant from the non-curdling parent culture, *Streptococcus* A described earlier[2]. Citrate dextrose broth was used as the growth medium.

Skim milk was sterilised momentarily at 15 lb followed by steaming on the subsequent day, while broth was sterilized at 15 lb for 20 min.

Skim milk was inoculated with an 18-hr test culture at 0.6 per cent level, while in case of broth 1 per cent inoculum of the same age was used. In another set of experiments, the level of inoculum was varied from 0.5 to 5.0 per cent.

The inoculated flasks were incubated at 30°C for 96 hr. In one set of trials involving the effect of incubation period on flavour production, the incubation was carried out for different periods. In another set the inoculated flasks were agitated on a rotary shaker at 193 rpm.

Citric acid was estimated by the method of Saffran and Densted as modified by White and Devis[3]. Diacetyl was estimated by both the methods of Westerfield[4] and King[5].

The effect of oxidation on the recovery of volatile compounds from the fermented mixture was studied by first steam distilling it without oxidation, and subsequently oxidizing the same sample with 40 per cent ferric chloride followed by steam distillation. A control experiment was also run by oxidising the fermented mixture as above followed by steam distillation.

The effect of rate of distillation on the recovery of flavour distillate was studied by varying the time taken to collect the distillate by controlling steam injection. The effect of temperature on condensate water temperature on the recovery of flavour compounds was also studied in another set of trials.

Vacuum distillation of the oxidised and unoxidised

fermented mixtures was carried out at 75°C according to Lindsay and Dey[1].

Results and Discussion

Cultures grown in skim milk and the citrated milk[6] were used for the preparation of flavour concentrate. The fermented mixture in each case was oxidised with $FeCl_3$, prior to distillation. By employing the modified procedure of Sandine[6], appreciable amount of diacetyl (39 mg/100 g) was recovered from citrated milk culture. Results on the effect of growth condition such as effect of inoculum, incubation temperature and aeration, on the production of flavour compounds have been recorded in Tables 1A, 1B and 1C. It may be seen that maximum amount of flavour components were recovered with the use of 2 per cent inoculum (317 mg/100 ml). Prolonged incubation of the fermented medium resulted in the loss of flavour compounds. However, after 96 hr of incubation, maximum amount of volatile compounds was recovered.

Continuous agitation of the fermented media adversely affected the production of flavour compound, while shaking for 4 hr towards the end of the incubation period significantly improved its yield (Table 1A, 1B and 1C). It is known that agitation during the growth of a culture stimulates oxidation of NAD[7]. It is likely that oxygen may deplete the production of lactic acid, thereby making more pyruvate available to the organisms for the production of acetoin and diacetyl. During later stages of fermentation i.e., at 96 hr, agitation did not show any appreciable effect on diacetyl production which may presumably due to the completion of the fermentation process by the utilization of all available pyruvate and CoA.

The effect of oxidation of the fermented broth on the production of volatile compounds has been recorded in Table 2. It may be seen that there is a substantial increase in the production of diacetyl and volatile acids in the first fraction of the distillate in both oxidised and unoxidised samples. Comparison of the data on production of flavour compounds from fermented broth prior to and subsequent to oxidation of the left-over residue, indicates that the release of flavour compounds is relatively faster in the oxidised than in the unoxidised samples. A similar trend may also be noted in case of fermented broth samples which were freshly oxidised and then steam distilled.

During the process of distillation, it is essential that the released compounds be condensed as completely as possible. The recovery of volatile compounds improves as the rate of distillation slows down by controlling the steam injection. The distillate collected after 90 min had a higher diacetyl content (290 mg/100 ml) as compared to that collected after 15 min (200 mg/100 ml).

It is generally known that the temperature of water used in the condenser affects the recovery of flavour compounds when chilled water was used in the condenser. A distillate with higher diacetyl content (292 mg/100 ml) was recovered as compared to that distilled by using water at 30°C (230 mg/100 ml). A similar trend was also noted in regard to recovery of volatile acids.

TABLE 1A. EFFECT OF INOCULUM ON THE RECOVERY OF FLAVOUR COMPOUNDS

Inoculum* %	Volatile acidity** (ml)	Diacetyl (mg/100 ml of distillate)
0.5	1.4	150
1.0	1.8	253
2.0	2.4	317
4.0	2.4	283
5.0	2.4	233

*18 hr old milk culture.
**ml of N/10 NaOH used to neutralise 10 ml of the distillate.
Incubation in each case was 48 hr.

TABLE 1B. EFFECT OF INCUBATION TIME ON THE RECOVERY OF FLAVOUR COMPOUNDS

Incubation period (hr)	Volatile acidity* (ml)	Diacetyl (mg/100 ml of distillate)
24	0.5	165
48	1.1	135
72	1.4	250
96	1.7	275
120	1.05	260

Inoculum 0.6%
*ml of N/10 NaOH used to neutralise 10 ml of the distillate.

TABLE 1C. EFFECT OF AERATION ON THE RECOVERY OF FLAVOUR COMPOUNDS

Experimental condition	Aeration time (hr)	Volatile acidity (ml)	Diacetyl (mg/100ml of distillate)
Control (no shaking)	48	1.5	198
	96	1.8	286
Continuous shaking	48	0.7	80
	96	—	—
4 hr shaking*	48	1.6	240
	96	1.8	294

*Shaking was done towards the end of incubation period.
**ml of N/10 NaOH used to neutralise 10 ml of the distillate.

TABLE 2. EFFECT OF OXIDATION ON THE RECOVERY OF FLAVOUR COMPOUNDS

	Dist. fraction No. (each 25 ml.)	pH of the fraction	Volatile acidity* (ml.)	Diacetyl (mg./fraction)
Steam distillation of the fermented mixture without prior oxidation	1	8.10	Nil	38.0
	2	7.70	,,	26.0
	3	7.20	,,	21.0
	4	6.90	,,	12.9
	5	6.90	,,	12.9
Oxidation of the left over residue followed by steam distillation	1	3.20	4.1	37.5
	2	3.25	3.7	12.5
	3	3.20	3.4	6.5
	4	3.30	3.0	3.2
	5	3.30	2.9	1.8
Freshly oxidised fermented mixture followed by steam distillation	1	3.00	4.6	70
	2	3.00	4.2	40
	3	3.10	3.4	28
	4	3.10	3.1	13
	5	3.30	2.7	4.5

*ml of N/10 NaOH used to neutralise 10 ml of the distillate

Vacuum distillation of the fermented mixture after oxidation yielded substantial amounts of volatile acids (16.0 and 9.0 ml of N/10 NaOH required to neutralise 10 ml of distillate in the 1st and 2nd fractions), whereas without the use of vacuum, the corresponding values for volatile acids for the 1st and 2nd fractions were only 7.0 and 3.2 ml respectively.

The yield of diacetyl was maximum by simple steam distillation (7,600 ppm) as compared to vacuum distillation (4,000 ppm), presumably due to use of higher temperature in the former case.

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An Active and Efficient Desiccant for In-package Desiccation of Dehydrated Foods

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Calcined lime from marine shells is found to be a very convenient and efficient desiccant for use as in-package desiccant for dehydrated foods. It should be used in bags made of a strong porous and permeable paper which is sift proof and is in a gussetted form.

The use of in-package desiccants for enhancing the shelf-life of dehydrated foods has been reviewed recently[1]. This technique gained application during the middle of the present century when it was realised that removal of moisture in some of the dehydrated products beyond a certain limit though desirable for better shelf life was not practicable in the conventional drying equipment. It could be however, readily brought about by using suitable in-package desiccants. In recent times when more sophisticated equipment and methods of drying have come into use, producing very low moisture products, it has been the experience of the present authors that unless specially maintained low-humidity packaging rooms are used, packaging of products such as accelerated freeze dried meat or beverage powder in unit packs usually results in moisture pick-up by the products from surrounding atmosphere to an extent that could prove detrimental to its expected shelf-life. In-package desiccants can play an important role in such cases by removing, during storage, the excess moisture thus adsorbed.

It has been reported that calcium oxide or lime[2] is a very convenient and effective desiccant for use with food materials. Its success on a practical scale, however, depends upon finding an economic and easy source of high purity product. The present investigation was therefore, carried out with several commonly available sources of lime and as a result, a promising source could be identified. The relative merit of this lime as a desiccant and other commonly used desiccants as well as suitability of different bag materials for its packing for placement inside a package was studied.

Materials and Methods

The different types of limes studied are:

(*i*) *Local market lime:* Several lime stone samples available in the local market were collected. These are made locally by calcination of lime stone brought from the neighbourhood.

(*ii*) *Lime from marble stones:* Marble chips available in the market were calcined in muffle furnace at 950°C for 3 hr, cooled and immediately transferred in airtight bottles.

(*iii*) *Lime from marine shells:* This is available in the local market packed in polyethylene bag. Part of it was regenerated by heating at 950°C for about 3 hr. The shell lime was also prepared by calcination of the shells in muffle furnace at 950°C for about 3 hr.

Other desiccants used are:

(*i*) *Silica gel:* An indigenous product of regular density marketted under the brand name "Amex gel" was obtained for this purpose.

(*ii*) *Activated alumina:* An imported variety was used. Both these desiccants were reactivated by heating at 150 –160°C for 4 hr.

Estimation of calcium oxide content: This was carried out by volumetric titration with standard acid[3].

Moisture absorption capacity: Weighed quantities of different desiccants were exposed in a vacuum desiccator over saturated lithium chloride solution. The desiccator was evacuated and placed in an oven at 37°C. Moisture absorbed was estimated from weight gain to a constant weight by different desiccants. This usually took 5-6 days.

Equilibrium moisture content at different humidities: Weighed quantities of desiccants were exposed inside evacuated desiccators containing saturated salt solution to give different humidities at 37°C as shown below[4,5].

The desiccators were placed inside an oven maintained at 37°C±1°C. The weight gain was observed periodically till it became constant.

Efficiency of desiccation: This was determined by using different weight ratios of product to be desiccated to desiccant inside sealed pouches made of laminate of

Saturated salt solution	Humidity (% RH) at 37°C
Lithium chloride	11
Magnesium chloride	31.6
Sodium dichromate	50
Sodium nitrite	62
Sodium chloride	75
Potassium chromate	86
Potassium nitrate	91

paper/0.04 mm aluminium foil/polyethylene and observing the rate of moisture loss in the product. The product used for this experiment was freeze dried mutton mince at a moisture content of 7.5 per cent. Weighed quantity of desiccant was used in gussetted bags made of grease proof paper. The bags were sealed with the help of polyvinyl alcohol adhesive. The pouches containing the product and the desiccant bag were stored under room conditions (27°–30°C).

Efficiency of different bag materials for enclosing desiccants: Grease proof paper (44-45 gsm), PT cellophane 300 and 25 micron low density polyethylene film were used for making desiccant bag. The rate of desiccation of AFD mutton mince at 7.5 per cent moisture, using lime from marine shells as the desiccant packed in bags of identical size made out of these materials, was determined.

Results and Discussions

Lime, which is a common material is available almost at every place and its main source is lime stone or marble from which it is obtained by burning in lime kilns. The purity of lime thus obtained, however, varies widely[6]. Certain varieties are nearly 90 per cent pure; the usually available lime stone lime in most places seldom exceeds 60 per cent of calcium oxide[6]. At Mysore several samples of lime, soon after they were received from lime kiln, were examined. A sample of locally available marble chip also was converted into lime by calcination. The lime content of these are not of a high degree, and their capacity for desiccation cannot be predicted even to a rough approximation without analysing the samples. Another potential source of lime is marine shells. Various types of shell bearing animals in inland areas like West Bengal and Assam which abound in rivers, ponds and marshy lands, are also the source of shells that are collected and burnt to yield lime. Samples of marine shells collected from Madras and Cochin beach were heated at 900°–950°C in muffle furnace and examined for lime content and moisture absorption capacity. Lime obtained from burning of shell is also available in the local market packed in polythylene bags. This variety with or without regeneration (at 950°C for 3 hr) was tested for purity and water absorption capacity. Table 1, giving these results, indicates that shell lime is in a high state of purity and though the market variety, because of moisture absorption and subsequent partial reconversion into calcium carbonate shows less activity, it can be regenerated by heating, prior to use, at 950°C for 2-3 hr. Thus the shells from shell bearing animals, which are available in plenty, provide a very convenient source of desiccant grade lime with predictable activity. Also, being in small solid pieces, shell lime has distinct advantage over powdery lime stone lime in use.

TABLE 1. PURITY OF LIME OBTAINED FROM DIFFERENT SOURCES AND ITS MOISTURE ABSORPTION CAPACITY

Source	CaO (%)	Moisture absorption capacity(%)
Market lime		
Sample 1	19.3	5.0
,, 2	22.6	6.7
,, 3	40.5	14.1
Freshly calcined marble	46.5	18.9
Freshly calcined marine shells		
Sample 1	94.2	32.5
,, 2	90.6	—
Commercial shell lime in poly bag	65.2	19.6
,, ,, after recalcination	81.1	31.2

Table 2 gives equilibrium moisture of different desiccants at different relative humidities, from which it is apparent that at lower humidities calcium oxide has a

TABLE 2. EQUILIBRIUM MOISTURE CONTENT OF DIFFERENT DESICCANTS AT HUMIDITIES RANGING FROM 11 TO 91% RH AND AT 37°C

Desiccant	Equilibrium moisture at RH of						
	11	31.6	50.1	62	75	85.5	91
Freshly calcined marine shell	31.8	32.4	32.4	32.9	35.6	37.9	38.0
Freshly calcined marble (CaO 46.5%)	18.9	19.6	20.8	22.3	23.6	26.1	30.6
Silica gel, regular density, indigenous	2.9	7.6	12.3	15.8	37.2	47.6	50.4
,, imported	8.4	17.6	23.1	26.9	31.0	32.6	34.4
Activated alumina, imported	8.0	13.1	17.2	20.0	31.4	37.0	36.5

TABLE 3. EFFICIENCY OF DESICCATION WITH DIFFERENT DESICCANTS USING AFD MUTTON MINCE AT 7.5% MOISTURE

Desiccant	Product : Desiccant (rati by wt.)	% moisture in the product estimated after (days)			
		4	8	15	21
Freshly ignited commercial shell lime	10 : 3.5	3.08	2.06	1.82	1.19*
Silica gel, regular density (indigenous)	10 : 3.5	—	5.02	4.74	—
	10 : 30	—	—	2.12	1.82
Activated alumina (imported)	10 : 3.5	—	5.56	4.39	—
	10 : 30	—	—	1.49	1.16

*After about 2 months moisture content becomes undetectable.

