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ห้องสมุดกรมวิทยาศาสตร์บริการ

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Development and Testing of a Hammer Mill with a Closed Circuit System of Grinding

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A closed circuit hammer mill with the elimination of the screen from the grinding zone and of the conveying fan was proposed with a conical screen introduced into a cyclone as an external separator. The novel mill was tested and the results compared with that obtained from an existing hammer mill drive motor of the same size. The performance of the mill was comparable to that of the existing mill with an energy saving of about 20%.

The production of cereal grains in certain parts of the world particularly the developing countries, has increased over the last decade[1]. Cereal grains such as maize, millet, rice, sorghum, wheat and Guinea corn are common food materials in Africa and Asia. Although some of these are consumed after cooking, they all undergo grinding for the preparation of various other menus.

The exact time the first machine for the grinding of agricultural grains was invented is not known. Today however, manual methods for the grinding of grains is practised only in villages of developing countries where small-scale grinding is still being done. The most commonly used modern mills for grinding agricultural grains are burr mill, roller mill, plate mill and hammer mill.

Power consumption is the major factor in deciding the type of mill for any grinding operation. Hall[2] reported that less power is required for fine grinding with hammer mill than with the plate mill. Power consumption is generally known to increase with moisture content in the grinding of cereal grain as reported by Ajayi[3], Jindal[4], and Baker and Farrell[5].

Design decisions: The detailed discussion on the conditions for deciding on the following summarized design criteria is reported by Ajayi[3].

1. A closed circuit type of hammer mill was proposed with external particle separation and the return of oversize materials into the mill for further size reduction.
2. The screen was to be eliminated from the grinding zone and replaced by breaker bars fitted to the wall of the grinding chamber to create more impact surfaces in the chamber.
3. The mill was to be fed from the Centre or eye and an outlet from the mill situated tangentially on the periphery.
4. The conveying fan was to be eliminated and the hammers used as a conveying fan.
5. A 2.2 kW variable speed motor the same size as that of an existing mill was to drive the mill.
6. A centrifugal classifier was proposed. This was a cyclone classifier with a replaceable conical screen fitted for separating particles of right fineness and returning the over size into the mill for regrinding.

It is known that as a classifier and grinding surface, the screen constitutes a high energy demand with power consumption increasing with decrease in screen size[5,6]. For the production of fine flour for human consumption therefore, a high energy demand is inevitable with the use of the screen in the grinding zone of the hammer mill.

The completed mill:

Fig 1 is the front view of the mill showing the re-circulation system consisting of the separator with the feeding and return links. An exposed portion showing the breaker bars and hammer are also indicated. The breaker bars, each 10 mm high and 15 mm wide were arranged at 20 mm spacing around the circular wall of the grinding chamber except the mill outlet. A tip clearance of 3 mm was maintained between the hammer ends and the breaker bars. The hammers were arranged in two rolls round the rotor with two hammers in opposite position in a roll.

Mill test: The terms used in testing and assessing the mill are defined as follows:
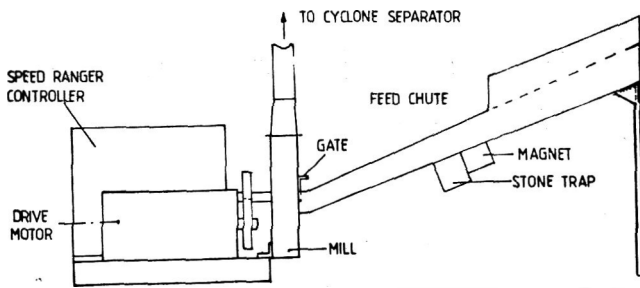
Fig. 1 Side view of mill assembly

(a) *Mill capacity (kg/hr):* Amount of feed produced by the mill per unit time at stabilized conditions.

(b) *Specific energy consumption* (kWh/t): Energy expended per tonne of material produced at stabilized conditions.

(c) *Stabilized/Condition:* Equilibrium state of operation when the amount of feed into the mill is equal to the amount of product coming out of the mill.

Grinding tests were conducted with clean and graded American No. 2 maize grains. Two conical screens of 1.6 and 2.4 mm apertures were used in the separator at 73° cone angle. The hopper was filled with grains at the start of each experiment. More grains were added to the hopper to maintain the level as grinding continued.

A single element Wattmeter was connected to the drive motor and measured the power drawn by the motor during the operation. Stabilized condition was considered to be reached when the Wattmeter reading remained constant. Trial experiments showed that the power demand of the mill was constant when the mill was running empty. An increase in power demand was noticed as grains were being fed into the mill for grinding. This rise continued until a state of constant power demand was reached. The constant power demand indicated a constant quantity of material present in the grinding chamber, implying that the rate at which material was entering and leaving the mill was the same. This was the stabilized condition.

Test procedure: The procedure for testing the mill was as follows:

(i) The mill was operated empty for a warm up period of about 15 to 20 min.

(ii) The mill speed was set by operating the speed ranger controller and measuring the speed with a mechanical Tachometer.

(iii) The power consumption of the mill with no load was read and recorded.

(iv) The vibrator feeder was set to vibrate at a desired rate and switched on to feed maize of known moisture content into the mill.

(v) The Wattmeter reading was observed and when a stabilized condition was reached, the diverter valve was operated to extract product sample for 1 min.

(vi) The Wattmeter reading at stabilized condition and the weight of sample extracted were recorded.

The above steps were repeated for different hammer tip speeds.

A comparative test was made with an existing conventional hammer mill which was also a 2.2 kW mill but had a 1 mm internal screen. The tests carried out were similar on the existing mill as well as for the new mill. Their energy demands per unit weight of feed produced and the feed size distribution were compared.

Calculation: The energy consumed in a stabilized operation is given by:

$$E_T = \frac{E_L t}{3600} \cdot \frac{\eta L}{100}$$

Where,

E_T = energy consumed in stabilized grinding condition, kWh

E_L = power drawn by motor during stabilized mill loading, kW.

t = time of stabilized operation, S.

η_L = efficiency of motor on load at stabilized condition, %

The mill capacity is a measure of the ground product per unit time and is given by

$$M_c = 3.6 \frac{W_w}{t}$$

Where,

M_c = mill capacity, kg/hr

W_w = weight of sample ground at stabilized condition, g.

The specific energy kWh/t of the mill is the energy required per tonne of ground product, given by

$$S_c = \frac{10^6 E_T}{W_w}$$

The geometric mean particle size of the feed produced is given by[7]

$$D_m = \text{Log}^{-1}\left[\frac{\Sigma W_i \, \text{LogD}_i}{\Sigma W_i}\right]$$

Where,

D_m = geometric mean particle size, cm

W_i = weight of particle on i^{th} sieve, g

D_i = size of particles on the ith sieve, cm

Results and Discussion

The three main parameters studied, that is, screen size, number of hammers and hammer tip speed affected the performance of the mill. When a 1.6 mm external screen was used, the geometric mean particle size varied from 265 to 295 μ depending on the speed and moisture content while the use of the 2.4 mm screen resulted in products of mean size ranging from 340 to 548 μ.

The throughput of the mill was higher for the 8 than for the 4 hammer operations with a higher production of fines, (Tables 1 and 2). The specific energy

TABLE 1. PRODUCT CHARACTERISTICS FROM THE NEW MILL WITH 8 HAMMERS AND 1.6 mm SCREEN

Mesh no.	Sieve aperture (μ)	Particle size on sieve (μ)	Wt of particle on sieve (g)	%particle on sieve by wt	WPC (% finer than)
4	4,750	5,641	0.0	0.0	100.00
6	3,350	3,989	0.0	0.0	100.00
8	2,360	2,812	0.0	0.0	100.00
12	1,700	2,003	0.0	0.0	100.00
16	1,180	1,416	0.0	0.0	100.00
20	850	1,001	0.31	0.31	99.69
30	600	714	5.38	5.37	94.32
40	425	505	22.85	22.81	71.51
50	300	357	23.41	23.37	48.13
70	212	252	18.23	18.20	29.93
100	150	178	11.10	18.08	18.85
140	106	126	8.49	8.48	10.37
200	75	89	6.48	6.47	3.90
270	53	63	3.44	3.43	0.47
PAN	—	44	0.47	0.47	—
Total			100.16	100.00	

Mean particle size=272 μ

S.D.±1.88

WPC=% wt finer than sieve aperture

Feeding rate=0.81 kg/min.

Hammer tip speed=67.0 m/sec

Moisture content=15.46 (%W.B.)

TABLE 2. PRODUCT CHARACTERISTICS FROM THE NEW MILL WITH 4 HAMMERS AND 1.6 mm SCREEN

Mesh no.	Sieve aperture (μ)	Particle size on sieve (μ)	Wt of particle on sieve (g)	%particle on sieve by wt	WPC (% finer than)
4	4,750	5,641	0.0	0.0	100.00
6	3,350	3,989	0.0	0.0	100.00
8	2,360	2,812	0.0	0.0	100.00
12	1,700	2,003	0.0	0.0	100.00
16	1,180	1,416	0.0	0.0	100.00
20	850	1,001	1.16	1.16	98.84
30	600	714	13.28	13.22	85.62
40	425	505	20.32	20.21	65.39
50	300	357	17.45	17.38	48.01
70	212	252	15.91	15.84	32.16
100	150	178	13.54	13.48	18.68
140	106	126	13.57	13.51	5.17
200	75	89	4.76	4.74	0.43
270	53	63	0.31	0.31	0.12
PAN		44	0.12	0.12	
Total			100.42	100.00	

Mean particle size=295 μ

S.D.=±1.88

WPC=% of wt. finer than sieve aperture

Feeding rate=0.91 kg/min

Hammer tip speed=67.0 m/sec.

Moisture content=15.45 (%WB)

consumption (kWh/t) revealed the 4 hammer operations to be less energy demanding. The operational cost of the mill when fitted with 4 hammers is thus lower than when it was fitted with 8 hammers. On the basis of energy consumption, operating the mill on 4 hammers is preferred to the 8 hammer operation when the 1.6 mm screen is used. This observation results from rigorous tests on the mill as reported by Ajayi[3].

Hammer tip speed affects the energy demand of the mill irrespective of the number of hammers or size of the conical screen. Fig. 2 and 3 show that an increase in the energy demand per tonne of product obtained with 2.4 and 1.6 mm screens, respectively. The increase in power per tonne of feed with speed is higher for the 8 than for the 4 hammer operations. An increase in the tip speed was observed to result in the production of more fines, indicating that overgrinding occurred with higher speeds.

Table 3 shows the results of tests with an existing mill with a 1 mm internal screen. The two mills were
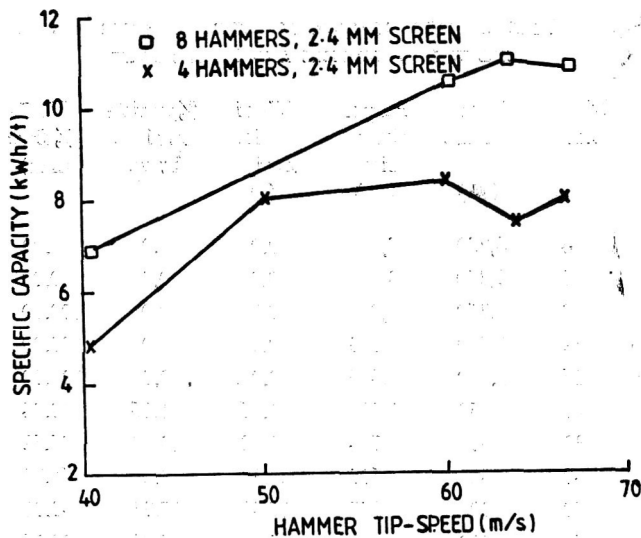
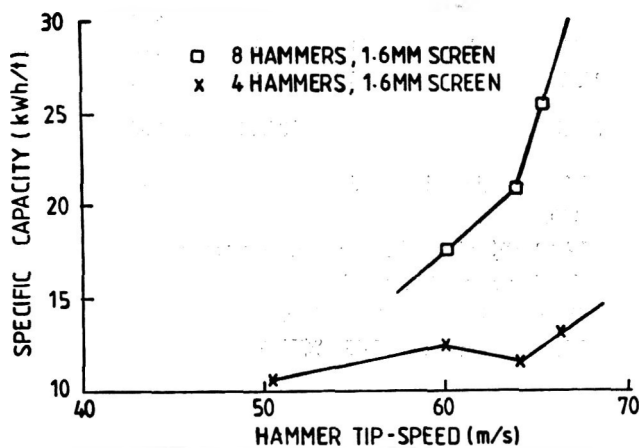Fig. 2. Effect of speed on energy demand with 2.4mm screen

Fig. 3. Effect of speed on energy demand with 1.6mm screen

TABLE 3. PRODUCT CHARACTERISTICS FROM THE EXISTING MILL

Mesh no.	Sieve aperture (μ)	Particle size on sieve (μ)	Wt of particle on sieve (g)	%particle on sieve by wt	WPC (% finer than)
4	4,750	5,641	0.0	0.0	100.00
6	3,350	3,989	0.0	0.0	100.00
8	2,360	2,812	0.0	0.0	100.00
12	1,700	2,003	0.0	0.0	100.00
16	1,180	1,416	0.0	0.0	100.00
20	850	1,001	0.0	0.0	100.00
30	600	714	8.14	8.11	91.89
40	425	505	26.92	26.83	65.06
50	300	.357	18.27	18.21	46.85
70	212	252	18.26	18.20	28.65
100	150	178	14.91	14.86	13.79
140	106	126	10.67	10.63	3.16
200	75	89	2.52	2.51	0.65
270	53	63	0.58	0.58	0.07
PAN		44	0.07	0.07	
Total			100.34	100.00	

Mean particle size=299 μ
S.D. =$\pm$1.76
WPC=% of wt finer than sieve aperture
Feeding rate=1.01 kg/min
Hammer tip speed=73.7 m/sec.
Moisture content=15.47 (% W.B.)

TABLE 4. COMPARISON BETWEEN THE NEW MILL AND A CONVENTIONAL MILL (1.6 mm SCREEN)

	Existing mill	New mill
Mill capacity (kg/hr)	65.16	52.38
No-load power (kW)	1.192	0.692
Stabilized power (kW)	2.02	1.3
Specific energy (kWh/t)	31.00	24.82

operating at their optimum settings to produce compartively fine flour (Tables 2 and 3). The geometric mean particle sizes are 295 and 299 microns with the new and existing mills respectively.

As seen from Table 4, the existing mill has a higher throughput but at the expense of energy demand. The energy demand per tonne of feed produced is also lower for the new mill than for the conventional mill tested, 24.82 and 31 kWh/t, respectively-an energy gain of 19.94 per cent per tonne of flour produced.

The closed circuit grinding adopted for the new mill with the elimination of the elevating fan and the external separation of particles have contributed to the energy saving. The no load power of mills with a fan increases with speed. The elimination of the fan is therefore an important factor contributing to lower energy demand of the new mill.

Particle separation outside the grinding chamber of the new mill is another factor that contributed to the lower energy demand of the mill. Due to the immediate removal of ground material from the grinding chamber, the residence time for the grain in the grinding zone is shorter compared with existing conventional hammer mill with internal screen. Material build-up in the mill causes an increase in the operational power as reported by Jindal[3]. The removal of the screen from
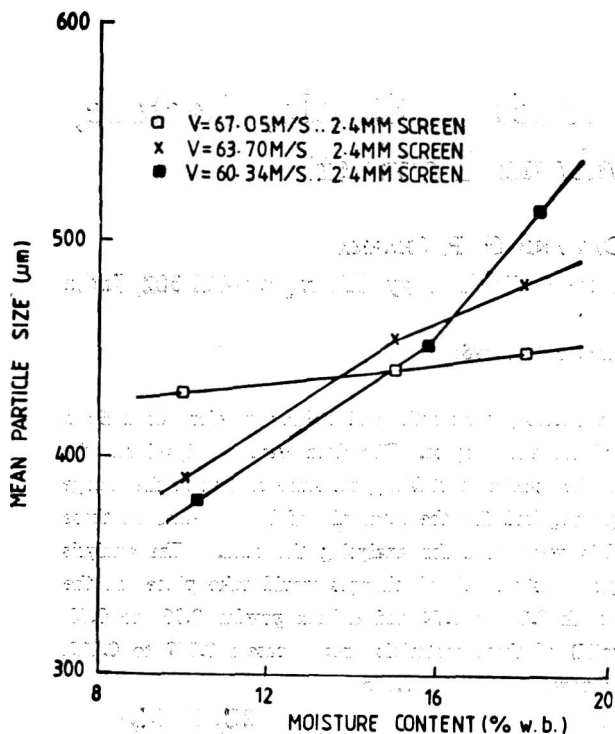
Fig. 4. Effect of moisture on the mean particle size

Conclusion

1. A simple design for a hammer mill has been accomplished with the elimination of the perforated screen from the grinding zone and incorporation of an external separator.

2. The elevating fan has been eliminated and the pumping effect of the hammers deployed to perform its duty thus saving energy and capital in construction.