TABLE 4. EFFECT OF PACKING MATERIAL ON RATE OF DESICCATION (*Product:* AFD mutton mince at 7.5% moisture) (*Desiccant:* Reactivated commercial shell lime)

In-package desiccation period (days)	% moisture in meat when desiccant is packed in		
	25 micron polyethylene bag	300 MST cellophane bag	Grease proof paper bag
7	4.92	5.09	2.31
16	4.41	4.19	1.79
34	2.94		0.85

far greater capacity for moisture absorption than the adsorbent desiccants like silica gel or alumina. Hence, dehydrated food materials which normally should have low equilibrium humidity over them in a closed container could be desiccated further more efficiently with the help of calcium oxide. This conclusion is well confirmed by experimental results given in Table 3. It is seen that with the same weight ratio of 10:3.5 of product to desiccant, moisture content is reduced from 7.5 to 1.8 per cent in 15 days when calcium oxide (reactivated market shell lime) is the desiccant, but it remains as high as 4.7 or 4.4 per cent when silica gel or alumina is used. Also, to bring about a comparable rate of desiccation with silica gel or activated alumina a too high proportion of the latter had to be used.

The transfer of moisture from the product to the desiccant takes place through its conversion into the vapour phase and the absorption of the moisture vapour by the desiccant. To hasten this process, it is necessary that the walls of the desiccant pack must be sufficiently porous and permeable to water vapour. It should also be sift-proof, because after moisture absorption calcium oxide is transformed into a fine powder of calcium hydroxide. The pack must also provide for the expansion in volume of the desiccant. A gussetted pouch is therefore, suitable. Results in Table 4 indicate that neither low gauge polyethylene nor MST cellophane is suitable for packing the desiccants since their permeability characteristics are hinderance to the quick moisture absorption by the desiccant. Grease proof paper, on the other hand is both sift proof and porous and permeable enough to facilitate quick moisture absorption. Polyvinyl alcohol has been found to be a very good adhesive for this purpose. Desiccants packed in grease proof paper can be kept in hermetically sealed moisture impermeable containers. However, if moisture absorption takes place as indicated by weight gain, then the pack has to be rejected since unlike silica gel, reactivation of lime cannot be carried out with the bag remaining intact.

The general conclusion of this investigation is that shell lime is a very convenient and efficient desiccant. For best results it should be prepared by burning of shells before use or where preformed lime is availabe, it should be reactivated by heating at 900°–950°C for 2 to 3 hr. For packing this desiccant a strong, porous and permeable but sift proof paper in the form of gussetted bags, should be used.

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New Approach and Apparatus to Control Relative Humidities in Test Environments

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A new approach to control relative humidities in test environments has been described. The method employs the principle of mixing dry and saturated air streams in the desired proportion by using a proportioning valve which is the heart of the system. It is possible to control relative humidity from 0 to 100% at an interval of 5%. An apparatus employing the above system has been described. The apparatus can be used, with appropriate additional test chamber, for sorption studies of food powders at temperatures ranging from ambient to about 80°C. The apparatus is simple in construction, and the equilibrium is achieved quickly-in about 10-12 hr.

Many experiments with food products require precise control of relative humidity in the test environments such as sorption studies, drying tests and shelf life determinations. Accurate RH control is necessary in these experiments since even a few per cent RH variation may affect the results considerably. At the same time the method should be simple in operation, the apparatus should be easily repairable whenever needed and should be in expensive. Very often a method or an instrument is not used because either it is very complicated or its cost is prohibitive and therefore accuracy is sacrificed. It was necessary, therefore, to design a dynamic method which was accurate, simple and inexpensive. The method described in this paper is believed to possess these desirable features.

The RH in a test environment can be controlled by employing any of the following three principles:

(*i*) mixing dry and saturated air in various proportions to obtain a flow of the desired RH, (*ii*) saturating air at one temperature and warming to another, and (*iii*) saturating compressed air and expanding to a lower pressure.

The first principle is used in the present method.

Basic Principle

The basic principle used for controlling relative humidity, (RH), in the method is, as stated above, to mix two streams of air-one dry and the other saturated-in desired proportions. The RH of the resultant stream of air will be roughly equal to the proportion of the saturated air stream. The difference between the apparent and the true RHs depends upon RH and temperature. The difference is less at extreme values of RH. The true RH is obtained as explained below using thermodynamic relationships.

Let us designate the dry air stream by 1, the saturated air stream by 2 and the resultant air stream by *r*. The true RH, i.e. that of resultant air stream, RH_r, at atmospheric pressure and constant temperature can be written as[1].

$$RH_r = \frac{100\, H_r}{\left(\frac{M_w}{M_a} + H_r\right) P_s} \qquad \ldots.(1)$$

where, H = absolute humidity of air, weight of moisture in air per unit weight of dry air

M_w, M_a = molecular weights of water and air, respectively

P_s = vapour pressure of water, atm.

The main inlet air stream is dried and then split into two streams: one which is maintained dry till mixing here called as 'dry air stream' and the other which is saturated before mixing, here referred to as 'saturated air stream'. Let the proportion of 'saturated air stream' be *x*. Then the absolute humidity of the resultant air stream, H_r, is

$$H_r = x\ H_2 = xH_s \qquad \ldots.(2)$$

where H_s is absolute humidity of air at saturation. Combining equations (1) and (2) we obtain

$$RH_r = 100 \times \frac{H_s}{\left(\frac{M_w}{M_a} + x H_s\right) P_s}$$

$$= 100 \times \frac{1}{\left(\frac{M_w}{M_a} + H_s\right) \frac{P_s}{H_s} - (1-x) P_s}$$

$$= 100 \times \frac{1}{1 - (1-x) P_s} \qquad \ldots.(3)$$

since $\left(\frac{M_w}{M_a} + H_s\right) \frac{P_s}{H_s} = \frac{P_s}{P_s} = 1$

The value of deviation factor $\frac{1}{1-(1-x)P_s}$ in equation (3) is always greater than 1, excepting at a limiting value of x of 1 when it is equal to 1, and depends upon the values of x and P_s. Since Ps increases with increasing temperature, it follows then, that the deviation will be more at high temperatures than at low temperatures. At moderate temperatures viz. 25° to 65°C, P_s is quite small fraction of 1, and therefore the deviation factor is little more than 1. The true RH is correspondingly higher than the per cent proportion of the 'saturated air stream'. Table 1 gives the values of corrected RH for various apparent RH values ranging between 0 and 100 at an interval of 10 at 25°, 35°, 45°, 55°, and 65°C.

Proportioning Dry and Saturated Air Streams

It is of paramount importance, for accuracy in the results, to be able to split the main dry air flow into the two air streams—dry and saturated—in desirable proportions accurately and quickly. It has been made possible by using a proportioning valve which functions on the principle of air flowing through capillary tubes in laminar flow regions, as explained below.

Under constant temperature and pressure conditions, the pressure drop for air flowing through a cylindrical capillary tube of uniform diameter, in laminar region, is given by Hagen Poiseuille equation.

$$\triangle P = \frac{32 L v \mu \rho}{d^2} \quad \ldots.(4)$$

where μ is viscosity of air, v velocity of air, ρ density of air, L length of capillary tube and d diameter of capillary tube. This equation assumes fully developed flow for laminar flow, the length required for fully developed flow, x_l, is given by[1].

$$x_l = 0.05\, d\, \mathrm{Re} \quad \ldots.(5)$$

where Re is Reynolds number. Now writing equation (4) for volumetric flow rate, q, following equation is obtained.

$$q = \frac{\pi d^4 \triangle P}{128 \mu L \rho} \quad \ldots.(6)$$

Since in equation (6) ρ, d and μ are constants, and if pressure drop, $\triangle$ P, can be kept constant, then

$$q \propto \frac{1}{L} \quad \ldots.(7)$$

that is, the flow rate will increase as many times as the length of capillary tube is reduced. If this principle is extended to a branched system, e.g., air flowing through two capillary tubes of same diameters, arranged in parallel, and having lengths L and xL, where x is is the length ratio, the flow rate in each branch will correspond to the proportion of its length. For the example considered, if pressure drops across both the tubes are same, i.e., $\triangle P_L = \triangle P_{xl}$, (achieved by employing manometers) the flow rate in the first tube will be xq while in the second one q. This will apply to any number of branches equally well.

For instance, if 4-capillary tubes are in length ratios of 1:2,5:2.5:5, the flow rate per cent in each would be 50, 20, 20 and 10 respectively; if 6 capillary tubes are in ratios of 1:1:1:2.5:2.5:5, the corresponding flow rate per cent would be 25, 25, 25, 10, 10 and 5, and so on. In the former example, it is possible to vary RH at an interval of 10 per cent, in the latter at an interval of 5 per cent. Figure 1 shows a proportioning valve having 4-capillary tubes in the length ratios of the first example.

Description of the Method

Air, obtained from a cylinder filled with compressed air, or other source, at constant pressure is dried in drying towers or cells containing silica gel. The dried air passes through a proportioning valve which is set to obtain desirable proportions of 'dry' and 'saturated' air streams. As explained earlier with the help of relation-

TABLE 1. APPARENT RELATIVE HUMIDITIES AND CORRESPONDING TRUE RELATIVE HUMIDITIES AT VARIOUS TEMPERATURES

Apparent R.H. %	True R.H. (%) at temperature of 25°C	35°C	45°C	55°C	65°C
10	10.30	10.5	10.9	11.6	12.8
20	20.5	20.9	21.5	22.8	25.0
30	30.6	31.0	32.0	33.7	36.2
40	40.7	41.4	42.3	44.0	47.0
50	50.8	51.2	52.2	54.0	57.0
60	60.7	62.3	62.2	64.0	67.0
70	70.6	71.0	72.0	73.1	75.9
80	80.4	81.0	81.6	82.5	84.1
90	90.2	90.3	90.9	91.3	92.2

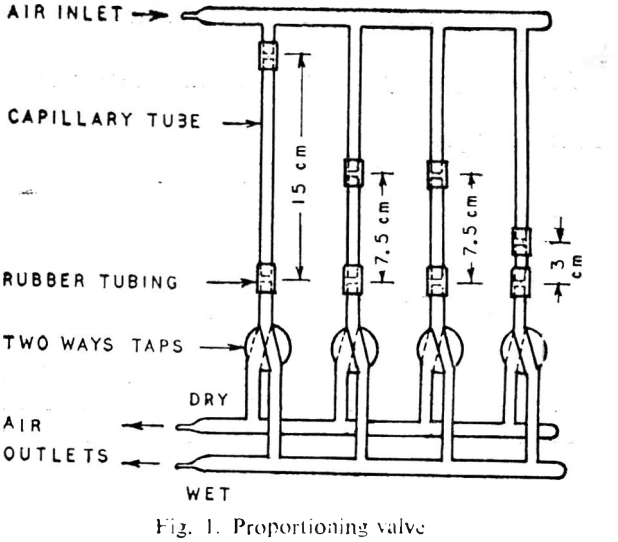

Fig. 1. Proportioning valve

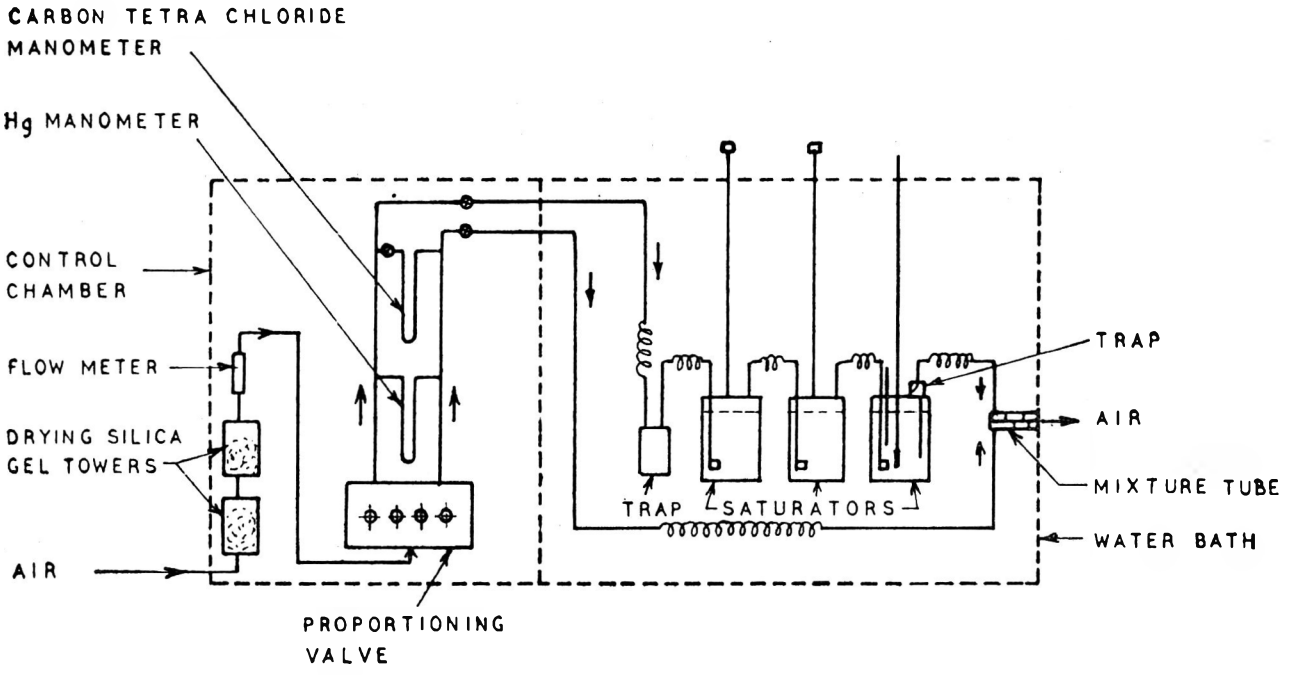

Fig. 2. Flow diagram of the relative humidity controlling apparatus

ship (7) that, to function a proportioning valve of this kind properly, pressure drops across 'dry air control branch' or branches and 'saturated air control branch' or branches must be same, that is, the upstream for both being common, the pressure difference between the branch must be zero, two manometers—one filled with mercury and the other filled with carbon tetrachloride—are brought into circuit, which are adjusted accordingly with the help of two fine control needle valves placed one each in each emerging air stream. The mercury manometer adjusts the pressures approximately and the second one—filled with carbontetrachloride-accurately. The second manometer is provided with a stop-cock to isolate it from the large transient pressure differences which occur when the air flow is started or stopped. It is brought into the circuit after balancing the first one. The saturated air stream is passed through two or three saturators, filled with distilled water, to saturate the air stream. Both the air streams are then mixed in a baffled mixing tube from which the emerging air stream will have desired RH. This air stream can be passed through the enclosed test environment to maintain the RH.

To maintain the desired temperature of the final air stream and in the test environment, the saturator assembly is placed into a thermostatically controlled water bath. The air streams are passed through tube coils submerged into the water bath so that their temperatures attain steady state quickly. The test chamber may also be placed in the water bath, if possible, for more accurate control. Water bath is not necessary for atmospheric temperatures. Fig. 2 shows the general system.

Brief Construction Details

All components of the instrument are conveniently arranged in the two sections-control chamber and water bath of a chamber. In the control chamber are placed drying towers, proportioning valve, manometers and needle valves. The silica gel towers, arranged in series, are conveniently held on one side with the help of clamps. An inlet is provided in the first tower for air to be passed from any suitable source. All connections are made with pressure rubber—tubing to eliminate any leakage. The water bath houses the saturator assembly, the tube coils, the baffled mixing tube which is also the outlet for the final air stream, an electrical immersion heater, a thermostat and a small fractional horse power agitator. If necessary the test chamber can also be placed in the water bath. As before, all connections are made with the help of pressure rubber-tubings. The saturator assembly is clamped to the wall, if necessary. Heater, thermostat and agitator are all rigidly fixed to the walls of the bath in such a fashion that the joints are leak proof, at the same time each one can be removed, if required. The water bath is made of rust-proof metal like copper and brass, with sufficient wall thickness to withstand the water pressure. All four sides may be properly insulated to minimize heat losses, if required. The cover should be removable.

The saturator assembly needs little greater detail. This assembly may consist of 2 or 3 wide mouth glass jars, each of about 1.5 l. capacity filled with water upto about 10 cm or more height. The mouth of each jar is closed by a rubber cork.

The cork of the first saturator has an inlet tube through

it, extended into the water and joined to a sintered glass gas bubbler to bubble the air through the water. The air is collected from the head space by a copper tube a coil above the cork. This coil restored the air to bath temperature after the slight cooling caused by the evaporation of saturator water. The cork is also provided with a brass tube with a rubber cork on the outer end, for releasing the pressure in the saturator headspace to the outer atmosphere. This hydrostatic pressure is necessary to be released when the air through the apparatus is stopped lest the saturator water should be driven backward into the heating coil and the proportioning valve. As an additional precaution, a trap bottle may be used between the preheating coil and the first saturator.

The second saturator is similar in design while the third one has some different features. The pressure releasing tube is extended to the bottom and does not have a rubber cork on outer end as it operates at atmospheric pressure. The air outlet has a trap so that any moisture carry over by air can be separated and returned to the saturator. A tube returns the separated moisture to the saturator.

The face of the control chamber where manometers are fixed should be made of perspex sheet, or any other transparent material to facilitate the manometer's adjustment. Other two faces may be made of any metal (preferably rust proof) or other suitable material.

Operation

The water bath is filled with clean water and a thin layer of any oil, say diesel oil, is spread on the water surface to present water evaporation from the surface.

The corks are put on the pressure releasing tubes on the first two saturators, the stop cock on the carbon tetra chloride manometer is closed, and the taps on the proportioning valve are set to give the desired RH.

The air flow is started through the apparatus and the pressures of the two streams coming out from the proportioning valve are balanced on the mercury manometer by means of the two needle valves. The air flow rate is then controlled to the required value. Then the pressures of the two air streams are balanced on the carbon tetrachloride manometer.

The heater of the thermostatic bath is then put on and the thermostat set at the required temperature. When desired to stop the operation, the carbon tetrachloride manometer is disconnected, the two corks are removed from the top of the pressure releasing tubes, and the air flow is stopped.

TABLE 2. SET AND OBSERVED RELATIVE HUMIDITIES

Apparent R.H. (%)	True R.H. (%)	Observed R.H. (%)	Deviation (%)
10	10.5	10.5	5.00
20	20.9	21.0	5.00
30	31.0	31.0	3.33
40	41.4	41.5	3.75
50	51.2	51.0	2.00
60	62.3	62.5	4.20
80	71.0	71.0	1.43
70	81.0	81.0	1.25

Test results have shown excellent agreement with the theoretically set values (Table 2). For tests, the air mixture after the mixer was passed through a test chamber is positioned on a tripod stand in the water bath. A hair hygrometer placed in an enclosed casing consisting a tube was fixed on the test chamber to indicate the relative humidity in the test chamber. In this experiment the proportioning valve used consisted of 6-capillary tubes of lengths 20, 10, 10, 4, 4 and 4 cm in the corresponding length ratios of 5:2.5:2.5:1:1:1. The results presented are at temperature 35°C. It may be observed that the maximum deviation in the observed RH from the set-RH is only 5 per cent and mostly it is less than 4 per cent. Therefore by using this method the required RH can be easily and accurately controlled and achieved without needing even hygrometer to read the RH. It is however desirable to use a hygrometer just as a check.

A similar apparatus employing the principle mentioned here has been used successfully in this laboratory for sorption—both adsorption and desorption—studies of dry milk baby foods[2] at relative humidities ranging from 20 to 25 per cent and at temperatures upto 60°C. The equilibrium time ranged between 8 and 16 hr.

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Isolation of *Streptomyces* having High Glucose-isomerase Activity and Assessment of their Efficiency in the Production of Fructose Syrups

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Two potent glucose-isomerase producing strains *S. fradiae*, SCF-5 and *S. cinnamonensis*, MFS-4 were isolated from natural source. The enzyme was cell bound and washed cell suspensions showed considerable activity at pH 7 to 8 and at temperatures of 60 to 80°C. SCF-5 cell suspension was more efficient having temperature optimum of 80°C for the enzyme activity. In a reaction mixture (of 20 ml volume) containing 1.5 M glucose maintained at 60°C, 59% glucose was converted to fructose in 24 hr by the washed cell suspension of SCF-5. The high fructose syrup after decolourisation, deionisation and flash evaporation yielded a water clear thick syrup having 65-70% solids.

Glucose-isomerase which catalyses conversion of dextrose to fructose has assumed considerable importance commercially in recent years for the production of "high fructose syrups"[1]. These syrups which are composed of nearly 50 per cent each of fructose and glucose have been obtained from mainly corn starch as the starting material and have been used as economic alternative sweeteners in countries lacking in cane or beet sugar. In view of the potential use of glucose-isomerase for converting low grade and less utilized starchy substances available in this country into high fructose syrups, the present work was undertaken to isolate, screen and select microorganisms for the production of glucose-isomerase.

The presence of glucose-isomerase as a cell bound enzyme was first reported in *Pseudomonas hydrophila* by Marshall and Kooi[2] followed by a large number of reports in other genera of bacteria which include *Aerobacter*, *Bacillus*, *Brevibacterium*, *Escherichia*, *Lactobacillus*, *Leuconostoc*, *Parcolobactrum*, *Pasteurella*[3], *Streptomyces*[3-5], *Arthrobacter*[6] and more recently *Actinoplane*[7]. In majority of these microorganisms, presence of xylose was essential for the formation of glucose-isomerase. Not much information is, however, available on the habitat of the glucose-isomerase producing microorganisms so that their isolation and screening can be done with some rationale. In the present work we report screening of microorganisms isolated from places where xylose or xylan containing natural substrates undergo decomposition and selection of two potent strains of *Streptomyces* cultures isolated one each from the sugarcane field and maize field soil samples. A comparative study of their cell suspensions as the source of glucose-isomerase in the production of high fructose syrup was made.

Materials and Methods

Cultures: Streptomyces cultures were isolated from compost and soil samples obtained from Mysore city and from the nearby agricultural farms and fields employing glycerol-aspargine agar[8]. The medium contained griseofulvin (625 μ/ml) and benzyl penicillin (500 unit/ml) to supress the growth of fungi and other bacteria. The *Streptomyces* cultures were recognised as powdery surfaced colonies which appeared after 8-10 days of growth in the petri dishes kept at 30°C. These colonies were picked and purified by replating. The cultures were identified by the methods outlined by Waksman[8] and Gordon and Smith[9].

Screening for glucose-isomerase production: The medium used for screening was of the following composition: Peptone, 0.1; yeast extract, 0.1; $MnCl_2$, 0.001; $FeSO_4.7H_2O$, 0.001; $CoCl_2.6H_2O$, 0.002; $MgSO_4.7H_2O$, 0.1; glucose, 1.0; corn steep liquor, 2.0 per cent; partially hydrolysed wheat bran, 16 ml per cent, and pH 7.0. Wheat bran hydrolyzate was prepared by suspending 500 g of bran in 2 lit. of water, adjusting its pH to 2.0 with concentrated HCl and autoclaving twice at 15 psi for one hour each. The slurry was cooled and filtered. Fifty millilitre of the above screening medium taken in 250 ml conical flask was inoculated with a loopful of culture or spore suspension taken from a 4-day old agar slope culture and incubated in rotary shaker at 28-30°C for 4 days. After this, the cells were harvested by centrifugation, washed twice with distilled water resuspended in a known volume of water and stored overnight at —4°C.

Enzyme assay: The frozen cell suspension was thawed and mixed in a tissue homogeniser for getting an uniform suspension. Enzyme activity was estimated

in 2 ml of the reaction mixture containing glucose (1.0 M), 0.2; phosphate buffer (0.2 M, pH 7.5), 0.5; $MgSO_4$ (0.1 M), 0.1; $CoCl_2$ (0.001 M), 0.1 and enzyme (cell suspension), 0.2 ml. Glucose isomerase from nearly all the sources reported so far required Mg^{2+} and Co^{2+} ions for activity as has been found in our studies also (unpublished). After 1 hr incubation at 60°C, the reaction was stopped by the addition of 2 ml (0.5 M) perchloric acid. In the controls the enzyme was added after the stoppage of the reaction. The enzyme activity in the cell suspensions of the most active cultures in these studies showed linearity upto 2.5 hr of incubation at 60°C. In this time about 10 per cent of glucose was converted to fructose. Fructose formed was estimated by the cysteine carbazole method[13] as difference between the test and the control samples. One unit of glucose-isomerase was defined as that amount which produced 1 mg fructose per hour under the above assay conditions, according to the definition of many earlier workers[4,11,12] which facilitated comparisons of potencies of our cultures with those of others.

Protein was estimated according to the method of Lowry *et al*[13]. Dry weight of the cells were measured by drying washed cell suspension to constant weight in an oven at 105°C. Brown colour of the sugar syrup was measured by absorbance at 420 nm in a Spectronic-20 spectrophotometer. Total reducing sugar was estimated by the method of Shaffer and Hartman[14]. Fructose was detected by spraying with ketohexose specific urea-HCl reagent[15] after paper chromatographic separation[16].

Results and Discussion

Screening and selection of glucose-isomerase producing cultures: 134 *Streptomyces* cultures isolated from 7 different locations from maize compost, paddy compost, sugarcane field soil, maize field soil and garden soil were examined for glucose-isomerase production. The isolates were differentiated on the basis of morphology, colour, shape and consistency of the colonies. The procedure of overnight freezing and thawing of the cell suspension prior to the enzyme assay was to facilitate partial lysis of the cells as this is one of the known methods for rupture and homogenisation of the microbial cells[17]; however several cycles of this procedure are necessary for a near complete homogenisation of cell suspension. Recent attempts in enzyme technology are to employ whole microbial cells as such as source of the enzyme, or those in which the enzyme is fixed by heat or by chemical treatment[18] or as immobilised whole cells. While comparing different cultures for the production of the enzyme, it must be reckoned, however, that absolute quantities of enzyme in each culture is rather difficult to determine because of the likelihood differences in the pH and temperature optima of the enzyme, the enzyme's location (i.e. whether cell surface bound or intracellular), mode of access of glucose to the enzyme as well as other influencing factors in the crude system in different cultures.

In Table 1 are listed the different samples and the number of microbial isolates from each, producing different quantities of glucose-isomerase. It was noteworthy that under the same conditions of screening 70 per cent of the total number of isolates were found to be producers of the enzyme. Three per cent of these could produce more than 5 units of glucose-isomerase per ml. Twelve of the high yielding isolates, were again screened in the screening medium containing 0.5 per cent xylose instead of wheat bran hydrolysate. In Table 2 are given the enzyme production by these cultures expressed as specific activity. Three isolates of *Streptomyces*, MFS-4, SCF-5 and MCI-2 isolated respectively from maize field soil, sugarcane field soil and maize compost were

TABLE 1. GLUCOSE ISOMERASE PRODUCTION BY MICROBIAL ISOLATES FROM COMPOST AND SOIL SAMPLES

Sample	Total no. of isolates	Number of isolates producing glucose isomerase (unit/ml) None	<1	Between 1 and 5	>5
Maize compost	64	22	26	15	1
Maize field soil	4	0	1	2	1
Sugarcane field soil	13	3	2	5	3
Paddy compost	39	9	30	0	0
Garden soil	7	3	4	0	0
Horse manure	7	2	3	2	0
Total	134	39	66	24	5

TABLE 2. GLUCOSE-ISOMERASE PRODUCTION BY STREPTOMYCES ISOLATES

Culture	Source/Isolation	Glucose-isomerase (Units/g dry cells)	(Units/mg protein)
Natural isolates			
MFS-4	Maize field soil	1348	5.5
SCF-5	Sugarcane field soil	1154	4.1
MCI-21	Maize compost	1287	3.3
MCIV-2	,,	750	3.0
MCIV-4	,,	900	2.9
SCF-10	Sugarcane filed soil	750	2.8
MCIII-7	Maize compost	453	2.2
MCV-10	,,	524	2.0
SCF-13	Sugarcane field soil	344	1.5
SCF-9	Maize compost	289	1.1
SCF-7	Sugarcane field soil	219	1.0

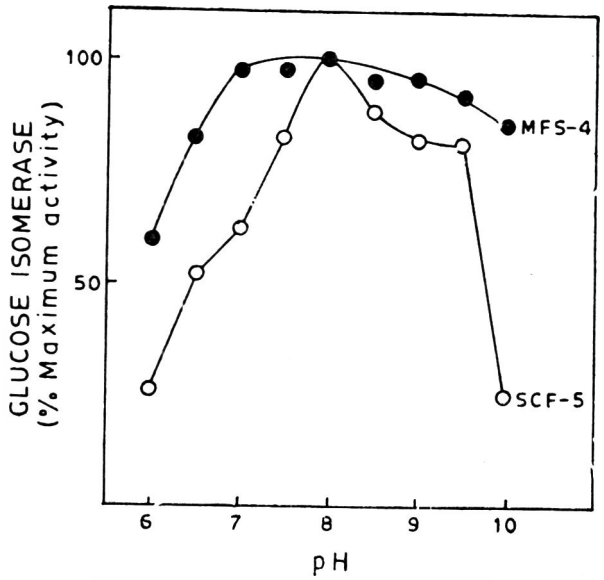

Fig. 1. Effect of pH on the enzyme activities of SCF-5 and MFS-4 cell suspensions
Reaction mixture: Glucose, 0.1 M; $MgSO_4$. 0.5 mM; $CoCl_2$, 0.05 mM; Na phosphate buffer (for pH 6.0 to 8.0) or Glycine-NaOH buffer (for pH 8.5 to 10.0), 0 05 M; enzyme, 0.5 mg; total volume, 2 ml; incubation at 60°C for 1 hr.