3. The performance of the new mill was comparable to an existing mill of same drive motor size in producing fine flour.

4. The recirculation system with particle separation outside the grinding zone and the elimination of the fan have proved advantageous with an energy gain of 19.94 per cent over the existing mill.

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the grinding zone also eliminates the problems of screen wear and frequent replacement.

The effect of grain moisture content on the geometric mean particle size is shown in Fig. 4. The mean particle size of the products increases with an increase in moisture content at all hammer tip speeds tested. The increase in mean particle size is, however, more rapid at lower than higher tip speeds. Grinding grains in the lower moisture range of 9-12 per cent resulted in the production of more fine particles than those at higher levels of moisture.

Moisture Adsorption Characteristics of Casein, Lactose, Skim Milk and *Chhana* Powder

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Water activity and equilibrium moisture content data of casein, lactose, skim milk and *chhana* powder at a fixed temperature of 50°C were obtained from an apparatus designed for the purpose. The data were analysed for the determination of the values of bound water, water activity for the maximum stability, moisture content at the change over points in the nature of moisture binding and the energy required for the removal of bound water in these materials. BET, Caurie, 'stability' and 'local' isotherm models were used for analysing the data. The analysis showed that the range of water activities at which the major moisture related changes would take place in the materials are: casein 0.08 to 0.56, lactose 0.20 to 0.73, skim milk 0.12 to 0.72 and *chhana* powder 0.06 to 0.60. The corresponding moisture contents (in kg water per kg dry solid) of these materials are: casein 0.049 to 0.112, lactose 0.020 to 0.092, skim milk 0.024 to 0.102 and *chhana* powder 0.019 to 0.086.

Moisture adsorption behaviour of milk products and their constituents are important for understanding the problems of caking, swelling, browning reactions, mould growth and development of rancidity. Moisture sorption isotherm equations give an insight into the moisture binding character of foods. Critical moisture contents which are of major concern to a food processor are bound water, energy required for the removal of bound water, water activity at the maximum stability and the moisture contents at the change over points in the nature of moisture binding in foods. This paper describes the methods of determination of these critical moisture contents in casein, lactose, skim milk and *chhana* (a product obtained from acid coagulation of milk) powder with the help of Brunaur *et al.*[1], Caurie[2], 'stability'[3] and 'local'[4] isotherm models.

The BET model is represented as

$$\frac{a_w}{m(1-a_w)} = \frac{1}{C_b\, M_b} + \frac{(C_b-1)a_w}{C_b\, M_b} \qquad .. (1)$$

The value of M_b in Eqn. (1) is known as the monolayer moisture and a food material is considered to be stable when it has this moisture content. The energy of bonding, E, over and above the latent heat of vaporization water is of obtained from the value of C_b.

$$E = RT\, \ln C_b \qquad .. (2)$$

Caurie's model is an improvement over the BET model and is expressed as:

$$\frac{1}{m} = \frac{1}{C_c M_c} \left[\frac{1-a_w}{a_w}\right]^{(2C_c/100M_c)} \qquad .. (3)$$

According to the Caurie's model, the moisture adsorption takes place in multilayers and the moisture content at which a food material becomes stable is the value of M_c obtained from Eqn. (3). The number, N, of the so called monolayers is given by

$$N = 100\, M_c/C_c \qquad .. (4)$$

'Stability' isotherm is obtained when the first derivative $\Delta m/\Delta a_w$ of the moisture sorption isotherm is plotted against the water activity a_w. The minimum of the resulting curve points to the value of a_w where the material shows the least inclination to adsorb moisture, i. e., a stable state with regard to the change in the relative humidity of the environment in which the material is kept. A food material would be considered to be stable when it would have the above mentioned value of water activity.

A 'local' isotherm results when the partial molal free energy, ΔF, ($\Delta F = -RT \ln a_w$) is plotted against the moisture content of the material. A combination of intersecting straight lines is usually obtained from such a plot. A change in the nature of moisture binding is expected to take place at the moisture contents corresponding to the points of intersection of the straight lines in the 'local' isotherm.

Materials and Methods

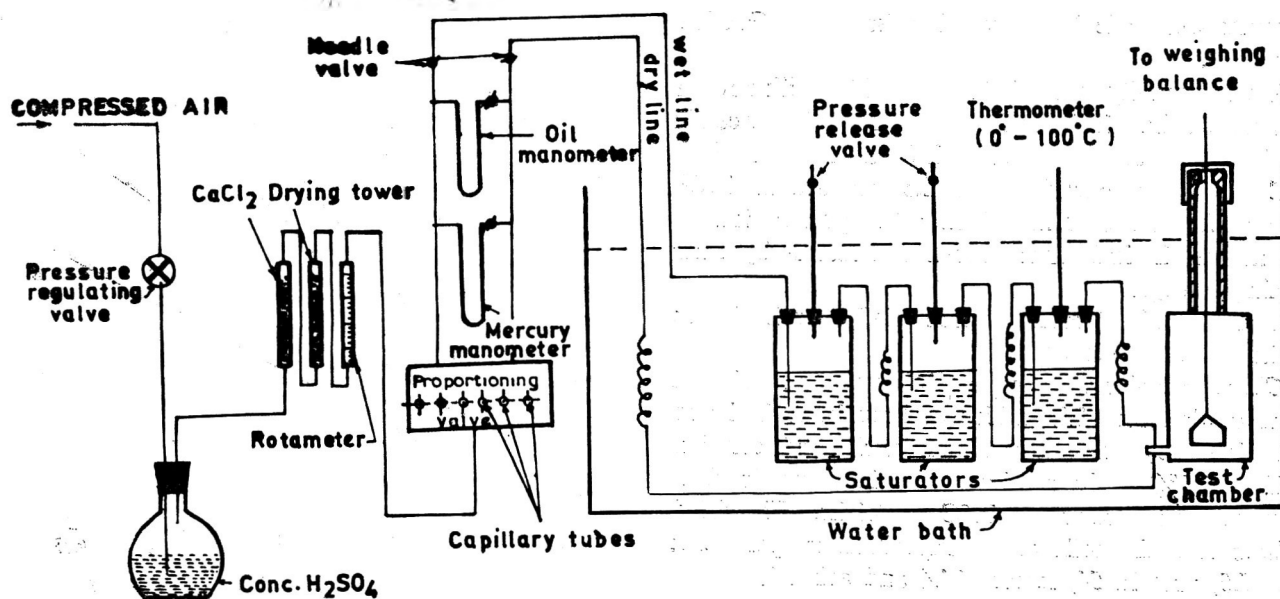The apparatus used for the determination of moisture

6

Fig. 1. Schematic diagram of apparatus developed for determination of water activity—equilibrium moisture content relationship.

content—water activity relationship is shown in Fig. 1. Based on the principle of working described by Smith[5], the apparatus was developed by Vaishney[6] and the present authors. The principle of dynamic method of attaining the equilibrium was adopted.

The working of the apparatus is that atmospheric air is first compressed and the compressed air with a regulated pressure of 5 to 35 kPa gauge is allowed to bubble through concentrated sulphuric acid for achieving partial dehydration. The air is then fully dehydrated by allowing it to pass through two calcium chloride towers arranged in series. The dehydrated air is then divided into two different streams by means of a set of capillary tubes which are mentioned in Fig. 1 as 'Proportioning Valve'. One of these streams remain dry and the other is saturated with water vapour by bubbling the air through distilled water contained in three saturators; they are designated as dry air and wet air respectively.

The proportioning valve consists of six capillary tubes having length 20, 10, 10, 4, 4 and 4 cm which can divide 0.05, 0.10, 0.10, 0.25, 0.25 and 0.25 fraction of the main air stream respectively into either dry or wet line. By suitable combination of the capillary tubes, any fraction of the main air stream ranging from 0.05 to 0.95 with 0.05 interval can be directed to either the dry or the wet line.

The temperature of the dry and wet lines was maintained at $50\pm1°C$ by controlling the temperature of water bath by means of a Thermins (Thermins Instruments, Cochin) temperature controller. Although a temperature of 25 to 30°C is normally experienced inside a storage space, a rather high temperature of 50°C was chosen to obtain an optimistic stability criterion of the materials in a worst possible storage condition.

The working pressures at the downstream side of the proportioning valve where the flow has been divided into either dry or wet stream are made equal by balancing two arms of the oil manometer connected across these two streams. The pressure equalisation was achieved by regulating the flow of air in the dry and wet lines by means of two needle valves fitted on these lines. The mercury manometer connected across the dry and wet lines helps in preliminary adjustment of pressure, whereas, the oil manometer is used for the final adjustment.

The dry stream and the wet saturated stream are mixed to give the mixed air stream a certain desired relative humidity, (RH) the value of which is given by[7]

$$RH = \frac{P(1-x)}{P-xP^*} \qquad ..(5)$$

The values of RH as calculated from Eqn. (5) for x taking values of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 are 0.112, 0.222, 0.328, 0.433, 0.533, 0.631, 0.727, 0.820, 0.911 and 1.00 respectively.

The mixed air stream is allowed to flow over a pre-weighed sample kept on the test pan hanging from a precision balance into the test chamber, until equilibrium is reached, as indicated by the balance.

Although in the present experiment, it was noted that about 7 hr were required to reach equilibrium for some of the samples, readings upto third hr only were recorded for other samples and the equilibrium moisture

contents were calculated by using Issacs and Gaudy's equation[8].

A commercially available brand of skim milk powder was used. It had the following composition (in per cent): fat 1, protein 36, lactose 52, ash 8 and moisture 3.

Casein was prepared as per the standard method[9]. The milk curd obtained during the preparation of casein was checked for the absence of lactose by using Fehling's reagent.

Edible grade lactose, commercially available as 'milk of sugar' was used.

Chhana powder was prepared[10] by acid coagulation from cow's milk. The milk was brought to boil and coagulated by using 2 per cent citric acid solution (pH to 4.6%). The resulting coagulant was dried in oven at a temperature of 80°C for 24 hr. The dried chhana had the following composition in per cent: fat 25.5, protein 54, lactose 14.4 and ash 6.1.

Results and Discussion

The water activity and equilibrium moisture content data of casein, lactose, skim milk and chhana powder were used to plot moisture adsorption isotherm (Fig. 2),

Fig. 3. Graphical representation of BET equation

'stability' isotherm (Fig. 5) and 'local' isotherm (Fig. 6). The data were also used to obtain the values of M_b and E of the BET equation and the values of M_c and N of the Caurie's equation. The graphical representation of the data fitted with the BET and Caurie's equation is shown in Fig. 3 and 4 respectively.

Moisture adsorption isotherm (MSI): Fig. 2 shows the moisture adsorption isotherm of the materials at 50°C. The MSI of casein shows that it adsorbs maximum amount of water at any value of water activity. A considerable amount of swelling was also observed when it was incubated in the test chamber at the relative humidity of 0.73. At low values of water
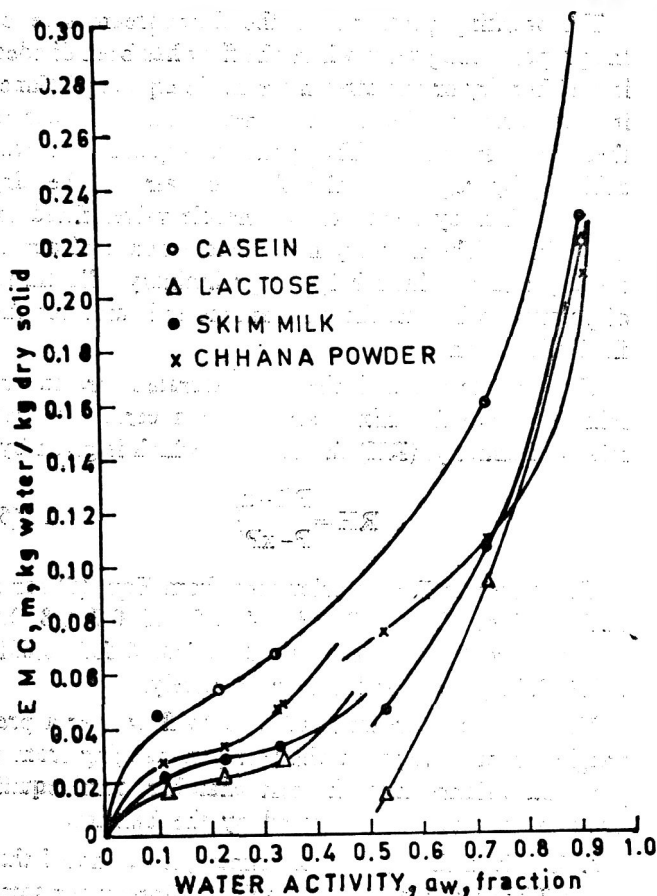
Fig. 2. Moisture adsorption isotherms of casein, lactose, skim milk and Chhana powder at 50°C

Fig. 4. Graphical representation of Caurie's equation
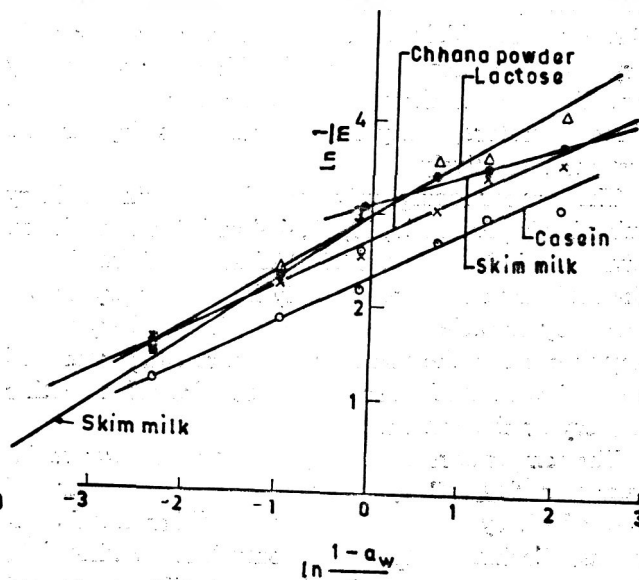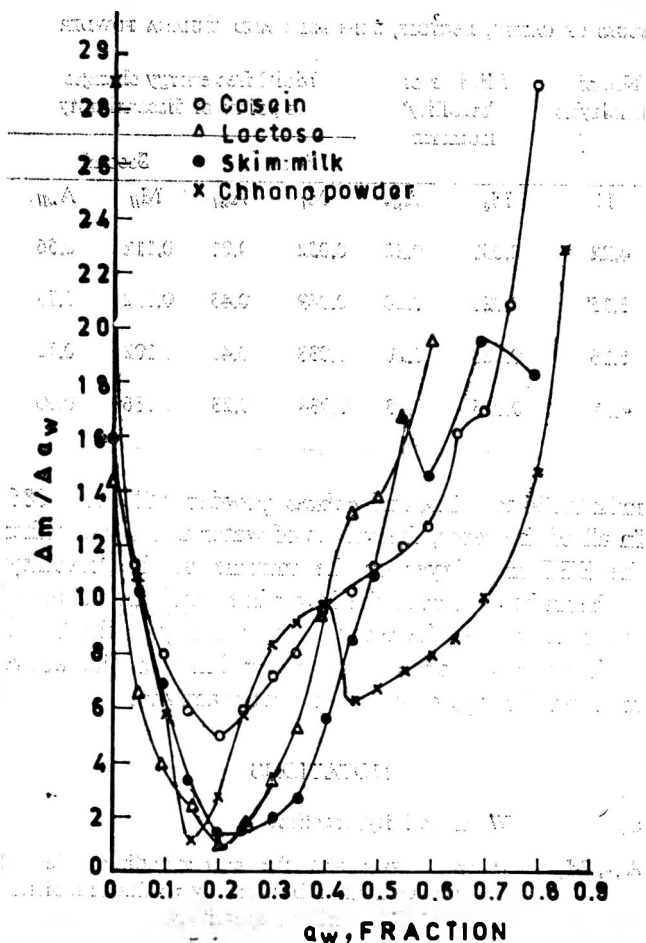
Fig. 5. Stability isotherms of casein, lactose, skim milk and Chhana powder
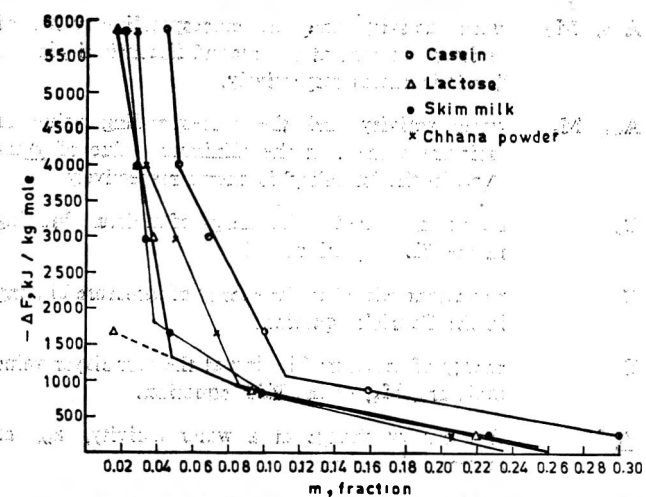
Fig. 6. Partial molal free energy change, $-\Delta F$ with change in moisture content, m for casein, lactose, skim milk and Chhana powder

activity, chhana powder was found to have higher moisture than the skim milk possibly due to its higher protein content.

The degree of discontinuity in the MSI of lactose (Fig. 2) is the highest followed by skim milk and chhana powder. Since lactose crystalises from the amorphous state within a water activity region of 0.4 and 0.5, the difference in the degree of discontinuity of the curves in Fig. 2 is possibly due to the difference in the lactose content in the materials, the amount of which is smaller in chhana than in skim milk powder. The experimental observations showed that lactose and skim milk developed grittiness and caking when they were incubated at relative humidities of 0.433 and 0.533.

Graphical representation according to BET and Caurie's equation: It may be observed from Fig. 3 that the BET equation holds good within the water activity range of 0 and 0.3, beyond which the data are found to be excessively scattered. Caurie's equation, on the other hand, is fitted to the whole range of water activity for casein, lactose and chhana powder, except for skim milk, the a_w–m data of which is fitted to two straight lines, above and below an a_w value of 0.38, where, In $(1-a_w)/a_w = 0.5$. The values of M_b and E of the BET equation and those of M_c and N of the Caurie's equation, as found out from the slope and intercept of the straight lines in Fig. 3 and 4, are given in Table 1. It is interesting to note that the monolayer value of casein ($M_b = 0.049$ and $M_c = 0.063$) is the highest of all the materials tested. Although chhana contains casein predominantly, the values for M_b and M_c ($M_b = 0.019$ and $M_c = 0.052$) are not so high, possibly because of its acidic nature and the product is separated from milk at its isoelectric point where the water holding capacity of casein is considered to be the minimum.

The energy of moisture binding, E, at the monolayer value of skim milk is found to be the highest. A striking similarity is observed between the E value of the BET equation and the N value of the Caurie's equation; the value of E increases with the increase in the value of N.

Stability isotherm and 'local' isotherm: The 'stability' isotherms of the materials are shown in Fig. 5. The values of water activity, A_{ws}, at the minima of these isotherms and the corresponding values of moisture content, M_s, are given in Table 1. These water activity values are found to be close to the BET monolayer value (i. e. $A_w \cong _s A_{wb}$) rather than the Caurie's multilayer value.

Local isotherms of the materials are plotted in Fig. 6. For all the materials, the isotherms resulted in a combination of three intersecting straight lines with two points of discontinuity. At low moisture contents, the free energy values are low (i. e., $-\Delta F$ value is high) indicating that the water is tightly bound to the material.

TABLE 1. CRITICAL WATER ACTIVITY AND MOISTURE CONTENT VALUES OF CASEIN, LACTOSE, SKIM MILK AND CHHANA POWDER

Material	Bound water				Energy of water adsorption (kJ/kg mole)	No. of monolayers	Minima of 'stability' isotherm		Molal free energy changes at points of discontinuity			
	BET equation		Caurie's equation						First		Second	
	M_b	A_{wb}	M_c	A_{wc}	E	N	M_s	A_{ws}	M_I	A_{wI}	M_{II}	A_{wII}
Casein	0.049	0.18	0.063	0.30	8979	4.22	0.051	0.20	0.052	0.21	0.112	0.56
Lactose	0.022	0.25	0.040	0.42	8786	3.15	0.020	0.20	0.049	0.45	0.092	0.73
Skim milk	0.024	0.12	0.053	0.49	10611	6.16	0.028	0.20	0.038	0.40	0.102	0.72
Chhana powder	0.019	0.06	0.052	0.35	8816	4.11	0.025	0.15	0.034	0.23	0.086	0.60

The steep slope of the straight lines at low moisture contents indicates that the free energy value rapidly decreases (i. e., $-\triangle F$ value increases) with the reduction of moisture content. The slope of the lines decreases with increasing moisture indicating that it becomes less tightly bound i. e. it becomes more free or unbound in nature.

The points of discontinuty in the 'local' isotherms indicate a change in the nature of moisture binding. The values of moisture contents, M_1 and M_{11} at which these two discontinuities have occurred and the corresponding values of water activity, A_{wl} and A_{wll} are given in Table 1. The first discontinuity for casein ($M_1=0.052$, $A_{wl}=0.21$) is found to be close to the BET monolayer value ($M_b=0.049$, $A_{wb}=0.18$) and also to the minima of the 'stability' isotherm ($M_s=0.51$, $A_{ws}=0.20$). The second discontinuity ($M_{11}=0.112$, $A_{wll}=0.56$) for casein may be attributed to the swelling of casein micella which probably starts from this water activity, although, this phenomenon has been found to be occurring when the casein samples have been incubated at a relative humidity of 0.73.

The first discontinuity in the case of lactose and skim milk (Table 1) has been found to lie in the region of water activity between 0.4 and 0.5, where the crystallization of lactose takes place. This region of water activity coincides with the Caurie's A_{wc} value for these two materials.

The two sharp discontinuities for chhana powder and the second discontinuity for lactose and skim milk cannot be fairly accounted for from the results of the present investigation.

The data in Table 1 therefore, have, shown that the major moisture related changes in the materials would take place in the following regions of water activity: casein 0.18 to 0.56; lactose 0.20 to 0.73; skim milk 0.12 to 0.72 and chhana powder 0.06 to 0.60. The corresponding moisture contents in these materials are: casein 0.049 to 0.112; lactose 0.02 to 0.092; skim

milk 0.024 to 0.102 and chhana powder 0.019 to 0.086. In all of the cases, the values of water activity at either the BET monolayer or the minima of the 'stability' isotherm have formed the lower limit and the values of water activity at the second discontinuity in the 'local' isotherm have formed the upper limit of the above mentioned range of values of water activity.

NOTATIONS

a_w	Water activity, fraction
A_{wb}, M_b	water activity and the corresponding value of moisture content at the monolayer value of moisture in the BET equation respectively.
A_{wc}, M_o	water activity and the corresponding value of moisture content at the multilayer value of moisture in the Caurie's equation respectively.
A_{wl}, M_l	water activity and the corresponding value of moisture content at the first discontinuity in the 'local' isotherm respectively.
A_{wll}, M_{ll}	water activity and the corresponding value of moisture content at the second discontinuity in the 'local' isotherm respectively.
A_{ws}, M_s	water activity and the corresponding value of moisture content at the minimum value of $\triangle m/\triangle a_w$ in the 'stability' isotherm respectively.
C_b	a constant related to the energy of moisture binding in the BET equation.
C_c	a constant related to the energy of moisture binding in the Caurie's equation.
E	energy of moisture binding at the monolayer value moisture, M_b, of the BET equation.
$\triangle F$	free energy change at a water activity, a_w, of material, kJ/kg mole.
m	moisture content, kg water/kg dry solid.
N	number of monolayers of moisture from the Caurie's equation.

P	atmospheric pressure, 101.33 kPa.
P*	saturation vapour pressure of water at the prevailing temperature of air in test chamber; 12.33kPa at 50°C.
R	universal gas constant, 8.314 kJ/kg mole K.
RH	relative humidity of air in test chamber, fraction.
T	absolute temperature of test samples, K.
x	fraction of dry air diverted to the dry line.

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Oil Expression Characteristics of Rapeseed *(Brassica campestris)* Under Uniaxial Bulk Compression*

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A 10 mm thick bed of rapeseeds was subjected to steady bulk compression under confined conditions. A 60 t capacity Universal Testing Machine was employed as the pressing device. Samples of 4.56, 6.46, 9.35 and 12.35% (w.b) moisture content were pressed at different rates of deformations 5.0, 10.0, 15.0 and 19.5 mm/min and 15 levels of maximum compression pressure ranging between 0.94 and 58.89 MN/M². Mathematical expressions were developed to correlate the effect of these factors on oil expression characteristics. Oil recovery efficiency for any degree of compression was found to be an exponential function of the composite variable $(P-P_o)\sqrt{1+R}$.

Besides being a concentrated source of energy in human diet, oilseeds constitute a very important component of agricultural production of the country. The annual demand of vegetable oils in the country is estimated at 5.5 million tonnes against a total production of 3.0 million tonnes. About 90 per cent of the oilseeds processed in the country are reported to be milled in low and medium powered oil expelling systems. The oil recovery in such a system is low at about 45 to 75 per cent[1,2,3].

Mechanical expression of oil from oilseeds is essentially a load deformation process; the oil bearing material is progressively compressed to physically expel the oil. *Ghanis*, hydraulic presses and screw expellers are the common techniques for effecting this solid-liquid separation[4]. A very careful application of heat, water and pressure is necessary for separation of oil from oil bearing materials. Published information on various aspects of oil expelling relates to solvent extraction and high pressure oil expeller systems and pretreatment effect. Very little information is available in respect of design data base for such systems, particularly about mechanical behaviour of rapeseed when it is subjected to an external load for oil recovery in pressure induced systems. The study reported here relates to investigations to evaluate oil expression characteristics of rapeseed under different conditions of load application in relation to moisture content and rate of deformation under confined conditions of uniaxial bulk compression.

Materials and Methods

Rapeseed (*Brassica campestris*) of commercial variety 'T-9' was used as the test material. Seeds were properly cleaned and passed through 1.18 mm opening sieve to ensure removal of shrevelled and immature seeds. The test cell consisted of 10 mm wide annular space formed between two rings of 160 and 140 mm diameter. A properly machined solid ring which fitted into the annular space was used as the loading piston. The cross section details of the test cell is shown in Fig. 1. For each test about 31 g of rapeseed was uniformly filled into the cell to form a 10 mm thick bed. In each case the seeds, which were originally at a moisture content of 6.46 per cent (wet basis) had been conditioned to a predetermined moisture levels.

Four levels of seed moisture content viz., 4.56, 6.46, 9.35 and 12.35 per cent (w.b.), 4 rates of deformation viz., 5.0, 10.0, 15.0 and 19.5 mm/min; and 15 levels of maximum compression pressure ranging between 0.94 and 58.89 MN/m^2 were used in the study. Each test was replicated thrice. Thus in all 720 runs were made. The loading was done on a 60 t capacity Universal Testing Machine (U.T.M.) A schematic diagram of the test rig is shown in Fig. 2. The perforations indicated in the figure were located on the periphery of the inner and outer rings. These perforations were made in the form of 100 mm wide and 6 mm high vertical slots. The outer ring had 65 such perforations with a pitch of 7.0 mm. The inner ring had 43 such perforations with a pitch of 7.0 mm as shown in Fig. 1. These openings ensured adequate drainage area of 0.14 cm^2/cm^3 of the test cell.
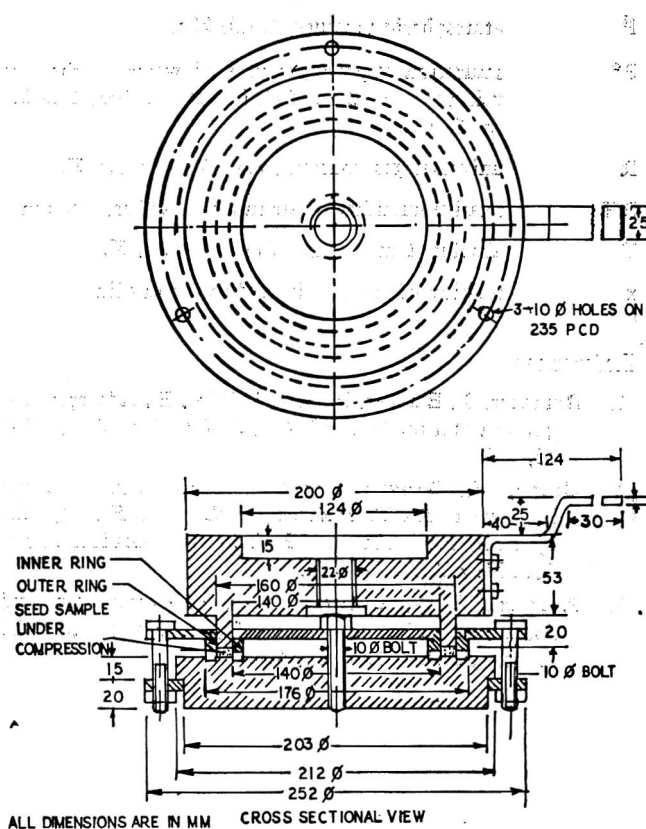
Fig. 1. An experimental test cell concentric cylinder piston system for uniaxial bulk compression in universal testing machine
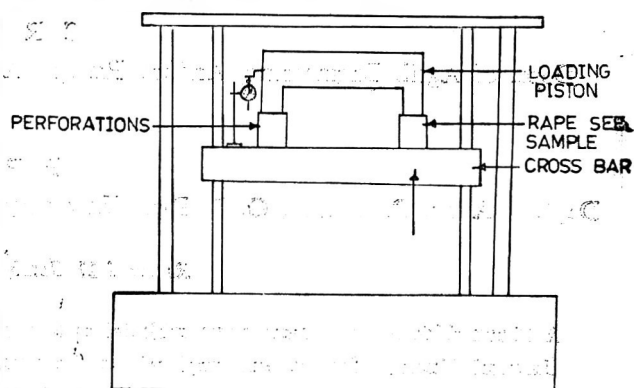
Fig. 2. A schematic view of the configuration of the loading arrangement

These rings perfectly fit into the grooves provided in the base plate. The rings and base plate assembly were suitably clamped with the help of bolts running through collar plates to prevent lifting up of the cylinder due to the pressure generated in the compression process. On the periphery of the piston a smooth extender plate was provided, which on touch with the dial gauge pointer during compression ensured measurement of the movement of the loading piston.

Experimental: The samples were filled uniformly in the annular space of the test cell kept on the base plate of UTM and the loading piston was put in the annular space over the sample. The piston was given 5 semi-circular turns in either direction to ensure uniform initial compaction and spread of the seeds. The cross-head speed was set to give a predetermined rate of deformation. The test cell loaded with specimen placed on the UTM base plate move up and touch the fixed cross bar. With the compression process initiated, since the sample holding test cell was rigid enough and loading piston unhindered, the process of compression was unidirectional. The compression was continued till the predetermined load was observed in the dial indicator of UTM. The UTM was then stopped and the pressed cake was collected and analysed for residual

oil[1,5]. The initial oil content of the sample was measured similarly. The difference between the initial and final oil contents gave the yield.

Results and Discussion

Oil recovery efficiency could be defined as the ratio of oil recovered to the oil present in the sample. Rape seed used in the study contained 0.704 of oil per g of oil free dry matter. The oil recovery efficiencies with rapeseed of 4.5 per cent moisture content at different rates of deformation and applied pressures are given in Table 1.