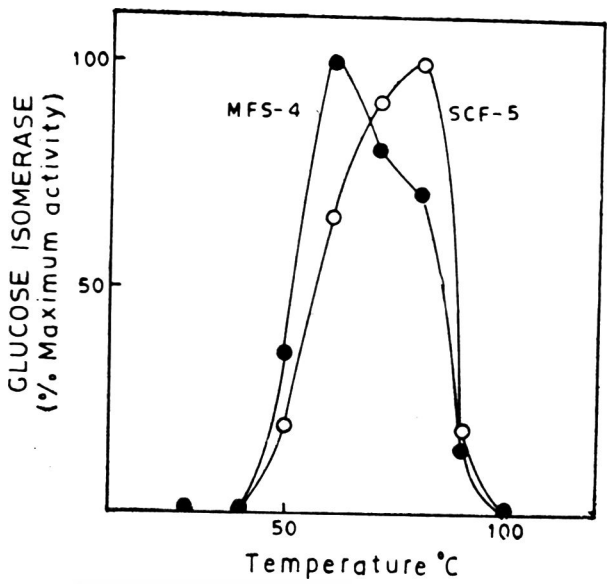

Fig. 2. Effect of temperature on enzyme activity of SCF-5 and MFS-4 cell suspensions.
Reaction mixture: Glucose, 0.1 M; $MgSO_4$. 0.5 mM; $CoCl_2$. 0.05 mM; Na phosphate buffer (pH 7.0), 0.05 M; enzyme, 0.5 mg; total volume, 2.0 ml.

found to give very high yields of the enzyme with high specific activities. They produced more than 1000 units of enzyme per gram of dry cells with specific activities comparable with values already reported in literature[4].

Taxonomic studies conducted on 2 of the best cultures, viz. SCF-5 and MFS-4 indicated their close relationship to *Streptomyces fradiae* described by Waksman and Curtis[19] and *Streptomyces cinnamonensis* of Okami *et al*[20]. respectively. The major deviation of MFS-4 from the typical culture was in its utilization of carbon compounds. MFS-4 utilised raffinose and did not utilise sucrose or mannitol while the reverse of this has been reported for the typical culture. Both MFS-4 and SCF-5 were not found to antagonize *Escherichia coli* K-12, *Serratia marcescens* Sa-3, *Saccharomyces cerevisiae* 101 (CFTRI isolate) and *Aspergillus niger* NCIM 594 in this study.

Relative efficiencies of SCF-5 and MFS-4 cultures in the production of fructose syrups: Optimum pH and temperature of glucose-isomerase activity in the cell suspension, final yield of fructose and the time required for the same, undesirable side effects such as browning and possibility of refining the syrup to get a water-clear product were the criteria examined to assess the relative efficiencies of the SCF-5 and MFS-4 cultures.

(*i*) *Effect of pH:* Fig 1 indicates the pH effect on glucose-isomerase activity of SCF-5 and MFS-4 cell suspensions. As it can be seen for both the cultures the pH optimum was 8.0. MFS-4 culture was, however, active over a wide range of pH values from 6 to 10 where nearly 60 and 85 per cent of the maximum activity were measured at both extremities respectively. SCF-5 culture had rather a sharp peak of activity at pH 8 and 6 at 10 its activity was only 25 per cent of the maximum measured at pH 8.0.

(*ii*) *Effect of temperature*: Samples of reaction mixture buffered at pH 8.0 containing SCF-5 and MFS-4 cell suspensions were incubated at 28, 40, 50, 60, 70, 80, 90 and 98°C for 1 hr. At 28 and 40°C only traces of enzyme activity were detected. At elevated temperatures, viz. from 70 to 98°C, the reaction mixture became highly coloured which interfered with fructose estimation. Therefore, the reaction was carried out at pH 7.0 where such interference was very much reduced. The enzyme activity as related to incubation temperature is shown in Fig 2. As can be seen the SCF-5 enzyme exhibited maximum activity at 80°C while that of the MFS-4 was at 60°C.

The details regarding the pH and temperature optima using cell suspension (Fig 1 and 2) are given only to indicate how a small shift in pH during incubation will affect the enzyme activity of SCF-5 culture and not that of MFS-4. However, the SCF-5 culture having a higher temperature optimum for glucose-isomerase (80°C) performed better, presumably it is also more heat stable. This point was further substantiated by the observations made in the following experiment.

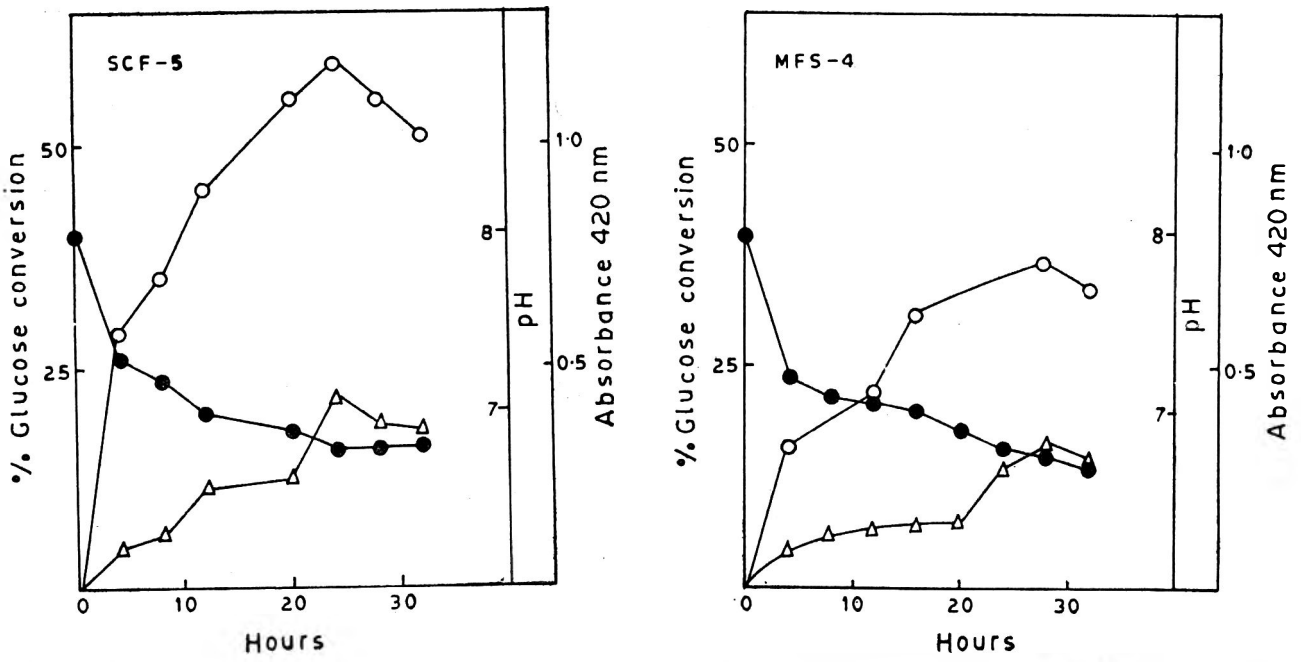

Fig. 3. Effect of incubation period on glucose conversion (O—O), pH change (● —●) and browning (△—△) in the reaction mixture.

Reaction mixture; Glucose, 1.5 M; enzyme, 125 units; Na phosphate buffer (0.2 M, pH 8.0), 4.0 ml; $MgSO_4$ (0.1 M), 1.0 ml; $CoCl_2$ (0.001 M), 1.0 ml; total vol. 20 ml; incubation at 60°C.

(*iii*) *Production of fructose syrups:* The capacities of SCF-5 and MFS-4 cell suspension to convert glucose to fructose were examined in 20 ml of the reaction mixture taken in a 50 ml conical flask; containing D-glucose, 5.4 g; phosphate buffer (0.2 M, pH 8.0), 4.0; $MgSO_4.2H_2O$ (0.1M), 1.0; $CoCl_2$ (0.001 M), 1.0 ml and enzyme, 125 units. The experiment was run in adequate number of replicates (flasks), so that two flasks containing the reaction mixture were removed at each interval from the incubator (maintained at 60°C) for the measurement of fructose concentration, pH and the brown colour. The mouths of the conical flasks had been covered with tin foil to minimise evaporation and estimations were made after making up the volume wherever necessary. The results are shown in Fig. 3. It was evident at the outset that SCF-5 enzyme was superior since it converted glucose to fructose more rapidly than MFS-4 did. SCF-5 cell suspension effected the highest conversion of 59 per cent within 24 hr of incubation. Sato and Tsumura[21] have obtained nearly the same level of glucose conversion (57 per cent) in 24 hr with *Streptomyces phaeochromogenes* but at pH 9.0. In case of MFS-4 only 37 per cent conversion was measured as the maximum after 28 hr under the same conditions of the experiment. Prolonged incubation of the reaction mixture after 24-30 hr brought about a reduction in the final yield of fructose in case of both the cultures. The changes in the pH value of reaction mixture was quite marked. From an initial pH of 8.0 it dropped to 6.8 and 6.7 in case of SCF-5 and MFS-4 respectively. Absorbance at 420 nm showed a gradual increase upto 24 hr of incubation after which very little change was noticed in both the cases. The reaction mixture was found to have developed a light yellowish brown colour at the end of the incubation period of 32 hr.

On the basis of the above observations, SCF-5 culture being superior to MFS-4 was selected for further work.

(*iv*) *Refining of fructose-syrup:* The fructose syrup was refined by the standard procedures of sugar decolourisation with activated charcoal followed by deionisation[7]. The following steps were adopted:

(*a*) Centrifugal separation of *Streptomyces* cells from reaction mixture at 3000 rpm for 10 min.

(*b*) Decolourisation of the syrup by adjusting pH to 5.5 and treating with 2 per cent activated animal charcoal (W/V) for 30 min. After this the syrup was filtered through paper pulp.

(*c*) After decolourisation the syrup was passed through Dowex 50-H^+ and Dowex 1-OH^- columns and the deionised syrup was concentrated by flash evaporation to a water clear thick syrup having 65-70° brix.

Acknowledgement

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Polyphenolases of a Local Variety of Mango

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Polyphenol oxidase was studied at intervals during the growth period of mango. The enzyme was specific for dihydroxyphenols and was active at pH 6.5-7.0. The order of specificity was dopamine-HCl, dopa, caffeic acid, chlorogenic acid and catechol and not specific for tyrosine and phenol. The Km value was 2.358×10^{-3}M catechol. The possible inhibitory effect of chemicals like ascorbic acid, cysteine, thiourea and sodium metabisulphite was studied.

Many fruits undergo rapid changes in colour following mechanical or physiological injury during harvesting and storage. Browning of tissues during the normal life cycle of plants is known as functional and after mechanical or physiological injury is termed as adventitious type. The latter shows the undersirable changes in flavour odour, colour and nutritive value. This browning has been attributed to the action of polyphenol oxidase (PPO) on the natural phenolic substances of plant tissues[1]. The phenolic substances are oxidised to quinones, which either react themselves or with other phenolics to form the brown pigments called melanin[2].

It is possible that enzymic browning continues in fresh slices of mango. The effect of sulphurdioxide on browning of mango during its dehydration was studied[3]. The polyphenol oxidase in mango fruits and also the changes in the enzyme activity induced by gamma irradiation were studied[4].

It was of interest, therefore, to study the possible role of polyphenol oxidase present in the fruit at the different stages of development. In this report, polyphenol oxidase in mango fruits both from peel and pulp were isolated and changes in the enzyme activity during different stages of development and its substrate specificity, as well as behaviour towards several inhibitors are investigated.

This work is a part of the thesis of Mr. P. R. Joshi for M.Tech. degree of Nagpur University.

Materials and Methods

Mangoes (*Mangifera indica* L) of the local variety were used for these studies. The fruits were collected at intervals of 20-25 days after fertilisation from the same tree which were tagged.

Preparation of PPO: Both pulp and peel tissues were selected separately. Diced tissues were frozen and stored at 0°C. Frozen tissues were macerated with chilled distilled water in a blender for 10 min. The homogenate was kept in freeze for 5-8 hr. Then it was thawed and centrifuged at -20°C at 10,000 rpm for 10 min. The supernatent was collected in a beaker and chilled acetone (1:1.6) was added until the enzyme precipitated. The enzyme precipitate was centrifuged at 10,000 rpm at -20°C and it was made free from acetone. Then it was dissolved in phosphate buffer of pH 6.8-7.0 and kept overnight below 0°C. The undissolved material after centrifugation was discarded and the clear supernatent was used as the enzyme preparation for the activity determination.

Polyphenol oxidase assay procedure: Enzyme activity was determined by measuring the increase in absorbance at 420 nm in a Klett-Summerson photoelectric colorimeter. The reference tube contained 4 ml of 0.2 M phosphate buffer (pH 7.0) and 1 ml of 10 mM catechol. Sample tube contained 3 ml of 0.2 M phosphate buffer, 1 ml of 10 mM catachol and 1 ml of enzyme solution. The reaction was carried out at room temperature. Under these conditions, linearity was maintained. The enzyme activity was calculated from the first maximum slope of the curve. The protein content of the enzyme sample was estimated by biuret method[5].