Effect of pressure: Fig. 3 shows the influence of applied effective pressure on oil recovery efficiency for different rates of deformation and moisture contents. Effective pressure is the difference between the total

TABLE 1. OIL RECOVERY AT VARYING PRESSURE AND RATES OF DEFORMATION IN OIL EXPELLING PROCESS OF RAPESEED OF 4.56% (w.b.) MOISTURE CONTENT

| Rate of deformation (mm/min) (R)	Applied pressure (MN/m^2) (P)	Oil points pressure (MN/m^2) (Po)	Effective pressure (MN/m^2) (P–Po)	Product $P-Po \times \sqrt{1+R}$	Oil expressed (%)	Oil recovery (%)
5.00	9.88	5.93	3.95	9.67	2.32	6.00
5.00	19.67	5.93	13.73	33.63	5.99	15.50
5.00	29.45	5.93	23.51	57.58	11.05	28.59
5.00	39.23	5.93	33.30	81.56	15.25	39.45
5.00	49.11	5.93	43.18	105.76	17.39	45.00
5.00	58.89	5.93	32.96	129.72	18.76	48.50
10.00	9.88	6.24	3.64	12.06	3.28	8.50
10.00	19.67	6.24	13.42	44.50	6.92	17.92
10.00	29.45	6.24	23.20	26.93	11.66	30.16
10.00	39.23	6.24	32.99	109.40	16.28	42.12
10.00	49.11	6.24	42.87	142.17	18.01	48.50
10.00	58.89	6.24	52.65	174.61	19.52	50.50
15.00	9.88	6.55	13.33	13.30	3.61	9.51
15.00	19.67	6.55	13.11	52.42	7.79	20.15
15.00	29.45	6.55	22.90	91.54	13.14	34.00
15.00	39.23	6.55	32.68	130.70	16.78	43.40
15.00	49.11	6.55	42.56	170.22	20.29	52.50
15.00	58.89	6.55	52.34	209.34	21.27	55.02
19.50	9.88	7.07	2.81	5.70	4.72	12.21
19.50	19.67	7.07	12.59	56.98	8.98	23.25
19.50	29.45	7.07	22.38	101.26	15.19	39.30
19.50	39.23	7.07	32.16	145.59	17.99	46.55
19.50	49.11	7.07	42.04	190.32	21.65	56.02
19.50	58.89	7.07	51.82	234.00	24.09	62.32
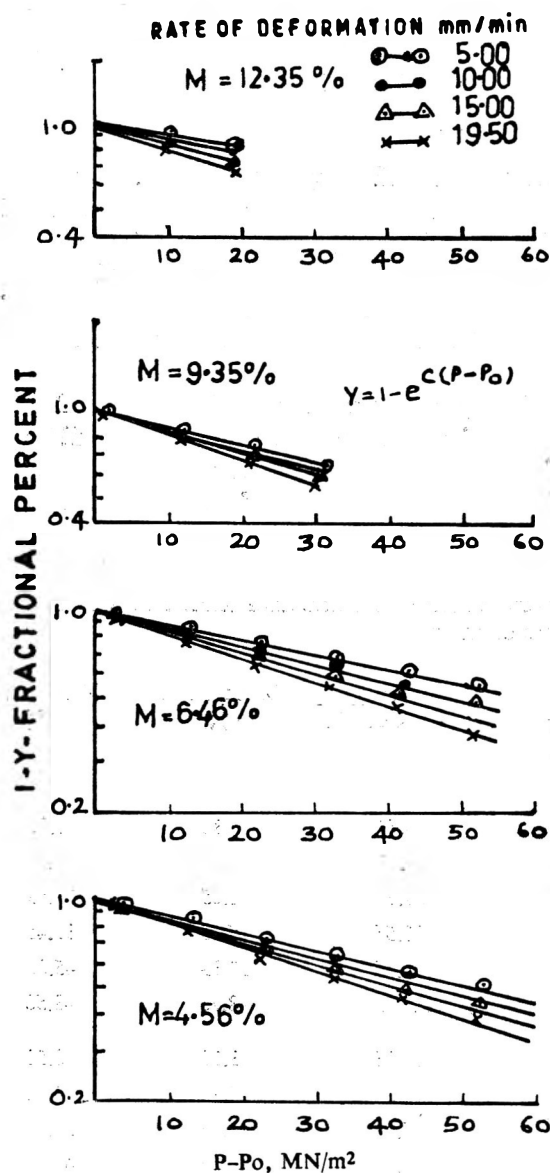
Fig. 3. Effect of pressure on oil recovery efficiency at various levels of rate of deformation and moisture contents

Fig. 4. Effect of rate of deformation on oil recovery efficiency for rapeseed of 6.46 % moisture content

pressure P and oil point pressure Po at which oil would just flow from the interior to the surface of the seeds[9,10]. As could be readily seen from Fig 3 the relationship yields a straight line on semilog plot such that,

$$Y = 1 - \text{Exp.} - C(P-Po) \qquad\qquad (1),$$

where, Y is the oil recovery efficiency expressed as a fraction. Oil recovery efficiency increases with increase in pressure at all moisture contents and rates of deformation.

At 4.56 per cent moisture and 19.5 mm/min rate of deformation, a maximum of 62.32 per cent oil recovery efficiency was achieved at a total pressure of 58.89 MN/m² (Oil point pressure was 7.07 MN/m²) compared to an oil recovery efficiency of only 12.21 per cent

at 9.88 MN/m² pressure. Similar trend was noticed at other moistures and rates of deformation.

Effect of rate of deformation: Fig 4 shows the effect of rate of deformation (R) on oil recovery efficiency for different levels of applied pressures at a moisture content of 6.46 per cent. It is readily seen that at any constant pressure, oil recovery increased linearly with increase in rate of deformation. The slope of the lines increased with applied pressure. Similar effect was found for other levels of seed moisture. For example, at a moisture content of 4.56 per cent the oil recovery increased from 48.50 per cent at 5.0 mm/min rate of deformation to 62.32 per cent at 19.5 mm/min rate of deformation for the same applied pressures of 58.89 MN/m². In all cases, 19.5 mm/min rate of deformation gave higher oil yields compared to other rates of deformation. Such effect of rate of deformation on oil yields found in the present case is in conformity with the results of Vinod Kumar and Satyanarayana[11], who have reported that rapeseed oil recovery increased from 20.00 to 23.30 per cent with increase in cross-head speed of pressing device from 6.5 to 17.00 mm/min at an applied pressure of 27.05 MN/m².

Effect of moisture content: Oil yields from rapeseed are considerably influenced by moisture content of the seed. Fig 5 shows the effect of moisture content at various pressures and at 10 mm/min rate of deformation. Similar effects were found at other rates of deformation. Increasing the moisture content of the seeds from 4.56 to 12.35 per cent resulted in the total oil recovery dropping from 39.30 to 29.50 per cent at an applied pressure of 29.45 MN/m² and 19.5 mm/min rate of deformation. The total bed deformation was 96.6 per cent compared to 67.5 per cent in case of 4.56 per
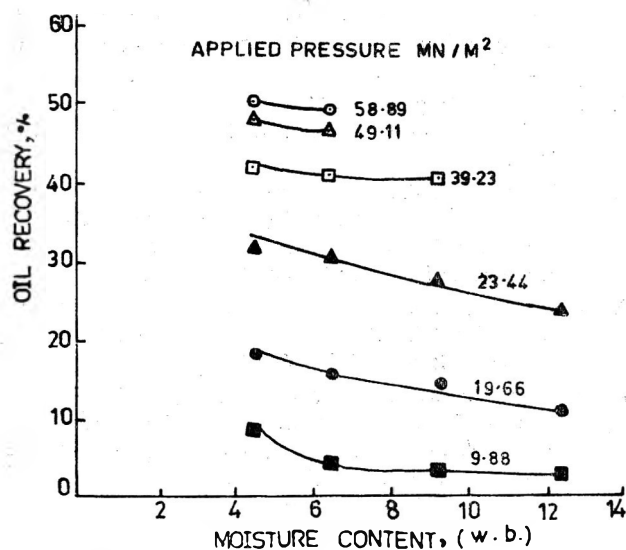
Fig. 5. Effect of moisture content on oil recovery efficiency for rapeseed at 10mm/min rate of deformation

Fig. 6. A general relationship for prediction of oil recovery efficiency

cent moisture seed. At 93.6 per cent of bed deformation for 4.56 per cent moisture seed a maximum of 62.32 per cent oil recovery was achieved at applied pressure of 58.89 MN/m^2 and 19.5 mm/min rate of deformation. Such a pronounced effect of moisture content on oil yields as noticed in the present study is similar to the reported results of Dedio et al.[6] on flax seed and Carter[7] on cottonseed.

Combined effects of pressure and rate of deformation: Analysis of data showed that individual effects of moisture content, pressure and rate of deformation on oil recovery that have been discussed could be emperically correlated such that.

$$Y = 1 - \exp.\ -0.0043\ (P-Po)\ \sqrt{1+R} \qquad ..(2)$$

Equation (2) could fit all the 76 data points corresponding to different pressures, the four levels of moisture content and rates of deformation, with a correlation co-efficient of 0.985.

Apparently, the moisture dependence of oil point pressure Po is adequate to account for the effect of moisture content on oil recovery efficiency of rapeseeds under uniaxial bulk compression. To check the hypothesis, the emperical constant of the prediction equation was evaluated separately for the data with different moistures. Four different values were obtained. They were 0.00442, 0.00420 0.00447 and 0.00372 respectively for moisture contents of 4.56, 6.46, 9.35 and 12.35 per cent. Correlation coefficients ranged from 0.982 to 0.988, the lowest being that for 12.35 per cent moisture. It is readily seen that the first three values of the constants are substantially the same, it is only at 12.35 per cent moisture that the value is somewhat different. Therefore at least over the moisture range of 4.56 to 9.35

per cent, the constant could be considered independent of moisture content. Taking the data of only the above three moisture contents the value of averaged constant comes to 0.00432 with a correlation co-efficient of 0.986. A comparison of those two values with the corresponding ones of equation (2) shows that at least to a first approximation, the equation is adequate to account for the effect of moisture content over the range of the variables of the present study.

In the earlier part of the discussion, it has been indicated that at constant pressure and moisture content oil recovery shows a linear increase with the rate of deformation. While equation (2) includes $\sqrt{1+R}$ explicitly and a function of the rate of deformation, which is through Po, implicitly. Variation of Po with R is given in equation (2). Apparently the combined effect of the two taken together is such that the resultant when plotted against rate of deformation at constant pressure tends to linearity in the region of 5 to 19.5 mm/min rate of deformation. Thus, equation (2) could be taken as adequate expression for predicting the oil recovery under uniaxial bulk compression. A data plot of oil recovery efficiency against (P-Po) $\sqrt{1+R}$ is given in Fig. 6.

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Carbohydrate and Fatty Acid Composition of Fermented Melon Seeds *(Citrullus vulgaris)*

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Sucrose and galactose were the predominant sugars in unfermented melon seeds. Fermentation decreased the total disaccharide and increased the total monosaccharides composition whereas it did not have much effect on the fatty acid composition except for a slight increase in the total unsaturated and decrease in total saturated fatty acids. During the 5-day fermentation, the pH, titratable acidity, soluble solids and soluble nitrogen increased.

The chemical changes which take place during food fermentation have not been fully investigated in Nigeria where fermented foods are in common use. Some act as staple and others as condiments in salads and as flavouring agents in soups. Fermentation transforms the raw material into a new product with characteristic texture, odour and flavour. It is a form of food processing as some fermented foods do not undergo further processing before use. The understanding of the chemical changes during fermentation may help the fermenter direct the process to the desired product. As part of an investigation on the biochemical and nutritional changes of indigenous fermented food, this study was aimed at investigating the effect of fermentation on carbohydrates and fatty acids of melon seeds. Melon seeds *(Citrullus vulgaris)* belong to the family Curcurbitaceae and are popular seeds in Nigeria They are early maturing short season (perennial) plants with very good yield. Many varieties of melon are grown and consumed in various ways; they are potential source of food nutrients[1-3]. They are used to prepare the popular 'egusi' soup, they are milled, wrapped in leaves and boiled "igbalo" or "mgbam" or they may be fried to produce "irobo"[3]. They are also fermented to produce "ogiri" and used as condiments to season or flavour soups. The seeds are high in proteins and essential amino acids[1,2].

Materials and Methods

Fresh, sun-dried melon seeds *(Citrullus vulgaris)* were purchased from the local market in Nigeria. The standard sugars and chemicals used were purchased from BDH England and trimethylsilylated sugars (TMS) were purchased from Sigma Chemical Company.

Preparation and fermentation: The seed coats were removed by hand pressure and the seeds were boiled, wrapped in aluminium foil for 2 hr and were ground in a mortar. Sodium chloride was added (1.0g/kg

sample) and the sample was inoculated with a previously dry fermented sample (3 g/kg sample). The inoculum in 5 ml distilled water was thoroughly mixed with the sample, wrapped in an aluminium foil and incubated for 5 days at 31 °C and freeze dried for analyses.

Total and soluble nitrogen, soluble solids, pH and titratable acidity were measured according to standard methods[4]. Procedures for the extraction of sugars and their derivatisation were the same as described by Li and Schuhmann[5] with minor modifications.

Preparation of stock solutions: The pyridine reagent was prepared in a 50 ml quantity with 25mg/ml hydroxylamine hydrochloride and 2mg/ml β-phenyl-D-glucopyranoside as the internal standard and stored at −5°C until required for use. Standard sugars were prepared with 20 mg of each sugar in 10 ml water and 1 ml was derivatised along with the sample after drying.

Extraction of sugar from the sample: Fat was removed from 5 g of freeze dried sample with n-hexane and the sample was extracted with 20 ml 80 per cent methanol for 24 hr with constant shaking (600 cycles/min) at room temperature. The sample was centrifuged (2000 rpm for 6 min) and the extracts concentrated to 1 ml in a vacuum evaporator at 30°C, then dried under nitrogen in a water bath at 50°C. The last trace of water was removed in a vacuum dessicator.

Derivatisation: The dried extract was heated with 1 ml of pyridine reagent for 30 min, the tubes were cooled and 0.5 ml of hexamethyldisilazane and 4 drops of trifluoroacetic acid added and vigorously mixed at room temperature. I μl of the sample and standard was injected into the GLC.

Gas chromatography: A Pye-104 gas-liquid chromatograph equipped with flame ionisation detector was used. The glass column (2.1m ×6.4mm i.d.) was packed with 3 per cent W/W OV-101 on 80/100 mesh

chromosorb W and preconditioned for 48 hr. The injector and detector temperatures were maintained at 300°C and the oven temperature was programmed at 5°C/min from 110 to 300°C with a 30 min hold at 300°C. Flow rates. N_2-30 ml/min, H_2-30ml/min and air 300 ml/min. Gas chromatographic peaks were identified by comparison of retention times with those of standard sugars. The area of the peak for each sugar was recorded and the sugar contents were quantified and expressed as mg sugar/g sample on dry weight basis.

Analysis of fatty acids: Fat was extracted from 2g of the sample with hexane under reflux for 6 hr. Component fatty acids were determined after methylation with a Pye - Unicam 104 GCV gas-liquid chromatography with flame ionisation detector. The column (2.7 m ×6.4 mm i. d.) was packed with GP 10 per cent SP2 300 on 80/100 supelcopot. Flow rates were N_2-30ml/min, H_2-33ml/min and air-330ml/min. Detector and injector temperatures were 240°C and 215°C respectively. The column/oven temperature was 215°C.

Results and Discussion

Table 1 shows the pH, titratable acidity, soluble solids, total and soluble nitrogen of the fermented melon seeds. There was a gradual increase in pH from 6.2 to 7.8 and an increase in titratable acidity. There was an increase in soluble solids (9-30%), soluble nitrogen (0.4-3.1g/100g) and not much change in total nitrogen. A pH increase despite an increase in titratable acidity was observed during a 72 hr fermentation of soybean substrate to produce tempeh[6,7] and during the fermentation of African oil bean seed (Achinewhu S.C. unpublished). This was attributed to active proteolytic activities and deamination of amino acids. A pH rise was also observed[8] during the fermentation of melon to produce *ogiri*. Increased proteinase activity, total

TABLE 1. pH, TITRATABLE ACIDITY, SOLUBLE SOLIDS, TOTAL AND SOLUBLE NITROGEN CONTENT OF THE FERMENTING MELON SEED

Days of fermentation	pH	Acidity (% lactic acid)	Soluble solids (%)	Nitrogen (g/100g)	
				Total	Soluble
0	6.2±0.03	0.11±0.002	9.0±0.05	5.6±0.06	0.4±0.001
1	6.3±0.05	0.18±0.002	9.5±0.03	5.6±0.02	0.5±0.003
2	6.8±0.02	0.45±0.001	11.0±0.08	5.3±0.05	1.8±0.01
3	7.3±0.08	0.63±0.001	27.0±0.03	5.4±0.05	2.6±0.02
4	7.6±0.03	0.09±0.0003	29.6±0.5	5.4±0.03	3.0±0.05
5	7.8±0.05	1.08±0.02	30.4±0.3	5.4±0.02	3.1±0.02

Values are mean ± SE of 4 determinations.

TABLE 2. CARBOHYDRATE COMPOSITION OF UNFERMENTED MELON SEEDS

Peak No.	Sugars	Concn. (mg/g dry wt)	% of total carbohydrate
1	Fructose	1.05±0.03	2.1
2	Galactose	5.0 ±0.40	10.0
3	Unknown	0.20±0.0007	0.4
4	Glucose	1.0 ±0.008	2.0
5	Glucose+galactose	0.4 ±0.001	0.8
	Total monosaccharides	7.65±0.44	15.3
6	Sucrose	42.35±1.5	84.7
	Total disaccharides	42.35±1.5	84.7
	Total carbohydrates	50.0 ±1.9	

* mean ± SE of 5 determinations.

Peak 1-5 comprise of total monosaccharides and 1-6 total carbohydrates.

TABLE 3. CARBOHYDRATE COMPOSITION OF FERMENTED MELON SEEDS

Peak No.	Sugars	Concn. (mg/g dry wt)	% total carbohydrate
1	Unknown	6.80±0.200	15.40
2	Unknown	7.64±0.500	17.30
3	Unknown	0.20	0.45
4	Fructose	7.84±0.300	17.80
5	Galactose	5.20±0.200	11.80
6	Unknown	0.20±0.001	0.45
7	Unknown	0.20±0.001	0.45
8	Unknown	0.20±0.001	0.45
9	Unknown	0.20±0.001	0.45
10	Unknown	0.20±0.001	0.45
11	Glucose	0.20±0.001	0.45
12	Glucose+galactose	0.20±0.001	0.45
13	Unknown	0.20±0.001	0.45
14	Sucrose	3.30±0.2	7.5
15	Maltose	11.56±0.5	26.2

* Mean±SE of 5 determinations.

Peak 1-13 comprise total monosaccharides and 1-15 total carbohydrates.

dissolved nitrogen and amino nitrogen has been reported[9] during the fermentation of soy sauce moromi.

Tables 2 and 3 show the carbohydrate compsosition of the unfermented and the fermented seeds respectively. The unfermented seed had a high content of surcrose (84. 7% of the total carbohydrate) which was reduced to 7.5 per cent after fermentation. There was not much change in the galactose content but an increase in the maltose content was noticed. The unidentified monosaccharides with high concentration appearing before fructose might be pentoses. Arabinose and ribose have been identified[10] at retention times before fructose in sugar standards containing them. Xylose in addition to ribose had also been identified[11]. The high concentration of maltose in the fermented seed was probably due to the breakdown of starch in the seed by α-amylases. Five varieties of melon seeds have been shown to contain between 10 and 13 per cent starch[12]. There was probably a further breakdown of maltose to glucose for microbial metabolism. Perhaps, more maltose was produced than was further broken down into utilizable glucose; hence the fairly high concentration of maltose. Fermentation decreased the total disaccharides to about 34 per cent and increased the total monosaccharides to 66 per cent of the total carbohydrates. Very high α-amylase activity had been demonstrated in fermenting melon[13]. Increase in α-amylase activity and an increase in total reducing sugar during the fermentation of soy sauce moromi had also been demonstrated[9].

Table 4 gives the fatty acid composition of the melon seeds. There was not much difference between the total fatty acids after fermentation except for the presence of trace amounts of C_8-C_{12} fatty acids. It is not known whether they might contribute to the characteristic odour and flavour of the fermented seed. During fermentation of peanuts, it was shown that micro-organisms did not appear to have utilized lipids[14,15] and there was no noticeable increase in lipase activity during the fermentation of melon seed[13]. Melon seed contained over 60 per cent linoleic acid which is an essential fatty acid and fermentation did not affect this acid.

Increase in soluble solids of the fermented melon observed in the present study indicated a general increase in the solubility of major and perhaps minor components produced by fermentation. Such increase will no doubt increase the food value of the product. Increase in soluble nitrogen and soluble sugars also indicated increased activities of the fermenting enzymes thereby leading to improved digestibility. The present study indicates that fermentation may improve the

TABLE 4. FATTY ACID COMPOSITION OF MELON SEEDS

Fatty acids	Per cent of total oil	
	Unfermented	Fermented
8:0 Caprylic	—	0.01 ± 0.00001
10:0 Capric	—	0.02 ± 0.0003
12:0 Lauric	—	0.05 ± 0.0001
14:0 Myristic	0.03 ± 0.0001	0.02 ± 0.0001
16:0 Palmitic	11.67 ± 0.3	10.78 ± 0.3
17:0 Margaric	—	—
18:0 Stearic	10.20 ± 0.4	10.04 ± 0.6
Total	21.90 ± 0.7	20.92 ± 0.9
18:1 Oleic	14.56 ± 0.5	15.21 ± 0.8
18:2 Linoleic	62.86 ± 1.2	63.51 ± 1.6
18:3 Linolenic	0.22	0.28
Total	77.64 ± 1.7	79.00 ± 2.4
Total fatty acids	99.54 ± 2.4	99.92 ± 3.3
% saturated	22.00	20.9
% unsaturated	78.00	79.1

* Values are mean $\pm$ SE of 4 determinations.

melon seed as a source of food nutrients. Further biochemical and nutritional studies are in progress.

Acknowledgement

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Flow Characteristics of Pulp, Juice and Nectar of 'Baneshan' and 'Neelum' Mangoes

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The power law model was used to calculate the rheological constants of pulp, juice and nectar of 'Baneshan' and 'Neelum' mangoes at various temperatures. The consistency coefficient values were considerably lower for pulp, juice and nectar of 'Baneshan' than those of 'Neelum'. Consistency coefficient decreased with increase in temperature, while there was no appreciable effect on the values of flow behaviour index. Arrhenius type equation indicated positive correlation ($r^2 \geqslant 0.97$) between consistency coefficient and inverse absolute temperature. Apparent viscosity decreased appreciably with increase of spindle speed and temperature in all the cases.

Mango (*Mangifera indica* L.) is known to be the king among the tropical fruits and greatly relished for its succulence, exotic flavour and delicious taste. India is the principal mango growing country sharing more than 70 per cent of the world's production of 11.8 million tonnes[1]. In India, mangoes are used for processing both at the raw and ripe stages. In ripe stage processing, the products manufactured are juice, nectar, ready-to-serve beverages containing fruit juice, squash and jam.

Knowledge of the rheological properties of fluid foods is essential for the proper design of equipment and for various unit operations as well as for understanding of the pertinent transport processes in the operations[2-5]. Several foods such as milk, filtered juices, vegetable oils and syrups are Newtonian fluids. For these foods, knowledge of the viscosity function and its dependence upon temperature and concentration is sufficient for engineering design[6]. However, a large number of fluid foods are non-Newtonian in nature. The power law model (Equation 1) has been employed extensively to relate the shear rate, γ and the shear stress, τ of these fluids[3,7].

$$\tau = m.\gamma^n$$

Typical magnitude of the consistency coefficient, m and flow behaviour index, n of food products can be found in the literature[3,8]. There is no literature on rheological properties of mango products. The objective of this paper is to report the effect of composition, shear rate and temperature on viscometric characteristics of pulp, juice and nectar of 'Baneshan' and 'Neelum' mangoes.

Materials and Methods

Preparation of material: Mangoes (cv. 'Baneshan' and 'Neelum') of uniform size and maturity were obtained from Parbhani local market. After washing, sorting and peeling, the pulp was extracted using a fruit pulper. It was heated to 85°C for 2 min, hot filled in sterilized glass jars having air tight stoppers.

The mango juice and nectar were prepared according to Krishnamurthy et al.[9] The juice contained 35 per cent pulp, 20 per cent total soluble solids and 0.3 per cent acidity, whereas mango nectar contained 20 per cent pulp, 18 per cent total soluble solids and 0.3 per cent acidity.

Chemical analysis: Moisture, titratable acidity, pH, reducing and total sugars, pectin (as calcium pectate) and insoluble solids were determined in pulp, juice and nectar according to the methods given in Manual of Analysis of Fruits and Vegetables[10]. Crude fibre was determined by AOAC method[11]. Total soluble solids (TSS) were read in a hand refractometer.

Rheological measurement: The Brookfield synchrolectric viscometer (Model, RVT) was used to measure the rheological characteristics of pulp, juice and nectar at various spindle speeds (0.5 to 50 rpm) and at temperature range of 40 to 80°C. The test sample was heated to the desired temperature in a water bath. It was then transferred into the measuring bowl which was surrounded by temperature regulating vessel. The product of dial reading on instrument and factor on chart gave the apparent viscosity in centiPoise. The values were converted to Poise.

TABLE 1. CHEMICAL COMPOSITION OF PULP, JUICE AND NECTAR OF 'BANESHAN' AND 'NEELUM' MANGOES*

Constituent	Pulp		Juice		Nectar	
	Baneshan	Neelum	Baneshan	Neelum	Baneshan	Neelum
Total soluble solids(°Brix)	17	19	20	20	18	18
Moisture (%)	81	74.2	78.1	78.0	81.1	81.2
Titratable acidity as citric acid (%)	0.6	0.4	0.3	0.3	0.3	0.3
Total sugars (%)	15.6	16.7	18.2	18.1	16.3	16.0
Reducing sugars (%)	6.9	5.2	5.7	4.5	5.2	4.1
Crude fibre (%)	0.89	0.95	0.16	0.17	0.03	0.06
Pectin as calcium pectate (mg/100g)	1.17	1.34	0.35	0.38	0.08	0.14
Insoluble solids (%)	2.41	5.96	1.40	1.43	0.83	0.89

*Each value is the average of three replicates.

Results and Discussion

In general, no considerable differences were observed in the composition of pulp, juice and nectar samples between the two varieties of mango except per cent moisture and insoluble solids in case of pulps (Table 1).

The values of apparent viscosity at various shear rates and temperatures are given in Tables 2 and 3. Pronounced decrease of apparent viscosity with increase in spindle speed was observed indicating pseudoplastic nature in all the cases. The pseudoplastic behaviour of mango pulp has been reported by earlier workers[12,13]. The apparent viscosity decreased considerably with increase in temperature at identical speed. Its values for pulp were considerably higher than those of juice and nectar in both the cultivars. Pulp, juice and nectar of 'Neelum' recorded higher values of apparent viscosity than those of 'Baneshan'. This may be attributed to the higher content of insoluble solids, dry matter, crude fibre and pectin for 'Neelum' than 'Baneshan' (Table 1). Effect of insoluble solids and pectin on rheological properties of various fruit pulps and juice concentrates has been reported[14,15]. The power law model was employed to estimate the rheological constants. The following equation was used.

$$\mu a = \left(\frac{1}{n} \right)^n (4\pi N)^{n-1} m,$$

or

$$\log \mu a = (n \log \frac{1}{n} + \log m) + (n-1) \log 4 \pi N \quad (2)$$

where, μa is the apparent viscosity in Poise; n, the flow behaviour index; m, the consistency coefficient, dynes-sec^n/cm^2 and N, the revolutions per second.

TABLE 2. APPARENT VISCOSITY (POISE) FOR PULP, JUICE AND NECTAR OF 'BANESHAN' MANGOES*

Temp. (°C)	Viscosity at indicated speeds (rpm)						
	0.5	1.0	2.5	5.0	10.0	20.0	50.0
			Pulp				
40	1300	830	500	300	180	110.0	56.0
50	1000	750	448	280	165	97.5	50.0
60	860	650	392	230	128	87.5	43.8
70	780	500	304	204	115	80.5	38.0
80	700	490	280	185	110	72.5	34.8
			Juice				
40	67.0	38.4	18.80	11.0	6.40	3.77	—
50	58.0	35.0	17.00	10.1	5.75	3.42	—
60	52.4	31.7	14.96	8.4	5.10	3.27	—
70	45.0	27.5	13.96	8.2	4.95	3.07	—
80	41.0	24.5	13.16	7.4	4.45	2.75	—
			Nectar				
40	9.2	5.9	3.0	1.80	1.10	0.60	—
50	8.6	5.2	2.5	1.50	0.90	0.56	—
60	8.0	4.8	2.4	1.40	0.87	0.51	—
70	6.8	4.0	2.3	1.25	0.83	0.43	—
80	5.5	3.5	2.1	1.20	0.76	0.40	—

*Each value is the average of two determinations.

TABLE 3. APPARENT VISCOSITY (POISE) FOR PULP,
JUICE AND NECTAR OF 'NEELUM' MANGOES*

Temp. (°C)	Viscosity at indicated speeds (rpm)						
	0.5	1.0	2.5	5.0	10.0	20.0	50.0
Pulp							
40	1500	1000	470	320	200	120.0	64.0
50	1300	900	440	310	185	110.5	58.0
60	1100	705	372	246	150	92.0	51.8
70	900	600	328	236	148	84.0	44.6
80	860	590	296	220	135	80.0	43.6
Juice							
40	89.0	48.5	23.5	13.7	8.0	4.75	—
50	85.4	46.5	22.1	12.6	7.3	4.30	—
60	82.0	45.0	21.3	12.0	7.0	4.10	—
70	66.0	41.0	18.4	10.7	6.2	3.70	—
80	59.0	37.5	17.4	10.1	6.0	3.40	—
Nectar							
40	13.0	8.0	3.88	2.30	1.41	0.87	—
50	12.4	7.4	3.76	2.22	1.36	0.82	—
60	11.8	7.0	3.68	2.16	1.30	0.74	—
70	10.5	5.2	3.20	1.90	1.15	0.70	—
80	8.8	5.1	3.00	1.80	1.10	0.68	—

*Each value is the average of two determinations.

Fig. 1. Influence of temperature on consistency coefficient for pulp, juice and nectar of 'Baneshan' and 'Neelum' mangoes

The consistency coefficient was considerably lower for pulp, juice and nectar of 'Baneshan' than that of 'Neelum' variety (Fig. 1). These values for juices of 'Baneshan' and 'Neelum' were 29 and 25 fold lower and for nectars 166 and 159 times lower respectively than those of their pulps. In general, consistency coefficient decreased considerably with increase in temperature. Similar observations for tomato concentrates have been reported by earlier workers[16,17]. There was no appreciable effect of temperature on the values of flow behaviour index, n; the variation in the values over the temperature range of 40 to 80°C were 0.309 to 0.343 and 0.314 to 0.354 for pulp of 'Baneshan' and 'Neelum' respectively. These values are in good agreement with those reported by Rao et al.[12] and Rao et al.[13] However, the consistency index, m is higher which might be due to variation in chemical composition of pulps.

The effect of temperature on consistency coefficient[5] can be described by an Arrhenius type equation.

$$\ln m = \ln A - \left(\frac{E}{R}\right) \frac{1}{T_A} \qquad (3)$$

Simple linear correlation was employed to estimate the values of Arrhenius rate constant and activation energy required for viscous flow. By obtaining data at fairly limited number of temperatures, its influence over a wide range can be predicted. Highly significant positive correlation ($r^2 \geq 0.97$) was observed between consistency coefficient and inverse absolute temperature (Fig. 1).

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Preparation of Foam-mat Dried and Freeze Dried Whole Egg Powder (Hen's)*

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Foam-mat dried and freeze dried hen's egg powder were prepared from egg melange of uniform composition and compared for various processing parameters and proximate composition. Glucose, total solids and β-carotene contents varied slightly from batch to batch. The material balance data for both types of egg powder were nearly the same. Though the drying techniques were different, the powders prepared by these techniques were similar in chemical and organoleptic quality. The bacterial load was brought down substantially by pasteurization and by hygienic handling.

Egg powder is an introduced Service item procured and supplied as per ASC and ISI specifications. This item has got several advantages over the fresh egg. Fresh egg is issued to troops in forward areas and on board ships and submarines. Being seasonal and fragile, they are difficult to supply to operational areas and cost more by way of packaging. transportation and losses due to breakage. Further, they are cumbersome to prepare into dishes especially where limited cooking facilities are available in operational areas. Under such circumstances, dehydrated egg powder offers several logistic advantages. Being dehydrated, it has

longer shelf life than the fresh eggs under ambient conditions and is therefore available throughout the year.

Drying reduces both weight and volume and is a very important consideration in handling eggs[1]. There are various methods of drying eggs like pan drying[2], belt drying[3], fluff or foam drying, foam spray drying[4], spray drying, accelerated freeze drying, lyophilization, etc. Some of these methods described in literature vary with respect to time and temperature and combinations are also used for drying. Only a few of these techniques like spray drying have been used commercially, but to a limited extent, freeze drying is

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also used. Freeze drying which is of comparatively recent origin, is a costlier process, although the product quality is superior. Foam-mat-drying has continued to remain a laboratory curiosity and one does not come across production of foam-mat dried egg powder on a large scale, although the method is much simpler and perhaps less costly. These different methods of drying yield products having different physico-chemical properties. The present study was undertaken to prepare foam-mat dried and freeze dried egg powders from the same melange and compare the physico-chemical properties, microbiological changes and organoleptic quality.

Materials and Methods

The eggs of "White Leghorn" birds were procured locally, washed with water and then with "Tween 80" to remove adhering material. The cleaned eggs were soaked in 2 per cent bleaching powder solution for 30 min. Finally, they were washed and dried at room temperature to remove surface moisture.

The eggs were then broken by hand and inspected visually for any spoilage. The egg white and yolk were homogenised with a mechanical stirrer. The mixture was filtered through muslin cloth.

Desugaring of egg melange was done as described earlier[5]. Baker's wet yeast was used at the concentration of 600 mg per 100 g of melange. After desugaring, the egg melange was pasteurised at 64°C for 3 min in a water bath, and then immediately cooled to 4°C with ice and salt.

Preparation of foam-mat dried egg powder: The egg melange was brought to room temperature (19-25°C) from 4°C. A 1000 g/batch of melange was made into foam with a stirrer rotating at the speed of 1000-1300 r.p.m. using a perforated ladle and the foam was spread on aluminium trays measuring 122×46×2.5 cm, with perforated mesh of 0.4 mm size. Each tray had about 350 g of foam. The trays were immediately transferred to a cross-flow drier, in which the velocity of hot air was maintained at 500–600 linear feet/min. The foam was dried at 50–60°C for 10 to 15 min. The dried powder was scraped by a stainless steel ladle and collected in an air tight tin container. Twenty to 22 batches of egg powder were prepared, each consisting of 100-150 eggs. The powder from all the batches was mixed and packed. Quantitative data are given in Table 1.

Preparation of freeze dried egg powder: The pasteurised egg melange was poured into aluminium trays (65×65×2.5 cm) at the rate of 5 kg per tray. Two such trays were kept in a blast freezer maintained at -20°C. After freezing at this temperature for 3-5 hr, the material was taken out and cut into 25 mm^2 blocks and placed on drying trays. The blocks were dried in a Socaltra freeze drier as per the method described by

Sankaran and Sharma[6]. Spray dried egg powder conforming to ISI specification[7] was procured from a commercial firm.

Moisture content, total lipids, total ash, acid insoluble ash, and β-carotene of raw egg melange and egg powder were determined by AOAC[8] methods. Total nitrogen and water soluble nitrogen were determined by micro-kjeldhal method. Glucose content of egg pulp and powder was determined by Folin Wu[9] method as described earlier[5]. Scrambled egg score/acceptability of the powders was determined by the method of Kline et al[10]. Standard plate counts and coliform counts in 10g samples were determined on standard plate agar and violet red bile agar respectively according to standard procedures[11]. Presumptive *Salmonella* detection[11] was carried out on 25 g samples by tetrathionate broth enrichment followed by plating on xylose-lysine-decarboxylase agar and biochemically confirming the presumptive colonies. Solubility was determined by the modified Haeenni method as described in ISI specification[7].