Phenolic constituents: The fruit tissue (5 g) was blended with 20 ml of 80 per cent ethyl alcohol in a blender for 5 min and centrifuged, and the phenols in the supernatent was determined[6].

Ascorbic acid: Tissue (20 g) was blended with 20 per cent metaphosphoric acid and water in a blender for 5 min and centrifuged. Ascorbic acid in the supernatent was estimated by titration against 2,6-dichlorophenol indophenol dye[6]. The total acidity was also estimated.

TABLE 1. MANGO PULP PPO ACTIVITY DURING GROWTH OF THE FRUITS

Days after fertilisation	Total acidity (%)	Ascorbic acid (mg/ 100 g)	Phenols (mg/100 g.)	Sp. activity (μg protein/ min)
12	1.781	135	456	V. little
40	2.973	52.8	356	3.51
54	3.019	47.3	191	19.20
66	3.119	45.6	171	26.06
85	0.750	43.5	110	V. little

TABLE 2. MANGO PEEL PPO ACTIVITY DURING GROWTH OF THE FRUIT

Days after fertilisation	Total acidity (%)	Ascornic acid (mg/100 g)	phenols (mg/100 g)	Sp. activity (μg protein/ min)
12	1.78	135.56	2260	26.25
40	2.978	103.00	1320	11.50
66	3.119	81.50	1200	9.50
85	0.75	69.85	690	V. little

Results and Discussion

Polyphenol oxidase activity during the development of the mango fruit: Experiments were carried out to assess the level of enzyme activity in both pulp and peel tissues during development of the fruit. The results are summerised in the Table 1 and 2.

The data in the Table 1 reveal that the pulp PPO specific activity increased as the fruit developed after fertilisation. The activity increased from the value of 3.5 units to 26 units and finally decreased to a negligible value at maturity. But the peel PPO activity was highest in the beginning i e. 26.25 units which decreased to negligible value at a mature stage. Very little or no activity was detectable in ripe mango pulp and peel tissues. The phenolase activity in tomato fruit reported by Hobson[8] rose continuously during growth phase. By the time maturation was complete, the activity was considerably low and there was significant reduction at the red stage of ripeness.

In the case of mango pulp the PPO activity was maximum at lower phenolic content and at higher phenolic content the activity had a negligible value.

Changes in phenols, ascorbic acid and total acidity: A significant decrease in the content of phenolic constituents was observed in both pulp and peel of the mango during developments and maturity. Also ascorbic acid content decreased rapidly during the development of the fruit. Acidity rose upto a certain stage and again rapidly decreased after maturation to ripe stage (Tables 1 and 2).

TABLE 3. PPO ENZYME SPECIFICITY

Substrate	Max substrate concentrate mm	Sp. activity (μg protein/min)
Dopamine-HCl	14-16	410.0
Dopa	12-14	352.5
Caffeic acid	10-12	347.0
Chlorogenic acid	10-12	333.0
Catechol	10-12	325.0
Tyrosine	—	0
Phenol	—	0

TABLE 4. MANGO PPO ENZYME INHIBITION

Inhibitor	Concn for complete inhibition (mm)
Ascorbic acid	2- 2.1
Cysteine	3.1- 3.3
Thiourca	10.5-11.0
Sodium bisulphite	12-12.5

The phenolic content of both pulp and peel practically followed the same pattern of decreasing in concentrations as the maturity increased. It was also found that the amount of vitamin C followed the same pattern as the phenolic content. The optimum pH for mango PPO was 6.7 to 7.0.

Effect of substrate concentration: The effect of substrate concentration on the reaction rate of mango PPO was determined. Michaelis constant (Km) was calculated by plotting the reciprocal of the PPO activity against the reciprocal of the substrate concentration. The Km value for PPO in mango was 2.358×10^{-3} M catechol.

Substrate specificity: Several phenolic compounds were tested for substrate specificity when oxidised by mango PPO. Table 3 shows that the effect of phenolic substrate on PPO activity. Among the substrates tested, mango PPO was most reactive towards dopamine HCl followed by dopa, caffeic acid, chlorogenic acid and catechol. The enzyme did not show any activity with substrates tyrosine and phenol. It is well known that the enzymes are quite specific for their substrates and mango PPO appeared to be specific for dihydroxy phenolic compounds.

Inhibition: To study the possible inhibition of mango PPO various chemicals used were ascorbic acid, benzoic acid, salicylic acid, p-amino benzoic acid, sodium bisulphite, thiourea, cysteine and sucrose. Out of these ascorbic acid, cysteine, thiourea and sodium bisulphite could inhibit the enzyme completely. The Table 4 indicates the effective concentrations required for complete inhibition. It shows that the ascorbic acid was found to be most effective and sodium bisulphite is the less effective.

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Pithecolobium saman Benth. (Rain Tree) Fruits as Raw Material for the Production of Ethanol

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Mature fruits of *Pithecolobium saman* Benth. (Rain tree) contained about 33% sucrose, 4.8% glucose, 0.8% fructose and the total protein, non-protein and ∝-amino nitrogen accounted for 9.6, 0.45 and 0.43% respectively on fresh weight basis. The free amino acid pool of the kernels were particularly rich in an unidentified amino acid which was similar to proline in some characteristics but distinctly separated from it in the amino acid analyzer. It also contained substantial amounts of glutamic acid, serine and threonine. A water extract of the kernels was found to be a complete medium for growth and alcohol production by *S. cerevisiae* var. *ellipsoideus*.

Rain tree (*Pithecolobium saman* Benth.) is a native of tropical America, but it is widely grown in many parts of the country especially in Karnataka, Andhra Pradesh, Tamil Nadu and Bihar as avenue trees and host trees for lac insect. A full grown tree generally yields about 200-300 kg of pods every year. The pods are relished by the cattle and horses and improvement in the quality and yield of milk from cows has been reported[1]. Although earlier reports[2,3] indicate that the kernels contain appreciable amounts of sugars, the detailed analysis for different sugars are not available. Further, there seems to be no information on the composition of the nitrogenous compounds in the kernels of *P. saman*. However, Airan and Desi[4] and Nigam *et al.*[5] have reported the qualitative data on the amino acid composition of fruits of *Pithecolobium dulce*. A knowledge of the sugar and amino acid composition of Rain tree kernels is needed for a better evaluation of their use in ethyl alcohol production. The aim of this work is, therefore, to report the composition of the kernels in relation to sugar, nonprotein and ∝-amino nitrogen and also to demonstrate the suitability of water extract of kernels as the substrate for producing ethyl alcohol.

Materials and Methods

Rain tree kernels having moisture in the range of 15-17 per cent were collected in one lot from the trees and used throughout the study. Seeds and woody portions which comprise 20 per cent of the whole fruit were removed manually. The shredded pieces of the kernels were dried at 70°C overnight and passed through Apex mill. Resulting ground powder was sieved through the Endecotts British Standard 40 mesh sieve and stored in a stoppered glass bottle at room temperature.

The microorganism used in this study was *Saccharomyces cerevisiae* Hansen var. *ellipsoideus* Dekker maintained in the Culture Collection Centre of the Institute. The stock culture was maintained on the slants of potato-dextrose agar.

Moisture, ash, fat, crude fibre, total protein and non-protein nitrogen were estimated by the AOAC[6] methods. Reducing and non-reducing sugars after inversion were estimated by the method of Shaffer and Hartmann[7]. Sugars were separated by paper chromatography[8] and analysed quantitatively by the phenol sulphuric acid method[9]. Total carbohydrate was also determined by the above procedure. Cellulose was estimated by the method of Viles and Silverman[10]. Organic acids were analysed by paper chromatography[11]. The ∝-amino nitrogen was estimated according to the method of Pope and Stevens[12]. For the determination of free amino acids, extract of the sample was prepared by blending for 5 min 100 g ground kernels with 450 ml of 75 per cent alcohol. The alcoholic extract was centrifuged at 2400×g for 10 min and the supernatant was flash evaporated to dryness under reduced pressure at 55°C. The residue was diluted in water and pH was adjusted to 2.4 with 0.1N HCl. It was then passed through Dowex-50H$^+$ (100-200 mesh) column which was washed with water and eluants were collected by washing with 2 N ammonia. The eluant was concentrated under reduced pressure and treated to remove colouring matter with the deactivated charcoal[13]. Amino acids were detected qualitatively[14] by high voltage electrophoresis at pH 1.9 and two dimensional paper chromatographic technique[15] using phenol treated with a pH 1.0 buffer in one direction and butanol-acetic acid-water (4:1:1) in other. Individual amino acids were determined quantitatively by an automatic

amino acid analyzer using M-72 resin and lithium buffers pH 2.8 and 4.1 at temperatures 39 and 60°C.

For the production of ethanol, laboratory fermentation method described by Stark *et al*[16]. was followed. Ethanol was estimated by the AOAC[6] method. The substrate for fermentation experiments was prepared as follows: Five liters of tap water was added to 1 kg of finely ground Rain tree kernels and the mixture was boiled for 30 min for the preparation of water extract. The extract and subsequent washings were combined and concentrated to get 20 per cent level of sugar. pH was adjusted to 4.5 with N/10 H_2SO_4 before autoclaving at 121°C for 10 min. After cooling the wort, 4 per cent inoculum was added and mixed thoroughly. Fermentation was carried out at room temperature (26-30°C). The data presented in this communication are the average values of three replicates.

Results and Discussion

The proximate composition of kernels and seeds of Rain tree was determined and it was comparable to the reported values[2] and hence not presented here. The major components of sugars, total protein and non-protein and ∝-amino nitrogen were analysed and results are presented in Table 1. Kernel extract contained only three major sugars of which sucrose accounted for about 85 per cent of the total sugars. The other sugars present were glucose and fructose. A very faint spot of raffinose was detected on the paper chromatogram. Total kernel protein accounted for 9.6 per cent whereas non-protein and ∝-amino nitrogen accounted for only 0.45 and 0.43 per cent respectively on fresh weight basis.

TABLE 1. CARBOHYDRATES AND NITROGENOUS CONSTITUENTS OF KERNELS

Constituent	g/100 g (Fresh wt basis)
Carbohydrates	
Total carbohydrate	72.0%
Non-reducing sugar	33.2
Reducing sugar	6.5
Glucose	4.8
Fructose	0.8
Sucrose	33.0
Nitrogenous substances	
Kjeldahl-N	1.53
Total protein (N×6.25)	9.60
Non-protein N	0.45
∝-amino N	0.43

TABLE 2. FREE AMINO ACID COMPOSITION OF RAIN TREE KERNELS

Amino acid	mg/100 g sample (Dry wt basis)
Lysine	6.79
Histidine	5.53
Threonine	27.70
Glutamic acid	51.66
Alanine	14.27
Serine	25.44
Valine	7.39
Proline	11.76
Unidentified amino acid*	200

*The approximate value given here for unidentified amino acid is by difference computed from the total ∝-amino nitrogen.

The free amino acid composition of the alcoholic extract of the kernels determined quantitatively by amino acid analyzer is presented in Table 2. In addition to those included in the Table 2, cysteine, cystine, phenylalanine, arginine, isoleucine and leucine were detected on the paper and electropherograms. The results show that the extract contained larger amounts of glutamic acid, threonine and serine. Two ninhydrin positive compounds showing maximum absorption at 440 nm preceding and suceeding proline peak were found in amino acid analyzer. The three peaks separated from each other distinctly. Presence of these compounds in the alcoholic extract of the kernels were also confirmed by paper chromatography and high voltage electro-

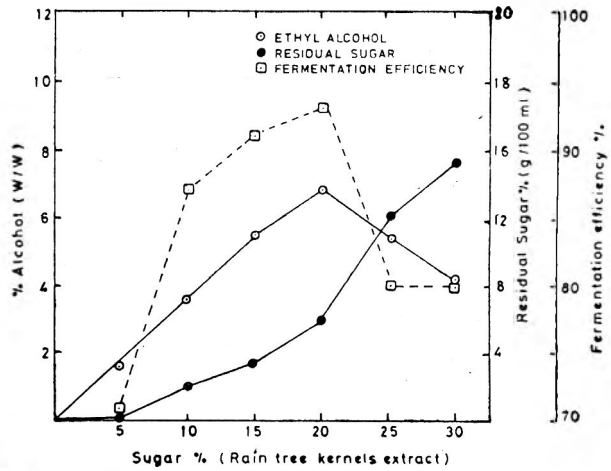

Fig. 1. Relationship between the concentration of sugar of Rain tree kernels and ethyl alcohol production (vol. 700 ml; inoculum, 4%; incubation period, 96 hr and pH 4.5).

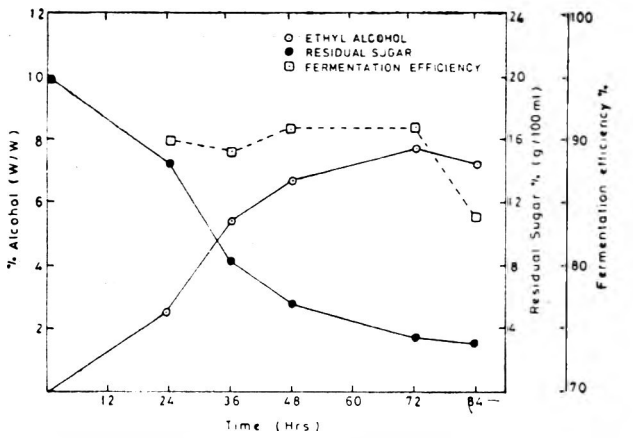

Fig. 2. Patterns of ethyl alcohol production, sugar consumption and fermentation efficiency in the fermentation of Rain tree kernels water extract at 20% sugar level, 4% inoculum and pH 4.5.

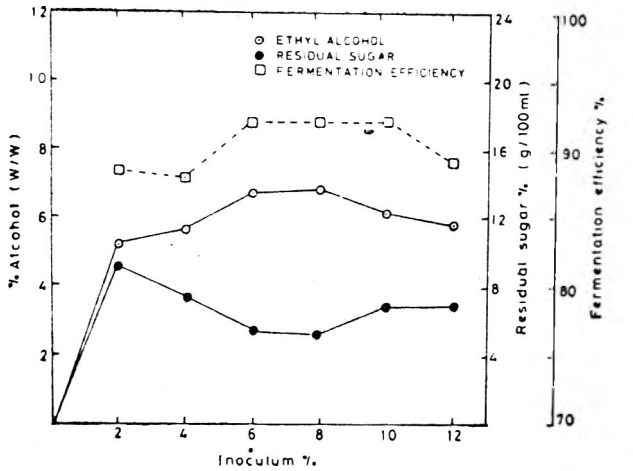

Fig. 3. Influence of size of inoculum on the amount of alcohol production and sugar consumption in the fermentation of Rain tree kernels water extract for 72 hr at 20% sugar level and pH 4.5.