Results and Discussion

The egg powders were prepared as per Iyengar et al[12]. The foam-mat-dried egg powder prepared under laboratory conditions requires further drying in a vacuum shelf drier for bringing down the moisture content to around 2 per cent. The freeze dried product does not require any further drying.

The data presented in Table 2 show the proximate analysis of liquid egg melange used for the preparation of both the types of egg powders namely foam-mat dried (FMD) and freeze dried (FD) egg powder. There is not much difference in the initial pH of egg liquid obtained from different batches of eggs. The variation was only 0.1 to 0.2 units. Freshly laid eggs generally have a pH around 7.7 to 7.9 and stored eggs have a pH of about 8.3.

TABLE 1. MATERIAL BALANCE DATA FOR THE PREPARATION OF FOAM MAT-DRIED (FMD) AND FREEZE DRIED* (FD) WHOLE EGG POWDER

Particulars	FMD	FD
Av. egg wt	58±4.00	59±1.41
Shell(%)	11.66±1.10	11.36±0.67
Wt. of melange (kg)	5.64±1.23	12.92±0.87
Filtration loss (%)	2.38±0.33	2.10±0.22
Recovery (%)	91.48±5.65	94.81±2.81

100-150 eggs in FMD and 200-275 in FD

TABLE 2. PROXIMATE ANALYSIS OF RAW EGG MELANGE USED FOR THE PREPARATION OF FOAM-MAT-DRIED AND FREEZE DRIED WHOLE EGG POWDER

Particulars	Mean$\pm$SD*
Initial pH	8.16$\pm$0.09
Glucose(g%)	0.4 $\pm$0.00
Total solids(g%)	25.22$\pm$0.77
Total lipids(g%)	10.42$\pm$0.56
Total protein (N$\times$6.68) (g%)	12.34$\pm$0.56
β-carotene (μg%)	0.05$\pm$0.10

*n=4

The data also show that the glucose content does not vary much from batch to batch. In the liquid egg, the glucose content varies from 382 to 396 mg per cent. A similar but greater variation (from 310 to 410 mg per cent) has been reported by Iyengar *et al*[12]. Since the range of glucose content in raw liquid egg is very wide, it is always advisable to standardize the conditions for desugaring with respect to the glucose content in the melange. This will help in optimising the quantity of desugaring agent. The total solid content of the egg melange ranges from 24 to 26 per cent and is important for calculating the yield. The total protein and fat contents of raw egg melange are in agreement with the reported values[13-15].

TABLE 3. PROXIMATE COMPOSITION OF FOAM-MAT-DRIED (FMD) AND FREEZE DRIED (FD) WHOLE EGG POWDER

Particulars	FMD	FD
Moisture (%)	2.37$\pm$0.71	0.19$\pm$0.04
Free fat (%)	36.29$\pm$1.06	38.65$\pm$0.06
Total ash (%)	3.21$\pm$0.08	3.42$\pm$0.13
Acid insoluble ash (%)	0.03$\pm$0.00	0.03$\pm$0.00
Total protein (%) (N$\times$6.68)	48.57$\pm$0.86	51.25$\pm$1.28
Water soluble nitrogen (%)	3.6 $\pm$0.22	4.07$\pm$0.06
Solubility (%)	96.64$\pm$1.39	96.84$\pm$1.5
Glucose (%)	0.07$\pm$0.01	0.06$\pm$0.0071
β-carotene (μg%)	0.05$\pm$0.0045	0.068$\pm$0.0205
Scrambled egg acceptability score	7.6$\pm$0.4	7.2 $\pm$0.22

Values are mean $\pm$SD (n=5)

TABLE 4. BACTERIAL LOAD IN LIQUID EGG AND FREEZE DRIED WHOLE EGG POWDER

Raw liquid egg		Pasteurised liquid egg		Whole egg powder	
Total count/ml	Coliform count/ml	Total count/ml	Coliform count/ml	Total count/ml	Coliform count/g
118$\times$10^2	214$\times$10^1	50$\times$10^2	Nil	100$\times$10^2	16$\times$10^1
100$\times$10^2	123$\times$10^1	—	Nil	109$\times$10^1	15$\times$10^1
112$\times$10^2	116$\times$10^1	20$\times$10^1	4$\times$10^1	125$\times$10^2	14$\times$10^1

Salmonella is absent in all samples

Table 1 shows the material balance data for the preparation of FMD and FD egg powders. It is seen that the weight of the extra large egg is between 53 and 63g. The average weight of the shell (10-13 per cent) and the loss of egg melange on filtration (2 to 2.5 per cent) are similar to those reported values[12].

Proximate composition as well as solubility (95-97%) and organ eptic scores of egg powder prepared by FMD or FD processes were comparable to the commercial spray dried sample (Table 3).

The aerobic bacteria and coliforms of raw liquid egg decreased considerably by pasteurisation (Table 4). Coliforms were totally eliminated (in two batches) although aerobic bacteria survived to the extent of 10^2-10^3. During dehydration, although destruction of bacteria occur to some extent, the elevated bacterial levels in the egg powder suggest post-processing contamination from equipment and handling.

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Bound Residues of Phosphine in Milled Products of Wheat

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Presence of the computed residues of phosphine (PH_3) at higher levels than the determinable free PH_3 residues and the absorption bands other than the one at 400 nm have provided evidences for the presence of chemically bound residues in three milled products of wheat. Bands due to chromophores at 560 and 600 nm are common to all the three milled products. Increase in intensity of bands suggests the continuous nature of the reaction while decrease would suggest the decomposition during storage. These bound residues were highly toxic to *Tribolium castaneum* adults. Depending on the levels of bound residues, per cent mortalities are 16 to 98 in whole wheat flour, 3 to 100 in *Maida* and 3 to 98 in semolina, while it was nil in control. Whole wheat flour holds higher levels of bound residue than the other two products.

On fumigation, cereals and their milled products absorb a certain amount of phosphine depending on their gas holding capacity[1-2]. The kinetics of desorption of phosphine residue from cereals and their milled products[3-5] have also provided circumstantial evidence for the presence of bound residues of PH_3. Physically bound determinable residues have been found to decrease significantly during 7 days of airing. The absorption band at 400 nm characteristic of physically bound phosphine residue-silver nitrate chromophore would decrease in intensity, as free PH_3 residue level decreases and ultimately disappears.[3-4] Due to stratification of desorbed PH_3 during storage, only small amounts of PH_3 could be discerned on certain days, followed by significantly large amounts on subsequent days[2-5] suggesting that some amount of PH_3 absorbed by the commodity would have been held in some chemically bound form, which would decompose and be responsible for such prolonged desorption.

The purpose of this paper is to provide evidences for the presence of bound residues in three milled products of wheat.

Materials and Methods

Whole wheat flour (*atta*), refined wheat flour (*maida*) and semolina (*soji* or *rawa*) were obtained from the International School of Milling Technology of this Institute. Experimental fumigation was done at 2 kg level as reported[5].

Immediately after fumigation, the initial PH_3 residue was determined[1] as reported[5] (Tables 1, 2 and 3). The same extracts (after dilution in the case of *atta*) were

TABLE 1. PH₃ AND PHOSPHORUS RESIDUES IN WHOLE WHEAT FLOUR AFTER DIFFERENT DAYS OF AIRING AND AFTER STORAGE

	PH₃ residue (p.p.m.)		Phosphorus	
	Determined	Computed	Whole wheat flour (mg/100g)	Side tube (μ g)
0 day airing				
	0.47±0.05	—	0.86±0.07 (9.46)	—
2 days airing				
Immediately after airing	0.25±0.13	0.39±0.08	1.09±0.14 (11.95)	—
After 60 days in storage	X	0.03±0.01	3.11±0.61 (34.10)	16.99±4.04 (0.19
4 days airing				
Immediately after airing	0.24±0.04	0.36±0.07	1.93±0.40 (21.20)	—
After 60 days in storage	X	0.03±0.01	3.31±0.33 (36.43)	9.83±2.93 (0.11)
7 days airing				
Immediately after airing	0.07±0.01	0.31±0.06	2.14±0.13 (23.47)	—
After 60 days in storage	X	0.03±0.02	3.98±0.78 (43.64)	5.01±0.42 (0.05)

Mean ± SD of 8 replicates
X = Below the estimatable limit of the method
Value in the parantheses is the equivalent amount of PH₃ in p.p.m.

TABLE 2. PH₃ AND PHOSPHORUS RESIDUES IN WHITE FLOUR AFTER DIFFERENT DAYS OF AIRING AND AFTER STORAGE

	PH₃ residue (p.p.m.)		Phosphorus	
	Determined	Computed	Refined flour (mg/100g)	Side tube (μ g)
0 day airing				
	0.20±0.01	—	0.49±0.20	—
2 days airing				
Immediately after airing	0.056±0.01	0.17±0.01	0.96±0.02 (10.56)	—
After 60 days in storage	XX	0.0	1.11±0.33 (12.17)	13.40±5.82 (0.15)
4 days airing				
Immediately after airing	0.09±0.04	0.19±0.03	1.29±0.50 (14.13)	—
After 60 days in storage	XX	0.0	1.81±0.30 (19.86)	9.06±2.86 (0.10)
7 days airing				
Immediately after airing	0.03±0.01	0.13±0.02	1.72±0.47 (20.94)	—
After 60 days in storage	XX	0.0	2.30±0.32 (23.17)	7.23±1.33 (0.08)

Mean ± SD of 8 replicates
XX : Below the estimatable limit of the method. Value in the parantheses is the equivalent amount of PH₃ in μ g.

TABLE 3. PH₃ AND PHOSPHORUS RESIDUES IN SEMOLINA AFTER DIFFERENT DAYS OF AIRING AND AFTER STORAGE

	PH$_3$ residue (p.p.m.)		Phosphorus	
	Determined	Computed	Semolina (mg/100g)	Side tube (μg)
0 day airing				
	0.13$\pm$0.06	—	0.40$\pm$0.24	—
2 days airing				
Immediately after airing	X	0.28$\pm$0.09	0.49$\pm$0.10 (5.43)	—
After 60 days storage	X	0.02$\pm$0.01	0.75$\pm$0.24 (8.22)	26.75$\pm$6.99 (0.29)
4 days airing				
Immediately after airing	X	0.26$\pm$0.08	0.71$\pm$0.13 (7.82)	—
After 60 days storage	X	0.04$\pm$0.01	0.9$\pm$0.31 (10.25)	23.07$\pm$5.79 (0.25)
7 days airing				
Immediately after airing	X	0.22$\pm$0.07	0.81$\pm$0.05 (8.84)	—
After 60 days storage	X	0.02$\pm$0.00	0.99$\pm$0.36 (10.90	15.30$\pm$3.79 (0.17)

Mean $\pm$ SD of 8 replicates

X = Below the estimatable limit of the method. Values in parentheses are the equivalent amounts of PH$_3$ in μg.
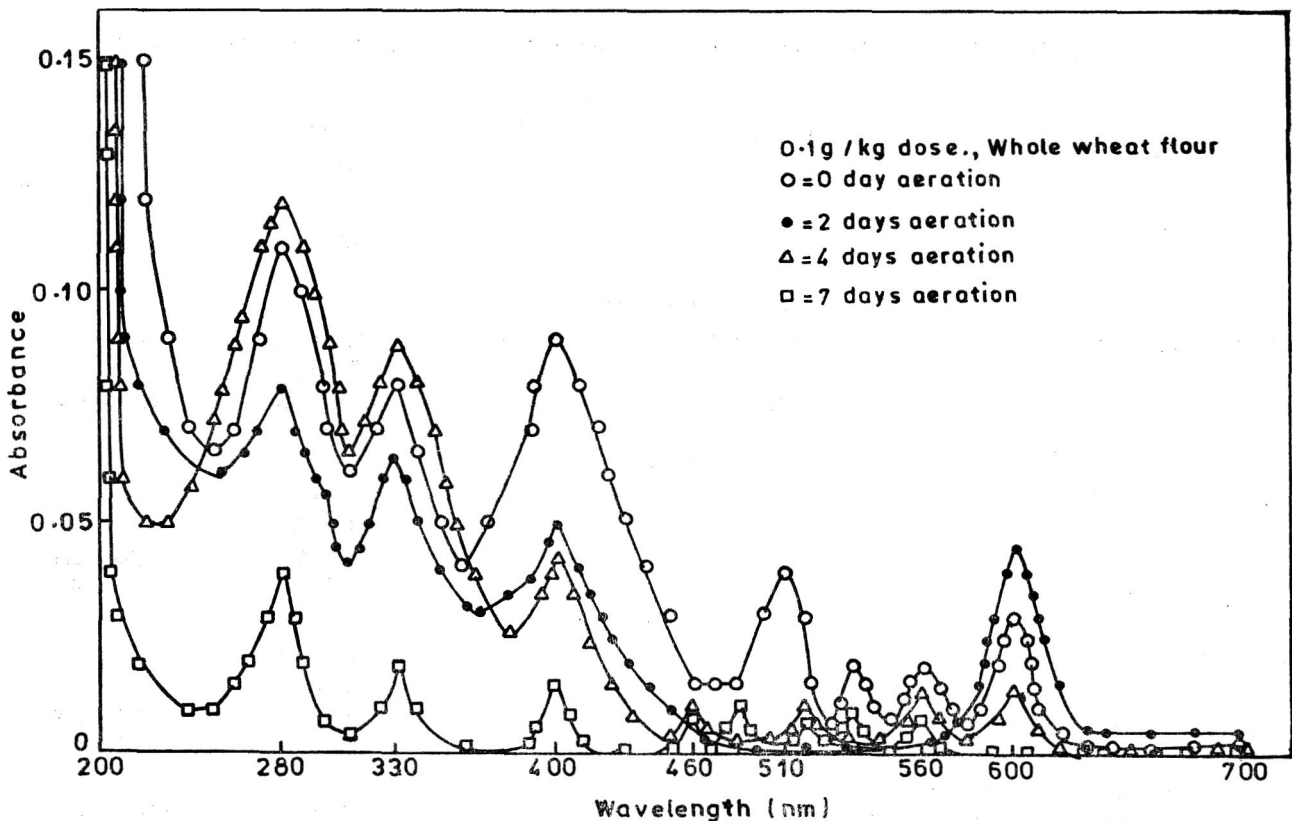

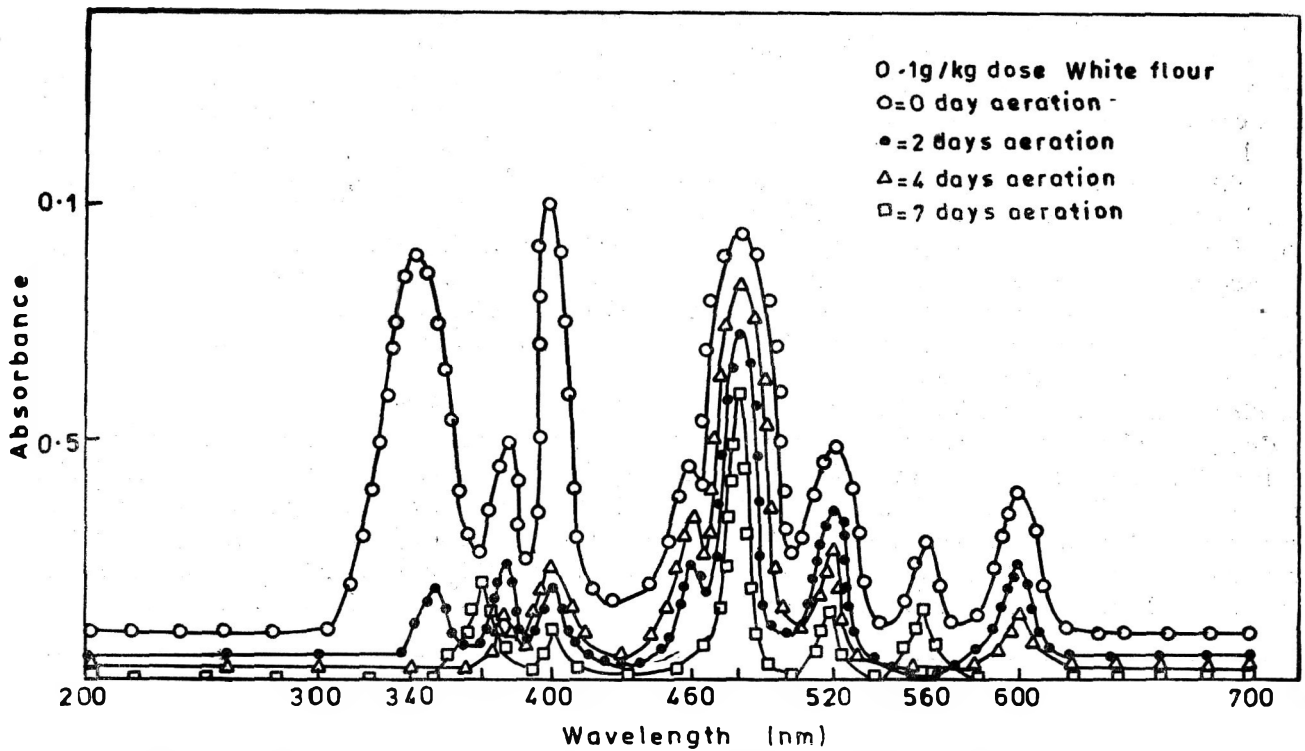Fig. 1. Absorption spectra of phosphine residue—AgNO$_3$ chromophores from whole wheat flour.

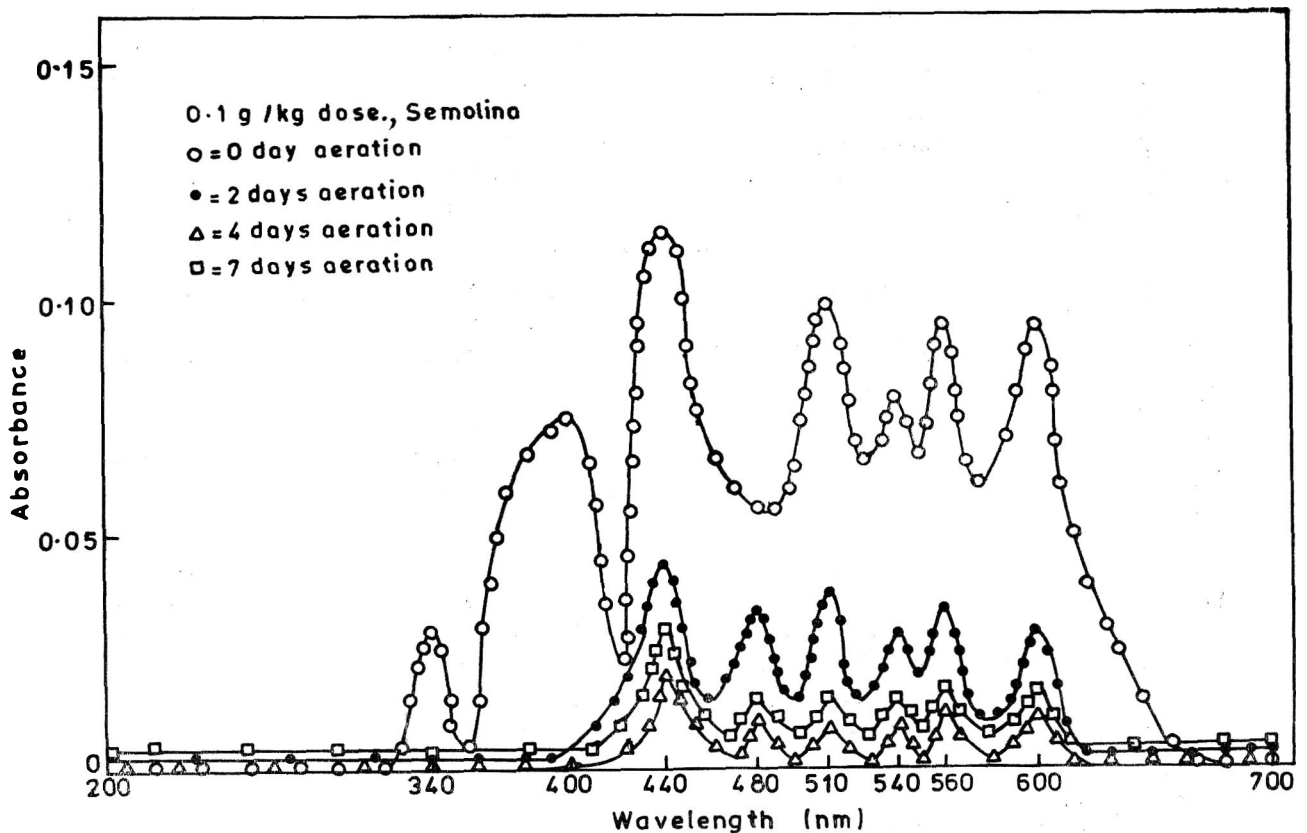Fig. 2. Absorption spectra of phosphine residue—AgNO₃ chromophores from white flour.

Fig. 3. Absorption spectra of phosphine residue—AgNO₃ chromophores from semolina.

used for scanning the spectra of PH_3 residue–$AgNO_3$ chromophore against the corresponding commodity control blank (Fig. 1, 2 and 3). Also, 10 g samples of each commodity were drawn for the determination of initial level of inorganic phosphorus as reported earlier[3,4] by the method of Bruce et al[6]. Airing of remaining commodities, determination of PH_3 residues, phosphorus levels, absorption spectra of bound PH_3 in these aired samples, and desorption experiments by storing 200 g aired commodities in gas-tight flasks having side spout were done as reported[2-5]. In all these determinations, an equivalent quantity of the corresponding commodity control was used as blank.

Toxicity studies: One hundred gram portions of each of the three commodities aired for 7 days were transferred to 250 ml conical flasks in 6 replicates. Five pairs of *Tribolium castaneum* adults (0-7-day old) were released and the flasks were closed with glass stoppers. Corresponding control experiments were

also done using non-fumigated commodities. After 7 days, the commodities were sieved to look for survivors. In control commodities after 7 days of oviposition, all the insects were sieved off and the larvae were allowed to grow. In case of fumigated commodities, after sieving off dead-insects, fresh pairs of insects were released at the end of 1, 2 and 3 weeks. At the end of the 3rd week, the commodities were aired for a further period of 2 weeks and another 5 pairs of insects were released. After one week, the insects were sieved off and the mortality data worked out (Table 4).

Results and Discussion

There is a fall in the initial physically bound residues by 50 per cent in *atta* at the end of 2 days airing (Table 1); the fall over 7 days of airing is concomitant with the decrease in the height of the peak (Fig. 1) at 400 nm due to free PH_3 residue-$AgNO_3$ chromophore. Airing for a further period of 48 hr did not cause any change

TABLE 4. TOXICITY OF BOUND RESIDUES OF PH3 TO TRIBOLIUM CASTANEUM ADULTS

Observation at the end of wk	Mortality (%)		Bound residues (p.p.m.)
	Control	Fumigated and aired for 1 wk	
Whole wheat flour			
1	6.66±12.1	98.3±4.1	0.27±0.001
2	—	93.3±12.1	0.23±0.0
3	Good no. of larvae	90.0±15.5	0.20±0.006
4	–do– and pupae	airing	
5	Larvae + pupae	airing	
6	–do–	5 pairs released	0.09±0.002
7	–do–	16.7±13.1	0.06±0.002
White flour (Maida)			
1	0.0±0.00	100.0±0.0	0.10±0.005
2	—	86.0±5.5	0.07±0.001
3	Good no. of larvae	63.3±12.1	0.06±0.001
4	–do–	airing	
5	–do–	airing	
6	–do–	5 pairs released	0.007±0.001
7	–do–	3.34±8.16	B
Semolina (Soji)			
1	0.0 ±0.0	98.0±4.5	0.18±0.001
2	—	82.0±8.4	0.16±0.002
3	Good no. of larvae	68.3±19.4	0.12±0.002
4	Larvae + pupae	airing	
5	–do–	airing	
6	–do–	5 pairs released	0.06±0.001
7	–do–	3.30±5.16	0.04±0.003

Mean ± SD of 6 replicates
B = Below estimatable limit of the method.

in the residue level (Table 1). Even after 7 days of airing, *atta* could retain as high as 0.07 p.p.m. free PH_3 residue (Table 1) which is higher than the FAO/WHO permissible limit of 0.01 p.p.m. At the end of 60 days of storage, free PH_3 residue (Table 1) in all the aired samples was below the level of determination by the method[1], whereas PH_3 residue computed from desorbed PH_3 is quite significant and is almost the same (0.03 p.p.m) in all the three samples of *atta*. The computed* PH_3 residue at the end of 2, 4 and 7 days airing is higher than the estimatable free PH_3 residues. This difference (0.237 p.p.m.) is significantly higher in 7 days aired sample as more of free PH_3 is lost during 7 days of airing. These values conclusively establish the presence of chemically bound residues, which in part form the source for such prolonged desorption of PH_3 from fumigated commodities. There is no significant difference among samples aired from 2 to 7 days in the values of computed residues (0.31 to 0.39 p.p.m.) (Table 1). This suggests the uniformity in the formation of chemically bound residues during the period of contact of PH_3 with *atta*.

The absorption spectra of PH_3 residue–$AgNO_3$ chromophore from *atta* aired for 0–7 days is shown in Fig. 1. Bands at 280 and 330 nm are shown by all the 4 samples, While 0 day aired sample shows peaks at 510, 530, 560 and 600 nm, 2 days aired sample shows only an intense peak at 600 nm. Samples aired for 4 and 7 days show peaks at 460, 530 and 560 nm, these two samples also show additional peaks one each at 600 and 500 nm respectively. Although the precise information conveyed by these absorption peaks is being studied from the pattern of change in their height during storage, it can be said that they represent interaction complexes between the reactive sites and PH_3. The absorption bands at 280 and 330 nm are very strong and their intensity in the spectra of 4-day aired sample is greater than that aired for 0 day. Also, the intensity of absorption of the chromophore at 600 nm from 2-day-aired sample is the highest indicating the continuous nature of reactions forming bound residues. The absorbance at 460, 530 and 560 nm continuously decreased as the duration of airing was increased indicating that these reaction complexes are more unstable and hence start decreasing in intensity during 7 days of airing. Reaction complexes formed in 7-day-aired *atta* also show absorption bands at all the wavelengths shown by 0-day aired sample except at 600 nm, but with lower intensity. A few newer bands have also emerged as a result of reaction at additional sites during the period of contact; for example, 4 and 7-day aired samples show

additional bands at 460 and 510 nm while 7- day aired sample shows one more at 480 nm.

Maida holds lower initial PH_3 residue than *atta* and a major portion of this was lost over 7 days of aeration (Table 2). *Maida* is unique in showing levels of computed residue below determinable limits at the end of 60 days storage. This may be due to either higher rate of decomposition of chemically bound residues or due to formation of irreversibly bound residues.

Absorption spectra of PH_3 residue–$AgNO_3$ chromophore from maida (Fig 2) aired for 0–7 days reveal the following points. Zero day aired sample exhibits eight peaks at 340, 380, 460, 480 520, 560 and 600 nm due to bound residues, the bands at 340 and 480 being highly intense. Intensity of bands at 400 460 and 480 nm of 4-day aired sample is more than that from the 2-day aired one which substantiates the residue values determined (Table 2). Contrary to the gradual but slow decrease in intensity of peaks at 480 nm over 0–7 days of airing, peak at 340 nm of 0-day aired sample has completely disappeared. Bands at 380, 520 and 600 nm show regular decrease in their intensities over 7 days of airing, suggesting a gradual decomposition of these bound residues. Two-day aired sample shows a new band at 350 nm while the original band at 560 nm is missing. Similarly, 7-day aired sample shows a new band at 370 nm, while the original bands at 380, 460 and 600 nm have completely disappeared. These observations suggest that the emergence of new peaks is due to the reaction of residual PH_3 with additional sites, while disappearance indicates complete decomposition of those bound residues.

Semolina (Table 3) holds the least amount of initial free PH_3 residue (0.134 p.p.m.) of the three milled products, and the residue after 2 days of airing was below the estimatable limit. Despite such low initial residues, it is surprising to find a large computed residue level (0.22 p.p.m.) in the 7-day aired sample, in addition to 0.02 to 0.04 p.p.m. at 60 days of storage. These values clearly demonstrate the formation of chemically bound residues.

Examination of absorption spectra of semolina aired for 0 to 7 days reveals that the band at 400 nm has disappeared completely at 2-day airing due to total loss of free PH_3 residue, which substantiates absence of determinable residues (Table 3). Similarly, the band at 340 nm has disappeared due to complete decomposition of the reaction complex on 2 days of airing. In 0-day aired sample, bands at 440, 510, 540, 560 and 600 nm show regular fall due to decomposition over 4 days of airing and then show a slight increase in intensity at the

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* The residue level during storage was computed after studying the total amount of phosphine desorbed during the next 60 days.

end of 7 days of airing. Such peculiar behaviour may be due to delayed reaction of desorbed PH_3. The new band at 480 nm in 2-day aired sample also behaves similarly.

Irrespective of period of airing, a commodity will hold a definite amount of computed residue of PH_3 and it appears to be constant for that commodity. Values of computed residues (Tables 1, 2 and 3) in 2, 4 and 7 days aired samples show that these values in *atta* are 0.31 to 0.39 p.p.m; in *maida* 0.13 to 0.19 p.p.m. and in semolina 0.22 to 0.28 p.p.m. Such computed residues which arise due to slow and continuous decomposition of bound residues indicate the inherent property of individual milled products to form bound residues.

Bands due to bound residues at 560 and 600 nm appear to be common to these three milled products. Although the band at 480 nm is also common, it appears at different stages of aeration as for example. 480 nm (7-day aired *atta*, 0, 2, 4, 7 day aired *maida* and 2, 4, 7 days aired semolina), 460 nm (4, 7 days aired *atta* and 0, 2, 4 days aired *maida*), this is totally missing from the spectra of semolina. Compared to the spectra of *atta*, those of *maida* and semolina have the first band at 340 nm, showing the absence of chromophores having the absorption in the far UV region. *Maida* and semolina are different from *atta* in that bran is totally absent in them. It is known that bran is rich in unsaturated oils and vitamins. So, the bands in the spectra of *atta* in the region of 200–300 nm may be due to bound residues formed as a result of reaction of these constituents of bran with PH_3.

After 60 days of storage, there is not much difference (Table 1) in phosphorus content of 2 and 4-day aired *atta*. Of the 3 milled products, *atta* shows the highest amount of phosphorus at the end of 60 days (Tables 1, 2 and 3). During airing, a regular increase in the amount of phosphorus formed has been observed in all the three milled products. Phosphorus content in 2 days aired *atta* is nearly trebled, and in 4 and 7 days aired, it is nearly doubled during 60 days of storage (Table 1); while the corresponding increases in *maida* (Table 2) and semolina (Table 3) are not that significant due to low initial residue of PH_3 in them. These levels indicate that the major portion of the free PH_3 residue is oxidised to phosphorus compounds in storage suggesting the bound residue formation during fumigation. Oxidation of PH_3 in the side tube is not significant due to the efficient and fast reaction of PH_3 with the detector strip.

The per cent mortality decreases as the bound residue level decreases (Table 4) in all the three products. At the end of 3 weeks, 90 per cent mortality is caused at

0.2 p.p.m. in *atta* while it is 63 and 68 per cent in *maida* and *soji* containing 0.06 and 0.12 p.p.m. bound residue respectively. Such variations depend on the amount of bound residue ingested with the food by the insect and also to some extent on the levels of PH_3 gas built up in the container as a result of decomposition of bound residues. At the end of 7 weeks, 16% mortality was observed in *atta* with 0.06 p.p.m. bound residues compared to only 3 per cent mortality in *soji* with 0.04 p.p.m. bound residue indicating that the mortality rate depends on the amount of bound residues ingested. It was also found that all the larvae fed on *atta* with 0.036 p.p.m. bound residue were dried up. Under similar conditions, the mortality is not only nil in control commodities but also population propagation was in good progress. These observations suggest the presence of the toxic chemically bound residue in PH_3 fumigated commodities. Further work on the mode of toxic action of ingested bound residues is in progress.

In conclusion, it can be said that the higher levels of computed residues than the physically bound residues, and appearance of absorption bands due to chromophore other than free PH_3 residue indicate the presence of chemically bound residues formed due to interaction of PH_3 with receptive sites in these commodities. For the first time in the history of phosphine fumigation, formation of bound residues of phosphine has been established.

Acknowledgement

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Evaluation of Processed Protein Fractions of Castor Bean (*Ricinus communis* L.) Meal with Rats

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Castor meal proteins were fractionated into salt-soluble and alkali-soluble fractions and these solvent extracted castor bean meal proteins were screened as a protein source for weanling rats. Salt-soluble and alkali-soluble fractions were found to be non-toxic. However, they did not prove to be growth stimulatory. Alkali-soluble proteins showed a better response.

Castor seed, an inedible oilseed, is an excellent source of oil (50%) and protein (20%) and about 0.91 million tons of castor bean are available per year. Castor meal finds its principal application as a fertiliser. Its utility as a protein source for feed is restricted due to the presence of water-soluble, nutritionally deleterious and toxic components. A great deal of information is available about the nature and mechanism of action of various toxic principles in castor seed meal[1-3], and several detoxification and de-allerginisation processes have been devised[4-5] for the defatted castor bean meal. But no acceptable preparation of the protein has been made available for animal or human consumption so far.