TABLE 3. EFFECT OF AMMONIUM SULPHATE ON THE AMOUNT OF ETHYL ALCOHOL PRODUCTION IN THE WATER EXTRACT OF RAIN TREE KERNELS*

$\%(NH_4)_2SO_4$	Fermentation period (hr.)	% ethyl alcohol (g. alcohol/w/w)
0.5	36	7.9
1	36	8.1
Control	36	5.5
0.5	72	8.8
1	72	8.5
Control	72	7.8

*Sugar, 20%; pH, 4.5; Inoculum, 8%; Volume, 700 ml.

phoresis. A clear cut separation of proline and two unidentified amino acids having different R_f values was observed on two dimensional paper chromatogram. One of these unknown amino acids, that followed proline peak in the amino acid analyser was found to occur in very large quantities among the free amino acid pool of the alcoholic extract of Rain tree kernels. Only L-proline, L-leucine, L-valine and asparagine were reported in the mesocarp of *Pithecolobium dulce*[5]. Among the organic acids, malic, citric and tartaric acids were present in the water extract of Rain tree kernels.

Preliminary experiments were done to determine optimal conditions for maximum alcohol yield in order to obtain highest fermentation efficiency. The maximum amounts of ethyl alcohol reached at 20 per cent sugar concentration (Fig. 1) whereas at higher and lower levels the alcohol yield decreased significantly. This is in general agreement with the observations of Al-Talibi *et al.*[17] who reported 25-30 per cent sugar concentration of Iraqui dates as an optimum range for ethyl alcohol production by *Saccharomyces cerevisiae*. The yield of alcohol from sugar in Rain tree kernels was found to vary during the course of fermentation the optimum being between 48 and 72 hr (Fig. 2). Hodge and Hildebrandt[18] also reported similar observations with black strap molasses of Puerto Rico and Java. An inoculum size of 6-8 per cent (Fig. 3) in the wort yielded maximum production of ethyl aocohol. It was also noted that the addition of different supplements such as ammonium sulphate and potassium dihydrogen phosphate in the wort did not improve the fermentation except for the former (Table 3) which shortened the period of maximum alcohol production. Further investigation on the method of preparing the substrate for fermentation of large batches showed that even crushed fruits or kernels gave the same amount of alcohol as the water extract (data not included). These observations point out that the fruits need only crushing and steaming to be ready for fermentation. Further in terms of alcohol yield, it is significant to note that an efficiency of 90-93 per cent is recorded at a level of 20 per cent sugar.

It may be concluded from these results that the water extract of Rain tree kernels could be directly used for the production of ethanol without any supplementation with either the phosphates or nitrogenous compounds. These results further show that the kernels can be an ideal substrate for alcohol production on a commercial scale.

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STUDIES ON AFLATOXIN CONTENT OF GROUNDNUT OIL IN ANDHRA PRADESH WITH REFERENCE TO CLIMATIC CONDITIONS AND SEASONAL VARIATIONS

Andhra Pradesh, being the largest producer of groundnut and a large consumer of the raw oil a survey was undertaken to find out the extent of aflatoxin contamination of the oil in different regions during different seasons. The overall toxin content of the oil varied from 0 to 5000 ppb. The samples from dry Rayalaseema area showed low toxin content, while that from humid coastal area showed large variation in their toxin content. Certain samples collected from dry regions of Telangana contained very high toxin which is due to the use of low grade seeds for oil extraction after separating by hand picking.

Groundnut oil—raw or refined is a major cooking medium in Andhra Pradesh. Average daily consumption of this oil in a middle class family is about 30-40 ml or 1 kg/adult/month.[1] The non-refined oil being cheap and largely available is used as a major cooking oil. It has been observed earlier[2] that considerable quantities of aflatoxin percolates during extraction of oil in the expellers. Further, it has also been reported[3] that some commercial samples contained very high amounts of aflatoxin (0.1 to 2.6 ppm). The toxin being heat stable, significant quantities are being carried over to the food materials in deep fat frying. Food materials fried in unrefined oils therefore have become an important source of aflatoxin toxicity.

Andhra Pradesh annually produces about 1 million tonnes of groundnut[4]. This crop is taken both in kharif and rabi seasons besides in summer wherever irrigation facilities are available. This survey was undertaken to collect information regarding the incidence of aflatoxin contamination in groundnut oils produced in different seasons in Andhra Pradesh.

Andhra Pradesh is divided into three distinct regions based on the climatic and economic considerations[5].

(*i*) *Coastal region:* Srikakulam, Visakhapatnam, East Godavari, West Godavari, Krishna, Guntur, Nellore and Prakasham districts constitute the coastal region. It receives both South West and North East monsoons with an average rainfall of 995 mm and very high humidity.

(*ii*) *Rayalaseema region:* This comprises four districts-Anantapur, Cuddapah, Kurnool and Chittor. The rainfall is about 670 mm. On an average there are only 42 wet days, in a year and the weather is generally dry.

(*iii*) *Telangana region:* Telangana comprises of nine districts—Hyderabad, Adilabad, Nizamabad, Medak, Karimnagar, Warangal, Khammam, Nalgonda and Mahbubnagar. Most of the area gets good rain from the South West and North West monsoon with an average rainfall of 894 mm. The weather is generally dry except for the four monsoon months.

The rainfall received in each district of the State is given in Table 1.

Crude groundnut oil samples were collected from the retail shops of the important towns covering the three regions of the State. It is presumed that the samples collected represent the commercially extracted oil in that particular season. Twenty oil samples were collected from 6-8 important towns, located in each region. The towns are located at not less than 20 miles apart; samples were collected both in kharif and rabi seasons. Samples from all the three regions were collected during October 1974—December 1975. The samples were assayed for aflatoxin as per the dilution to extinction method[6,7].

The regional and seasonal variations in the aflatoxin content of groundnut oils collected are given in Table 2. It is evident from the Table that some samples contained very high amount of toxin. On an average 44.2 per cent

TABLE 1. NORMAL RAINFALL (mm) IN DIFFERENT DISTRICTS OF ANDHRA PRADESH

Months	Coastal region	Rayalaseema	Telangana	State Av
June	102.0	58.0	130.6	105.9
July	152.0	79.6	227.9	170.7
August	147.1	95.4	177.7	150.4
September	162.5	136.0	183.3	165.4
Total	563.6	369.0	719.5	593.4
October	184.3	110.5	67.0	120.0
November	116.2	80.0	25.1	70.3
December	22.1	20.5	4.1	14.1
Total	322.6	211.0	96.2	204.4
January	10.3	9.1	4.5	7.6
February	13.0	5.5	14.1	12.0
Total	23.3	14.6	18.6	19.6
March	12.0	6.7	11.5	10.8
April	24.3	19.2	23.1	22.8
May	49.9	49.9	25.1	39.3
Total	86.2	75.8	59.7	72.9
Grand total	995.7	670.4	894.0	890.3

South West monsoon : June–September
North East monsoon : October–December
Winter period : January–February
Hot weather period : March–May

TABLE 2. REGIONAL AND SEASONAL VARIATIONS IN THE AFLATOXIN CONTENT OF GROUDNUT OIL DURING 1974–75

Region	Season	Samples analysed (nos)	Samples without toxin (nos)	%	Samples showing toxin <100 ppb (nos)	%	>100 ppb (nos)	%	Max. toxin content (ppb)
Rayalaseema	Kharif (Sept. '74)	75	58	77.4	15	20	2	2.6	170
	Rabi (Jan '75)	100	85	85.0	10	10	4	4	670
	Rabi (July '75)	100	89	89.0	11	11	1	1	330
	Total	275	232	84.6	36	13.1	7	2.6	670
Telangana	Kharif (Oct. '74)	140	51	36.4	49	35.0	40	28.6	670
	Rabi (Mar. '75)	140	85	60.7	15	10.7	40	28.6	670
	Rabi (Sept. '75)	140	88	63.8	31	22.1	21	15.0	670
	Total	420	224	53.3	95	22.6	101	24.1	670
Coastal	Kharif (Dec. '74)	154	47	30.5	28	18.1	79	51.3	1000
	Kharif (Dec. '75)	180	37	20.6	29	16.1	114	63.3	5000
	Rabi (May '75)	180	126	70.0	44	24.4	10	5.6	170
	Total	514	210	40.9	101	19.7	203	39.5	3000
Grand total		1209	666	55.1	232	19.2	311	25.7	—

The months mentioned indicate the time of collection of samples.

samples contained toxin, and in this 25.7 per cent contained more than 100 ppb (0.1 ppm) of toxin. A few samples collected from kharif crop from coastal district contained as much as 5000 ppb (5 ppm) of aflatoxin.

When the nuts could not be dried properly after harvest due to humid conditions, they offer favourable condition for the growth of the fungus[8]. Incessant rain and humid weather, is common in coastal districts and in Telangana, when the rainfed groundnut crop comes to harvest. The results have shown that 59 per cent of the samples collected from coastal districts indicated contamination with the toxin, out of which 39.5 per cent of the samples contained more than 100 ppb of toxin. In Telangana area, over 46 per cent of the total samples had toxin, out of which 24 per cent had more than 100 ppb of toxin. The Rayalaseema being a dry area, showed a low percentage of toxin contaminated samples (15 per cent), out of which only 2.6 per cent had more than 100 ppb of toxin.

Effect of seasonal variations: Groundnut is cultivated mostly as a rainfed kharif crop, being sown from April-May to June-July depending on the receipt of monsoon rains. In some areas it is sown as late as August or early September (Rabi). Irrigated crop is grown to a limited extent either in January-March or May-July (hot weather).

The groundnut crop harvested in kharif season is found to be associated with high percentage of toxin (about 65 per cent) in their oil samples. This may be due to the prevailing North-East monsoon during the harvest period followed by improper drying of the pods. Although the rabi crop has shown less number of samples containing toxin (28 per cent), but still considerable amount of toxin was present which was above the safe limits.

It is observed that in some places of coastal districts and also in Telangana area, the samples have indicated high content of aflatoxin upto 5000 ppb. On enquiry it was found that good seeds are being separated and used as HPS for export and the remaining inferior grade seeds are being used for oil extraction. This results in high toxin content in the extracted oil.

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FRACTIONATION OF PALM OIL

Palm oil has been fractionated with and without solvent. In conventional process the yield of the liquid fraction is low. In solvent fractionation the variables studied were miscella concentration with solvent hexane and acetone at different temperatures. The yield of the liquid fraction in hexane fractionation was 90% with a cloud point of 8°C. A concentration of 1:1.5 miscella (oil to hexane ratio) 3 to 5 hr crystallization time and a temperature of 5°C were found to be the optimum conditions for fractionation of palm oil. Also, hexane was found to be a better solvent than acetone for the preparation of salad oil from palm oil.

To-day palm oil is one of the important sources of fat for the world. In India, palm oil is mainly imported and mostly utilized in vanaspati industries after controlled hydrogenation. Also, palm oil contains 43 per cent disaturated glycerides[1], the saturated fatty acid being essentially palmitic acid. Therefore, if this disaturated glyceride fraction could be obtained in a concentrated form, such a product (cocoa butter substitute) should have properties desired in a number of food industries like confectionaries. In an attempt to make such a product from palm oil, preliminary studies were made in the laboratory on fractionation of palm oil.

Considerable amount of studies has been made in abroad on the fractionation of palm oil which involves different technological processes like conventional winterization, winterization by solvents and fractionation by surface active agents[2-5]. Their studies have indicated low yield of liquid fraction in the conventional winterization and very high output of fluid oil in solvent fractionation. In conventional winterization, under most favourable operating conditions, the fractionable output generally exceeds 50 per cent and in our studeis it was about 60 per cent. By using special crystallization methods with hexane solution of the oil, Bernardini and Bernardini[3] have obtained the yield of liquid and solid fractions as 80 and 20 per cent respectively. Bailey[4] has given a typical time temperature sequence for winterization of fatty materials. Detergent fractionation of fatty materials was described by Fratelli Lanza[6]. Hartman and Stein[5] and Stein[7] have shown that the separation of the suspension of crystals in the water phase from the liquid fatty acids can be industrially carried out by means of centrifugal separators and that any other detergents such as lauryl sulphate could be used. Ben Biae[8] reported that the results obtained by detergent fractionation of palm oil can be compared to those obtained by acetone fractionation. He stated that a good yield of an olein with high iodine value (IV. 65) can be obtained by re-fractionating the olein from the first pass. The yield of olein in each pass is about 80 per cent and the increase in iodine value is about 5 units for each pass.

Fractionation of palm oil: The fractionation experiments of both wet and dry processes were done in an ice box surrounded by a thermocool insulation. Two chambers were provided in the ice box. One chamber has been used for crystallization and the other for separating the fractions through suction filtration. Both the chambers were covered with ice. The solution to be fractionated has been slowly chilled to the desired temperature and with occasional stirring by a glass rod. After keeping the oil/miscella for a prescribed period at the desired temperature, the fractions were separated by suction filtration. Filtrate has been collected in a suction flask. In solvent fractionation, the solid fraction was washed with pre-cooled solvent (5°C). The solvent in both the solid and liquid fractions were distilled off. After removing the solvent by vacuum distillation, the yields of the solid and liquid fractions were estimated.

In conventional winterization, neutralized, bleached and dried palm oil has been slowly chilled from 40 to 18°C in a period of 18 hr. The fractions were separated at that temperature through vacuum filtration.

In solvent winterization, the variables studied were, miscella concentration with two solvents, (hexane and acetone), and at different temperatures. The concentrations of the miscella studied were oil to hexane ratio of 1:1, 1:1.5, 1:2, 1:2.5 and 1:3 (w/v.) Refined, bleached and dried palm oil (500 g) (mp. 38°C and I.V., 55) after mixing with the respective solvents was slowly chilled from 40 to 5°C in 3 hr. The fractions were separated at 3°C under vacuum. The conditions of winterization, the percentage yields and characteristics of the fractions like iodine value and cloud point which were determined according to the AOCS official methods are given in Tables 1, 2 and 3.

TABLE 1. ANALYSIS OF THE WINTERIZED PALM OIL FRACTIONS

Winterization method	Yield of fractions Liquid (%)	Soild (%)	I V of fractions Liquid	Solid	Cloud point(°C)
A. Non-solvent winterization					
Slowly chilled to 18-20°C in 6 hr	64.0	36.0	63.0	18.8	12
and temp. maintained for 12 hr.	60.0	40.0	61.0	19.3	12
B. Solvent winterization*					
Fractionated at 5°C for 3 hr.	90.5	9.5	63.5	18.8	9
Fractionated at 10°C for 3 hr.	92.0	8.0	63.7	18.0	8

*Oil to hexane ratio is 1:1 (W/V)

TABLE 2. SOLVENT WINTERIZATION OF PALM OIL

Fractionation Temp (°C)	Time (hr)	Yield of fractions Liquid (%)	Solid (%)	I V of fractions Liquid	Solid	Cloud point °C
5	4	90.0	10.0	63.4	18.9	8
10	5	91.0	9.0	61.2	19.2	12
15	5	92.0	8.0	60.0	19.0	14
20	5	93.0	7.0	57.3	20.0	17

Oil to hexane ratio is 1:1.

TABLE 3. SOLVENT WINTERIZATION OF PALM OIL

Oil to solvent ratio (W/V)	Yields of fractions Liquid (%)	Solid (%)	I V of fractions Liquid	Solid	Cloud point °C
1:1.5*	90.0	10.0	63.3	18.8	8
1:2.0*	90.0	10.0	63.6	18.9	8
1:2.5*	90.0	10.0	63.4	18.4	8
1:3.0*	90.0	10.0	63.6	18.8	8
1:1.0**	80.0	20.0	58.5	24.5	14
1:2.0**	78.0	22.0	58.2	22.2	12
1:3.0**	80.0	20.0	62.8	19.5	8
1:5.0**	82.0	18.0	63.6	19.7	8

*Hexane as solvent.

**Acetone as solvent.

In non-solvent winterization, the yield of the liquid fraction varied from 60 to 64 per cent with an average of 62 per cent (Table 1). The iodine values of liquid fractions were in between 61 and 63. The yield of solid fraction varied from 36 to 40 per cent with an average value of 38 per cent. The cloud point of the liquid fraction was 12°C.

In solvent fractionation, (Table 1B) winterization at 5°C for 3 to 5 hr has given optimum yields of liquid fraction with satisfactory cloud point of 8°C and no advantage has been noticed when the temperature of cooling was raised to 10–20°C (Table 2). When hexane was used as solvent, 1:1 or 1:1.5 (wt/vol) has been found to be optimum miscella concentration. In solvent winterization the yield of the liquid fraction was 90 per cent. It is much greater than that obtained by non-solvent winterization (about 62 per cent). The time required for crystallization in solvent fractionation has been much less, being about 3 hr than for non-solvent winterization (18 hr). Liquid fractions obtained by solvent winterization have considerably lower cloud points (8 to 9°C) than in non-solvent winterization (12°C).

When acetone was used as solvent in the ratio of 1:2 or 1:3, optimum results were obtained. The yields of the liquid fractions were about 78 to 80 per cent while the solid fractions were 20 to 22 per cent (Table 3). In salad oil preparation, solvent hexane is perferable to acetone, because yields of liquid fraction are higher.

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EVALUATION OF SYNTHETIC JUVENILE HORMONE ANALOGUES FOR THE CONTROL OF RICE MOTH *CORCYRA CEPHALONICA* STAINTON.

The population build up of rice moth, *Corcyra cephalonica* Stainton., in *Sorghum vulgare* was effectively suppressed by treating the grains with the JH analogue, 'Altozar' at a dose level of 2 ppm. A higher dose level of 10 ppm 'Altosid' was required to bring about the same effect.

Juvenile hormone analogues (JHA), which do not have any toxic effect on insects or on other organisms[1] are ideally suited for the control of stored product insect pests and the availability of a number of synthetic JHAs has led to many evaluation studies on some of these pests[2-4]. Strong and Diekman[5] had evaluated 15 JHAs against 12 species of stored grain pests and among the compounds tested, the dienoates, 'Altozar' (ethyl 3, 4, 11-trimethyl-2, 4-dodecadienoate) and 'Altosid' (isopropyl 11-methoxy-3, 7, 11-trimethyl-2, 4-dodecadienoate) were found to be most effective in controlling all these pests. Present communication relates to the evaluation of these two compounds against rice moth, *Corcyra cephalonica* Stainton., a serious Lepidopterous pest of stored products in the tropics.

Insects were grown on broken *Sorghum vulgare* grains at 27°C and 70±5 per cent R.H. Required amounts of 'Altozar' and 'Altosid' (a gift from Zoecon Corp., USA) were dissolved in acetone and thoroughly mixed

TABLE 1. NUMBER OF *Corcy a cephalonica* ADULTS EMERGED FROM THE MEDIUM TREATED WITH VARIOUS CONCENTRATIONS OF JH ANALOGUES

Compound	Treatment concentrations (ppm)				
	10	5	2	1	0
Altosid	0	69 (18)	215 (56)	353 (92)	384 (100)
Altozar	0	0	0	138 (36)	

*The values in the brackets are percent adult emergence corrected to control.

with 1 kg of broken sorghum grains to give a final concentration of 10, 5, 2, and 1 ppm (W/W). For rearing the control insects grains were treated with acetone without juvenile hormone analogues. After evaporating the acetone, the treated media were transferred to beakers containing 0-24 hr old eggs. Forty days after the eggs were introduced, adult emergence was scored every alternate day and the experiment was terminated at the end of 75 days. Each treatment was replicated 3 times with 200 eggs per replicate. Taking the total number of adult emergence in all the three replicates of control as 100 per cent the per cent emergence of insects in treated samples was computed.

Juvenile hormone analogue, 'Altozar' completely inhibited adult emergence at a concentration of 2 ppm; and even at 1 ppm, the inhibitory effect was discernible. However, 'Altosid' was effective only at 10 ppm in completely suppressing the adult emergence (Table 1). The dose level of 'Altozar' required to control *C. cephalonica* is less than the reported amount required in the treated medium for the suppression of the population build up of other Lepidopterous stored product pests, *Cadra cautella* (Walker)[5], *Poldia interpunctella* (Hubner)[5] and *Anagsta kuehniella* (Zeller)[4].

Examination of 'Altozar' and 'Altosid' treated media at the end of 75 days revealed the presence of few larvae and pupae, the latter being invariably deformed and dead. The surviving larvae from the 2 ppm Altozar treated medium were transferred to fresh untreated medium to test whether they could undergo normal metamorphosis. Thirty eight per cent of the transferred larvae developed into normal adults and the eggs laid by these moths showed normal viability. This observation stresses the need for the continued presence of JHA in the treated medium until the pest population is completely eliminated. Similar observations were also reported in the case of *Trogoderma granarium* Everts[6] and *Anagasta kuehniella* (Zeller).[4]

To ascertain the period for which the JHA treatment would be effective against infestation, grain samples were treated with 'Altozar' at a dose level of 5 ppm and stored for different periods after which the potency of this compound was tested by releasing the eggs of rice moth. Treated medium stored upto a period of 6 months completely inhibited development of rice moth. Only 6 per cent of the insects completed their life cycle in the treated medium stored for 9 months. These results indicate that the juvenile hormone analogue, 'Altozar' persists in the treated medium in sufficient quantities and can effectively prevent fresh infestation of rice moth, at least for a period of 6 months.

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BOOK REVIEWS

Catastrophe or New Society? A Latin American World Model: By A.O. Herrera and others, International Development Research Centre, P.O. Box 8500, Ottawa, Canada K1G3H9; 1976; pp: 108; price: $ 5 in Canada, Europe, USA and Australia; Microfiche edition: $ 1.

The dire predictions of the Club of Rome in its famous book *Limits to Growth* were based on the fact that human society, through exponential increases in consumption and population and growing pollution, was moving towards a self-destructive ecological catastrophe. Such outstripping of the physical and material limits to growth had raised serious world-wide concern, yet many have privately wondered whether doomsday was indeed closing in upon us, and whether man could not avert disaster. Interestingly enough the present book also emerged from a meeting sponsored by the Club of Rome to discuss the earlier study. The new book was prepared by 10 Latin American scientists led by Amilcar Herrera as Director, and as its title implies it offers a new direction to human society based on mathematical models resolved using a computer. The conclusion is squarely that the fate of man depends in the last instance not on insurmountable material limitations, but on social and political barriers that man himself must modify. A span of just one generation is computed to be sufficient to achieve the human values laid down.

And what are these values? After eliminating numerous candidate criteria, the three values adopted are equality, participation and non-consumption, and these arise in turn from four fundamental needs which are the basis of the study. The first is nutrition, defined as 3000 calories and 100 grams of protein a day. By Asian standards these may seem outrageously high figures, but they were adopted when it was found that varying the calorie and protein needs for each geographic area made little difference to the results obtained. The next fundamental need is housing, defined as 50 square metres of shelter with adequate sanitation for a family of five. The third is education and a new view is taken than education should be defined as means of helping people participate in their society and its evolution; on this reckoning minimum basic education should involve 12 years of schooling for all from the age of six, with continued special schooling only for certain population groups. The fourth and last need is health and this has been linked with demography; the criterion used is the achievement of a high life expectancy at birth, and this is believed to be a composite index sensitive to numerous other features of the total environment.

Several other features evolved as the model took shape. Thus population increase is seen not as the cause but as the result of poor life expectancy, so that a high birth rate in underdeveloped countries becomes inevitable; as basic conditions of life improve a self-corrective mechanism will depress the birth rate. Again the basic philosophy is radically different from the one adopted in the first Club of Rome study. What is now predicated is a production system that is determined not by consumption needs and by profit, but by social needs. And consumption is not viewed as an end in itself, private ownership of the means of production becomes replaced by concepts of the use and management of these means. The political system that prevails is not particularly relevant according to this definition, and the main function of the system itself is to allot resources to each sector so as to maximise its return. Again the single criterion of this return adopted for the mathematical operation is that the highest life expectancy is operative at birth.

The model concentrates on third world countries. It concludes that these cannot follow the path already traversed by the developed countries. They must base their efforts on the creation of a society which is intrinsically compatible with the environment. To achieve early results a redistribution of income from the present glaring inequalities must be brought about. The model shows that when this is done, one generation is sufficient to achieve the targets laid down. Without such income redistribution, as many as two generations more may well be required.

The model itself is based on a production system aimed solely at satisfaction of basic human needs. The world is divided into five regions based on 8 socio-economic indicators. Two of these incidentally are per capita calories per day and per capita proteins per day. The accuracy of the model was first checked by feeding into it the statistical data available for 1960, and checking the results which emerged for 1970 against actual data. The excellent tally suggested that the model was reliable.

The chapter on food is the most complex, much more so than those on health, housing and education and a brief resume will serve to indicate the style of the whole study. The basic question set was whether it will be possible to feed everybody in future and how this should be done. The total system was divided into three sub-sectors, agriculture, livestock and fisheries. Factors which influence each of these were thoroughly identified and developed in the form of flowchart of inter-relationships. Thus for the agriculture sub-system alone 21

factors are identified. Even after each sub-system had been developed, eventual optimisation of the total system employed as many as 39 inter-relating factors which covered population, labour force, capital, inputs, land areas, calories and proteins, food processing losses and so on.

The conclusions that emerged from the computer are different for each of the five global regions. Predictably, developed countries satisfy their needs in the first few years of the run of the mathematical model. For the third world countries it is shown that technical development is essential if they are to be liberated from underdevelopment and misery. But such development should be of a type different from that in the past, which demonstrably succeeded only in widening the gaps between groups. The model reveals that relatively higher inputs for education and housing will be required in underdeveloped countries.

A few side-issues are also of interest. External aid is shown to be useful only if conditions of social equality prevail in the recipient countries; otherwise it assists only the already-wealthy segments. The best form of aid which affluent nations can give would be to fix fair prices for the products which they import from the underdeveloped countries. Non-conventional foods like algae, bacteria, novel proteins and the like are shown by the model to be unnecessary in the foreseeable future.

A critical apparaisal of the study would appear at first sight to be a formidable task. Yet what one has really to accept are the four fundamental predicates, which are so simple as to seem unexceptionable. Once this is done the mathematical logic is probably inevitable, wherever it may lead. Written in simple, straight forward language that is formal and yet lit with passionate social concern, the 100-odd pages of this volume should be required reading for policy-makers in rich and poor countries alike. One may argue that the social and political philosophy of sharing that it espouses will never be adopted on a voluntary basis. The very fact that such a philosophy is expressed with so much practical logic should give it greater thrust. The title sets the choice bluntly; is it to be catastrophe or a new humanity?

K. T. ACHAYA
PROTEIN FOODS ASSOCIATION, BOMBAY.

Cooling Technology in the Food Industry: by Aurel Ciobanu, Gabriela Lascu, Vasile Bercescu, Lidia Niculescu, Laboratory of Refrigeration, Research Institute for the Food Industry and Food Chemistry, Bucharest Abacus Press, Abacus House, Speldhurst Road, Tunbridge, Kent, England, 1976.

This first publication by Abacus Press in English is much needed by all those engaged in cold chains of food products.

The second chapter in Part I "Effects of Low Temperatures on Foods" deals in detail with the main physical and physico-chemical changes in food systems, biochemical changes in living fruits and vegetables, post-mortem biochemical, physico-chemical and physical changes in muscular tissues, chemical and biochemical changes in food fats, nutritional changes in refrigerated foods, and thermal properties of food.

The second part "General Systems Applied in Food Refrigeration" contains the chapters on chilling, freezing, air-conditioning, additional treatments to refrigeration and freeze-drying.

The authors have put in their maximum efforts in referring maximum number of books in furnishing the technical details. The chapter on chilling contains preparatory treatments, chilling down processes, storage of chilled foods, and tempering.

The fourth chapter 'Freezing' in detail deals with the preparatory operations and treatment, freezing process, thermal calculations, packaging, storage and thawing of frozen foods.

The next chapter is about air-conditioning, which is needed most by all the personnel engaged in cold chain. This Chapter deals with psychrometrics, control of air humidity in food refrigeration, mass loss of the products and odour controls.

The following chapter is about modified atmospheres used for fruit, vegetable and animal products preservation. This also deals with vacuum packaging, cehydrofreezing, thermal inactivation of enzymes, chemical treatments, antibiotic treatments and irradiation. The freeze-drying chapter gives a special emphasis for the quality obtained by the product through the above process when compared to other processes. The Eighth & Ninth Chapters—'Meat and Meat Products'—deal with the processing of meat products and different methods of storing them and their comparative merits. This also deals with the slaughterhouse by-products and meat products quality control.

Tenth Chapter contains the necessary handling, preservation by different methods and quality control of fish and fish products. In the next three chapters, milk, milk products, eggs, fruits and vegetables have been considered. Here the author explains clearly as to how the quality of the products vary under various storage conditions and disorders encountered.

Later the author has discussed critically the specific requirements of refrigeration for ice cream not only for storage and distribution but also for hardening ice

cream. The chapter "Prepared Foods" contains the summarised general methods of processing, preservation and quality control of the food products.

Also the author has furnished sufficient information on the requirements of refrigeration for beer industry with a specific emphasis to Must and Bakery.

Ultimately the importance of cold chain in food industry has been presented.

The last chapter includes the practical aspects of Industrial Cold Storages, viz., capacity, building systems, space allotment, handling, stacking, etc. The storage conditions and transport conditions for different products are indicated.

The Book "Cooling Technology in the Food Industry" is an essential tool for all engaged in the cold chains of food products, research engineers/technologists, students and teachers. I recommend this as a good addition to libraries.

M. RAMESH BABU
C F T R I, MYSORE.

Principles of Food Science—Part I: Food Chemistry: edited by Owen R. Fennema, Marcel Dekker Inc, New York and Basel, 1976, pp. 792.

This is one of the volumes of a series of monographs on food science edited by Dr. Owen R. Fennema of the Department of Food Science, University of Wisconsin (Madison), Wisconsin. This particular volume deals with various aspects of food chemistry written by various accredited workers and is organised into 17 chapters ranging from an introductory chapter on the history of food chemistry; water and ice and their relation to foods; chemistry and functional properties of carbohydrates, lipids, amino acids, peptides and proteins, vitamins and minerals; pigments; flavours; desirable or undesirable constituents of food; enzymes; characteristics of a few typical food products as milk, eggs, edible plant tissue and muscle tissue, and finally a short chapter on integration of chemical and biological changes in foods and their influence on quality.

In the chapter on water and ice, Dr. Fennema deals with the basic concepts of water activity as the most important controlling factor in the preservation of food against chemical, enzymic and microbiological deterioration. Further he has given an excellent treatment on the nature of bound water, and relationship between water activity and the moisture contents of various food materials at different temperatures. In conclusion, Dr. Fennema has said that water certainly contributes greatly to the desirable native qualities of food and associated with non-aqeous food constituents in such a complex manner that when these relationships are disturbed by some such means as drying or freezing they can never again be completely reinstated and therefor he has urged more study of this frustrating and poorly understood complex.

The chapters on carbohydrates, lipids, amino acids, peptides and proteins, vitamins and minerals deal with general chemistry, structure, nomenclature, chemical reactions and their functional properties and the changes undergone in these components of food under various processing conditions including processing by ionising radiation. The chapter on proteins also describes chemistry, distribution, amounts and functions of the protein in various foods—both of animal and plant origin—and also some unconventional protein sources such as single cell protein—unicellular algae, bacteria, yeasts—leaves and fish protein concentrates. Lastly, for each of these dietary constituents, nutritional attributes and human requirements and various factors influencing these have been discussed.

In the chapter on pigments, FM Clydesdale and FJ Francis have given a very authoritative treatment on colouring materials present in various plant and animal tissues and their chemistry, significance in fresh and processed food stuffs and the changes undergone by them on various food handling, processing and storage conditions. The section on the pigments present in meat, viz., myoglobin and haemoglobin, has been extremely well written and the various chemical, physical and biochemical reactions undergone in these pigments on processing such as cooking and curing have been well documented. Though significance of the presence of plant pigments, e.g., chlorophyll and anthocyanin, in foods have not been extensively investigated, nevertheless, the importance of flavonol aglycones, quercetin, kaempfarol and myricetin, which are found in considerable amounts in instant tea powders where they contribute to astringency have been well studied. A detailed description has also been made in this chapter on chemistry and food uses of leucoanthocyanins, tannins, betalains, quinones, xanthones, carotenoids and synthetic colours.

Before concluding this chapter, the authors have raised the interesting theoretical possibility of utilising more fully the inherent optical characteristics of natural pigment and unquestionally other synthetic colourants for use in foods, by taking advantage of certain physical phenomena which occurs when light strikes an object or internal diffusion of light which is dependent on the randomly oriented interfaces between materials of different indices. They hope that a start in this direction can be made by controlling the particular size and the refractive index of 'lakes' which are made by absorbing

the pigments of substrates such as alumina hydrate which produces an insoluble pigment which can be finely ground in order to achieve this possibility, in different media and maximum colour intensity. Lastly, the authors hope that this will stimulate thinking concerning new ways of utilising existing and new food colourants to maximum advantage.

In the chapter on flavours, the authors RM Pangborn and GF Russel have sub divided the whole approach of research on food flavour into two main component parts, viz., (1) flavour chemistry including analysis of volatile constituents primarily by separation of components by gas liquid chromatography and subsequent identification by spectroscopic and other methods and (2) sensory analysis—use of human subjects to quantify perceptual experiences emphasising quality evaluation, i.e., examination of the flavour quality of specific products by experienced judges like expert wine taster, tea taster or perfurmers. They have also indicated areas of fundamental research in three major fields (1) physiological, (2) molecular and (3) behavioural aspects.

Synthetic flavours occupy an important place in food industry and have generated a considerable amount of legislation to regulate the use of a great number of FDA listed flavour substances many of which are synthetic in an easily assimilar form the results of extensive research into isolating the chemical components that are the components of flavour formulation in foods. The process by which flavours are generated and methods of stabilisation to minimise physical and chemical changes during storage are selectively abstracted. Types of flavours covered include fruit and vegetable flavours, bread, dairy and meat products. The various delivery systems, as well as flavour potentiators or modifiers have been described and the physiological aspects pertaining to sensory perception and the interaction of the various flavour chemicals with that of taste buds, olefactory sensitivity and receptor function and theories of olefaction have been well characterised.

In the chapter on desirable constituents of food RC Lindsay gives an account of the various chemicals which are used as chelating agents, stabilisers and thickeners, polyhydric alcohols, anticaking, clarifying agent, flour bleacher and bread improver, antioxidants, artificial sweeteners and a number of anti-microbial agents which are used in the various food systems. The mode of action of these chemicals, their level at which these are to be used and the various food materials in which these can be used have been brought out in this paragraph. In the chapter on undesirable or potentially undesirable constituents of food, GN Wogen listed various toxic constituents of applied food stuffs, their chemical nature, the main food source and their major toxicity. Particular emphasis has been given in this chapter on the use of nitrites and nitroso compounds as curing agents for meat and meat products and the hazards of microbial growth resulting in formation of mycotoxins in human food stuffs. Attention has been drawn to the need of evaluation of control measures with a view to ensure the safety of the food materials which are recommended for human use and which would be possible only by multidisciplinary research efforts involving the joint participation of chemist, biologist, microbiologist, toxicologist, and epidemiologist.

WD Powrie and MA Tung in the chapter on food dispersion have given the basic physico-chemical concepts of the formation and stability of various food dispersion systems including food sols, gels, emulsions and forms. The role played by various food components and synthetic chemicals to form food emulsions and dispersion systems as exist in natural and a few food products have been well brought out and their application in solving industrial problems have also been suggested. Lastly methods of preventing undesirable foams during the manufacture of many food products, viz., concentrated fruit juices, coffee extracts, vegetable oils etc., have also been described.

Characteristics of a few typical food of animal and vegetable origin such as milk, egg, edible plant and muscle tissue have been described in four chapters of this book and a detailed discussion has been made on the chemical, physical and biological problems pertaining to stability of each category of foodstuffs. Apart from chemical composition, structure, physiology and technological aspects pertaining to these products, the future avenue of research and development in some of these products have also been indicated. Intensive studies have been suggested in the field of plant tissue cultures, suitable product flavours, nutrients, pigments and other food additives with a view to supplement the food availability which is bound to diminish under pressure of population.

The last chapter in this volume is on the integration of chemical and biological changes in foods and their influence on quality by SR Tannenbaum in which causes of deterioration of a few food products under various conditions of treatment such as oxidation of lipids leading to further changes in proteins, bleaching of carotenoid pigments and enzymic as well as non-enzymic browning have been listed and the various factors such as sex, age, season of catch and harvesting type of food in relation to these causes of deterioration listed. This particular chapter is more or less an analytical approach as applied to spoilage of specific food commodity which have reference to discussions in the previous 16 chapters of the book.

In conclusion, the reviewer feels that the present volume Food Chemistry, Part I of the series on Principles of Food Science, fills a long standing need for a text book in food chemistry that is suitable for students with the background in organic chemistry and biochemistry as well as teachers of food science and those who have been initiated in research in this subject. It also serves as an excellent reference source for scientists involved in food research, food product development, quality control and food processing as well as for nutritionists, biologists and biochemists. The book is free from printing errors and the diagrams and figures have been excellently presented. However, an important omission in the book is discussion on one of the major food crop, cereals like wheat, rice and millets, e.g., triticale, sorghum etc. It is hoped that information on these classes of foodstuffs which are of great importance to the develpoing countries will be furnished in one of the next volumes of the series.

H. NATH
D F R L, MYSORE.

The Journal of Environmental Sciences—Part B., Executive editor, J. G. Shah, published by M/s Marcel Dekker Inc., Quarterly Subscription: US £ 35.

Environmental Science and Health Part B, which covers Pesticides, Food Contaminants and Agricultural Wastes, is born out of Environmental Letters published by M/s Marcel Dekker Inc. It is heartening to note that contributions in this Journal are published free of charge and are evaluated and scrutinised by a strong Editorial Board consisting of eminent persons working on Environmental Science. The Executive Editor Dr. J. G. Shah and Associate Editor Dr. S. U. Khan of the Chemistry and Biology Research Institute, Research Branch, Canada Agriculture Ottawa (Ontario), Canada are wellknown for their contributions in the area.

The new Journal is devoted to all aspects of pesticides, food contaminants and agricultural wastes. Most of these involve inter-disciplinary investigations. The articles have appeared in diverse journals over the past two decades. The present journal would give an opportunity to provide an outlet for original research reports on pesticide residues and food contaminants, natural and additives and their metabolites in the ecosphere. Original factual reports on the persistence, binding, translocation, chemical and biodegradation, metabolic fate of the chemicals, their factual contamination of the biosphere, methods of detoxification and the improvements in the analytical techniques are to be included freely in the Journal.

The present issue of the Journal under review contains articles on Radiobiochemistry of Phytodrugs from Italy; Metabolism of 0, 0-dimethyl 1-01(3, 5, 6-trichloro-2-pyridyl) phosphorothioate in sheep and rats and of 3, 5, 6-trichloro-2-pyridinol in Sheep from USA; Contributions to Ecological Chemistry CVII. Fate of Lindane-14C in lettuce, Endives, and soil under outdoor conditions from West Germany; Formation of Ethylenethiourea from 5, 6-Dihydro-3H-imidazo (2, 1-c) 1, 2, 4-dithioazole, -3-thione by Microorganisms and Reducing Agents from The Netherlands; The Evaluation of a Radio Gas-Chromatographic System for the Detection and Trace Amounts of Labelled insecticides from Venezuela; and Residues of Lindane and its metabolities in Eggs, Chicks and Body Tissues of Hen, Phesants after ingestion of Lindane—14C via Treated Wheat Seed or Gelatin Capsules from Canada; indicate the international acceptance of the Journal. The key words for the contents of the research papers have made the computerisation of abstracting quite easy.

As a research worker in the field I feel this is a welcome addition to the Journal of Environmental Sciences which would specifically include pesticides, food contaminants and agricultural wastes.

The Journal of Environmental Sciences and Health Part B will have four issues per year and the subscription is US $ 35.

S. K. MAJUMDER
C F T R I, MYSORE.

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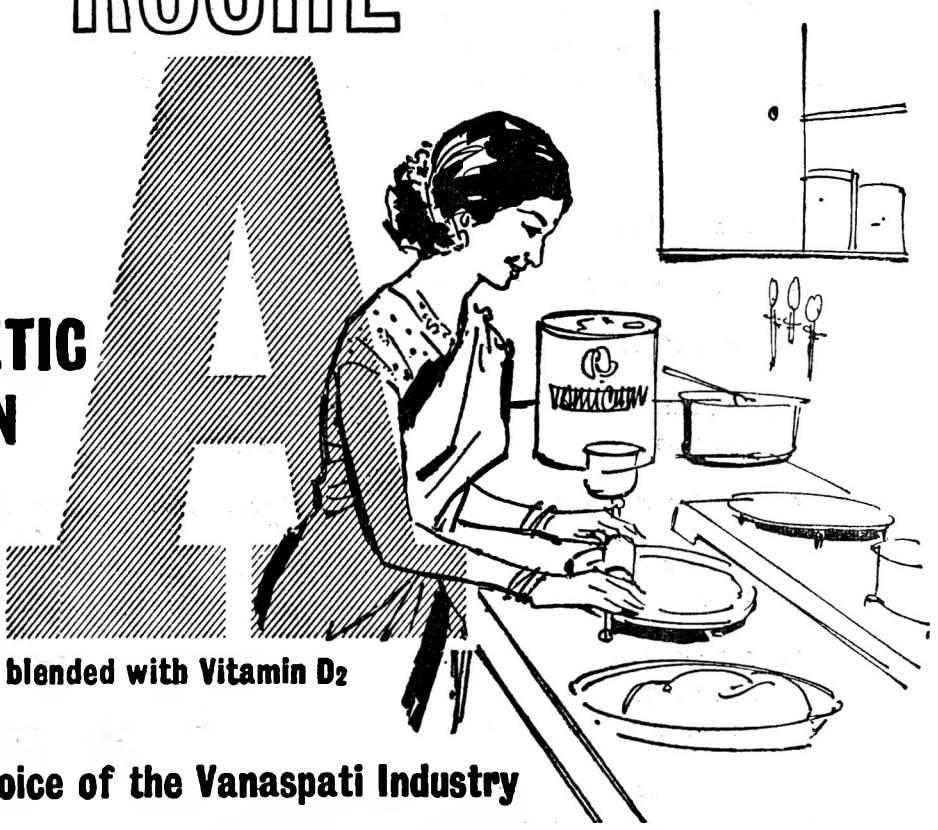

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SUBJECT INDEX

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AUTHOR INDEX

กำหนดส่ง

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

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

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