The aims of the present investigations are to make suitable preparations of castor meal proteins and to explore the possibility of utilising these protein fractions as a feed for rodents instead of the total detoxified meal proteins reported previously.[6-8] After removing the water-soluble toxic proteins, the castor bean meal is processed into salt-soluble and alkali-soluble fractions by a simple procedure under ordinary laboratory conditions instead of conventional methods adopted earlier[4-5].

Materials and Methods

Castor bean meal (CBM) under study was a commercial mixed meal; it had been partially detoxified during hydraulic pressing of the castor beans to extract oil. The last traces of oil were removed by extraction with petroleum ether (40–60°C) for 18 hr.

Preparation of protein samples (A and B): Defatted castor bean meal was first extracted exhaustively with distilled water to remove the water-soluble proteins. The residue was then treated with salt and then with alkali to get salt-soluble (A) and alkali-soluble (B) proteins respectively. The extraction procedure is described below–
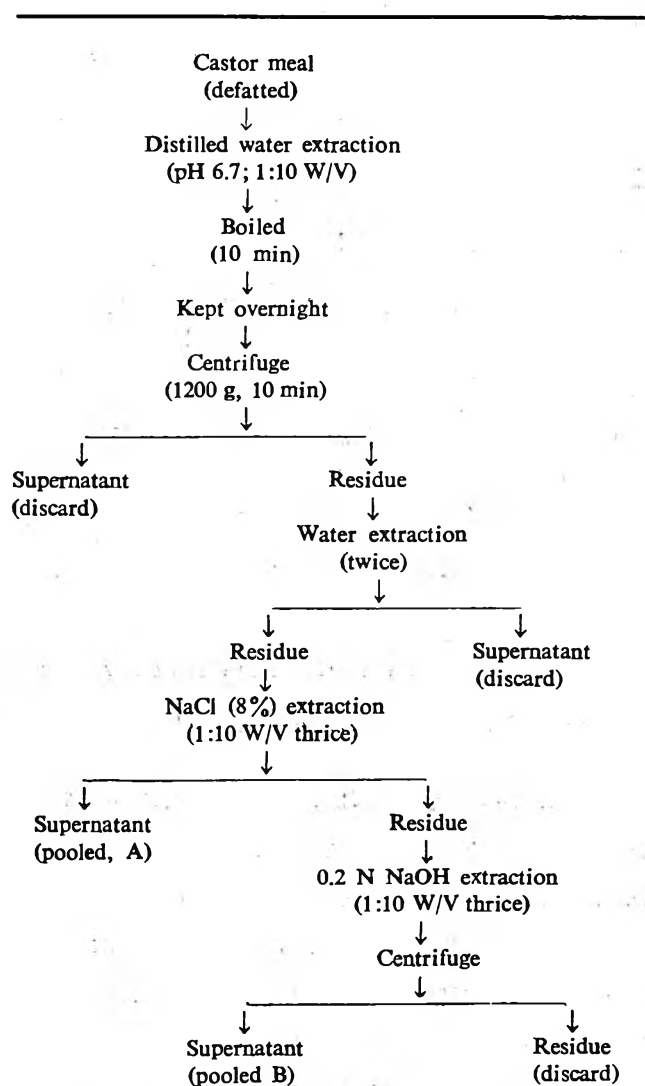

Fig. 1. Flow-sheet diagram for preparation of protein fractions A and B from castor bean meal.

Supernatant A and B were exhaustively dialysed against distilled water (1:10 v/v) for 48 hr with repeated changes of water and centrifuged. The precipitates so obtained were washed with acetone and ether twice and the air dried material was powdered and stored in the desiccator. Protein in these fractions was estimated by biuret and Kjeldahl methods[9-10].

Biological experiments: Three-week old weanling albino rats of either sex were randomly allocated to six groups of six rats each.

The Laboratory control (LC) group was fed the Hind Lever Laboratory feed.

Casein prepared freshly from milk and dried and fed at 10 per cent level to the experimental control (C) group. Test groups A and B were fed the salt-soluble (A) and alkali-soluble (B) proteins at 10 per cent level respectively. Supplemented groups, A_s and B_s were maintained on diets containing A or B at 10 per cent level and 1 per cent lysine and 0.5 per cent tryptophan.

The rats were fed a diet prepared by the method of Muramatsu and Ashida[11] using salt mixture prepared according to Osborne and Mendel[12] and vitamin mixture as described by Chapman *et al.*[13] The rats were housed individually in metal cages. The assigned protein diet and water were offered *ad libitum*. Left over diet was collected and weighed daily. Change in body weight of rats was noted after every seven days. Feed efficiency ratio was calculated from this data.

On the 31st day, blood was collected from these rats under mild ether anaesthesia and they were sacrificed immediately. Liver, kidney, heart, brain, spleen, intestine and lungs were dissected out and washed with ice cold normal saline. Ten per cent homogenates of liver, brain and intestine and five per cent homogenates of kidney and heart were made in ice cold distilled water. The samples were centrifuged at 10,000 r.p.m. at 4°C and supernatants were taken for all the biochemical estimations. The protein was precipitated from each sample with 10 per cent TCA and estimated by the biuret method[9].

Acid (EC 3.1.3.2) and alkaline phosphatase (EC 3.1.3.1) activities were measured routinely by estimating the release of phenol[14] and inorganic phosphorus[15] respectively. Glutamate oxalo-acetate transaminase (GOT; EC 2.6.1.1) and glutamate pyruvate transaminase (GPT; EC 2.6.1.2) activities were measured according to Rietman and Frankar[16]. Xanthine oxidase (XOD; EC 1.2.3.2) activity was measured by the method of Bauer and Bradley[17] and succinic dehydrogenase (SDH EC 1.3.99.1) activity according to the method of Srikantan[18].

Results and Discussion

Physico-chemical studies: Fractions A and B were found to be 100 per cent proteins when estimated by biuret and Kjeldahl's methods. Both protein fractions were insoluble in normal saline acetate buffer (0.1M, pH 5.0), tris-maleate buffer (0.1M, pH 6.0), phosphate buffer (0.1M, pH 7.4) and veronal buffer (0.1M, pH 8.6), but were soluble in 0.01N NaOH

A probe into the amino acid composition revealed that fraction A was almost compatible with the whole castor bean meal (CBM) fraction reported in literature[19], whereas fraction B was deficient in some of the essential amino acids. The work of Vilhyalmsdottir and Fischer[19] with CBM on chicks suggested that castor bean meal

TABLE 1. FEED EFFICIENCY RATIO AND HAEMOGLOBIN CONTENT OF RATS ON DIFFERENT DIETS

Parameter studied	Lab. control	Casein control	Salt-sol. Protein (A)	A+lys+tryp	Alkali sol. Protein (B)	B+tryp.+lys.
Wt gain/loss in 4 wk (g)	68.00*±2.68	41.00*±1.15	-11.00*	-10.33*	-8.33*	-9.50*
Av. daily food intake (g)	9.1*±0.25	5.6*±0.15	3.0*±0.05	3.6^a±0.19	4.5*±0.13	4.0*±0.07
Av. daily gain in wt (g)	2.27*±0.11	1.37*±0.09	-ve	-ve	-ve	-ve
Food efficiency gain/feed (g)	0.249	0.245	-ve	-ve	-ve	-ve
Growth depression (%)	0	39.7	116.2	115.2	112.9	113.9
Mortality (%)	nil	nil	43	nil	nil	nil
Av. haemoglobin content (g%)	11.6*±0.22	10.4^a±0.13	7.9 ±0.11	10.0^a±0.06	9.8^a±0.07	10.2 ±0.09

Mean ±SE of 6 rats; ^{a} not significantly different from group Bs
-ve=negative.
*Significant at $P<0.05$ from all the groups.

can be further processed so as to provide a much improved source of protein for monogastric animals after supplementation with limiting amino acids, lysine and tryptophan. Keeping this point in view, castor bean meal was processed in the present investigation into (A) salt-soluble and (B) alkali-soluble fractions, and these fractions were fortified with lysine and tryptophan for feeding.

Acute toxicity studies on proteins did not show any evidence of toxicity to mice. However, mortality was observed in group A animals (Table 1) but postmortem examination did not reveal any abnormality or symptoms of castor bean intoxication as described in the literature[20].

Rat growth studies: Food intake was decreased with all four castor bean meal fractions, with concomitant decrease in growth rate (Table 1). However, alkali-soluble protein groups (B & B_s) showed a better response than salt-soluble groups (A & A_s). Feed efficiency ratio on different diets showed a trend similar to the growth response, and negative feed efficiency values were obtained with CBM proteins. The reduced intake of food resulted in producing a condition similar to that of

protein-calorie malnutrition in these animals[21]. The low intake of food by rats in the present studies indicate that inspite of fortifying the protein fractions with lysine and tryptophan, the diet is still imbalanced as regards the amino acid make-up. The amino acid imbalance of any kind results in depression of growth, decrease in food consumption and in some cases certain pathological lesions in young rats[22].

The haemoglobin content of the rats of groups A and B (Table 1) evidenced the presence of mild anemia. The amino acid supplemented groups had haemoglobin content somewhat very similar to that of casein fed group of rats. Nutritional quality of vegetable proteins is often quoted as a factor determining iron utilisation both in man and animal[23].

The malnourished state of rats fed the CBM proteins is further reflected in the significantly lower weight of all vital organs except brain (Table 2) and total protein in these organs. This decrease was proportional to the low body weight of these animals. Similar relationship between body weight and liver, spleen and other organs, and protein has been observed in several amino acid imbalance and protein-energy deficiency investigations[24].

TABLE 2. ORGAN WEIGHTS AND PROTEIN CONTENT OF ORGANS OF RATS

Organs studied	Dietary regime					
	Lab control	Casein control	Salt sol. protein (A)	A + lys + tryp	Alkali sol. protein (B)	B + lys + tryp
Average weight (g)						
Liver	4.00 ± 0.23	3.28 ± 0.23	$1.50^{ab}\pm0.16$	$1.11^{ab}\pm0.19$	$1.49^{ab}\pm0.32$	$1.43^{ab}\pm0.18$
Kidney	0.92 ± 0.06	0.83 ± 0.01	$0.16^{ab}\pm0.06$	$0.54^{ab}\pm0.06$	$0.44^{ab}\pm0.07$	$0.40^{ab}\pm0.07$
Heart	0.36 ± 0.01	0.35 ± 0.05	$0.17^{ab}\pm0.02$	$0.14^{ab}\pm0.02$	$0.18^{ab}\pm0.03$	$0.18^{ab}\pm0.04$
Spleen	0.32 ± 0.03	$0.19^{a}\pm0.01$	$0.09^{ab}\pm0.02$	0.08 ± 0.01	$0.09^{ab}\pm0.02$	$0.14^{ab}\pm0.01$
Lungs	0.58 ± 0.04	0.62 ± 0.07	$0.38^{ab}\pm0.05$	$0.36^{ab}\pm0.05$	$0.34^{ab}\pm0.05$	$0.38^{ab}\pm0.04$
Brain	1.40 ± 0.08	1.37 ± 0.10	1.29 ± 0.05	1.28 ± 0.08	1.20 ± 0.10	1.25 ± 0.12
Average protein content (mg/100 mg wet tissue)						
Liver	20.00 ± 0.00	20.63 ± 1.43	$13.15^{ab}\pm1.39$	$15.40^{ab}\pm0.23$	$18.25^{c}\pm1.25$	$20.50^{cd}\pm1.19$
Kidney	15.33 ± 2.80	10.50 ± 1.85	10.25 ± 1.18	10.00 ± 0.82	$16.00^{bcd}\pm1.08$	$15.00^{cd}\pm1.47$
Heart	14.25 ± 0.15	$11.00^{a}\pm1.08$	$8.35^{a}\pm0.85$	$10.00^{a}\pm0.82$	$10.75^{a}\pm1.03$	$11.00^{a}\pm1.00$
Brain	9.25 ± 0.45	$13.65^{a}\pm0.62$	$9.81^{b}\pm0.29$	11.25 ± 2.46	$9.75^{b}\pm1.11$	$10.75^{b}\pm0.64$
Intestine	19.00 ± 1.18	$4.35^{a}\pm0.62$	$5.36^{c}\pm0.24.$	$5.00^{a}\pm0.00$	$8.75^{ab}\pm1.49$	$10.75^{abc}\pm2.21$

Mean $\pm$ SE of 6 rats

Significant difference at $P<0.05$; [a] from Laboratory Control (LC) group; [b] from Casein Control (C) group; [c] from Salt soluble group (A) and [d] from Salt-soluble group supplemented with lysine and tryptophan (A_s).

The growth inhibitory effect of the CBM protein fractions A and B and especially that of lysine and tryptophan supplemented fractions was quite unexpected. Studies on chicks suggested that partial or complete removal of fibre will increase the potential use of CBM protein for monogastric animals[19]. However, the present data indicate that protein fractions obtained without fibre did not improve food consumption or gain in weight. To further assess their nutritional status, their response at the tissue level was studied by measuring the activities of certain enzymes known to be affected by the dietary state of the organism.

Data in Fig. 2 indicate that, in general, the specific activity (activity/mg protein) of the transaminases, namely GOT and GPT decreased in liver, kidney, heart and brain when compared to the casein (C) group. Acid and alkaline phosphatase activities (Fig. 3) increased in liver, decreased a little in kidney and remained more or less same as the casein (C) group in heart and brain. The specific activities of succinic dehydrogenase and xanthine oxidase (Table 3) remained almost same in all the organs with different diets. These findings are quite similar to those observed in severe protein, energy and protein-energy malnutrition states[25]. But inspite of all these factors, the animals were still able to survive. This shows that some other factors besides the protein level and the protein adequacy can influence enzyme activities. Barrow[26] and Barrow and Chow[27] showed that the difference in the activities of liver XOD
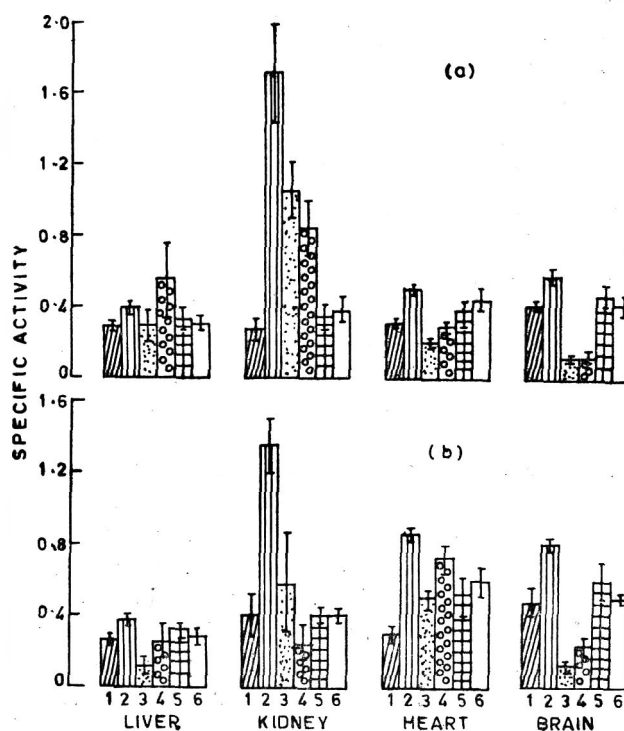

Fig. 2. Specific activity (activity/mg protein) of GOT (a) and GPT (b) in different organs of rats.

1. Laboratory control group (LC); 2. casein control group (C); 3. Salt-soluble protein (A); 4. Salt-soluble protein supplemented with lysine and tryprophan (As); 5. Alkali-soluble protein (B); 6. Alkali-soluble protein supplemented with lysine and tryptophan (Bs).

TABLE 3. SUCCINIC DEHYDROGENASE AND XANTHINE OXIDASE ACTIVITIES IN DIFFERENT ORGANS OF RATS

Organs studied	Lab control	Casein control	Salt-sol. Protein (A)	A + lys + tryp	Alkali sol. Protein (B)	B + lys + tryp
SDH activity						
Liver	0.03±0.006	0.02±0.004	0.03 ±0.008	0.02 ±0.007	0.03 ±0.012	0.01 ±0.001
Kidney	0.04±0.011	0.05±0.012	0.05 ±0.015	0.04 ±0.013	0.06 ±0.013	0.04 ±0.010
Heart	0.05±0.007	0.03±0.002	0.02^{ab}±0.001	0.03^{c}±0.005	0.08^{bcd}±0.002	0.04^{c} ±0.006
Brain	0.01±0.002	0.01±0.003	0.02 ±0.002	0.02^{a}±0.002	0.02 ±0.009	0.01^{d}±0.002
XOD activity						
Liver	0.01 ±0.008	0.01±0.003	0.01±0.003	0.01±0.001	0.00±0.00	0.00±0.00

Mean ± SE of 6 rats

Significant difference at P±0.05;   [a] from laboratory Control (LC) group;   [b] from Casein Control (C) group;   [c] from Salt-soluble group (A) and [d] from Salt-soluble group supplemented with lysine and tryptophan (As).

SDH activity=Succinic dehydrogenase activity, μ moles TTC reduced/30 min/mg protein.

XOD=Xanthine oxidase activity.
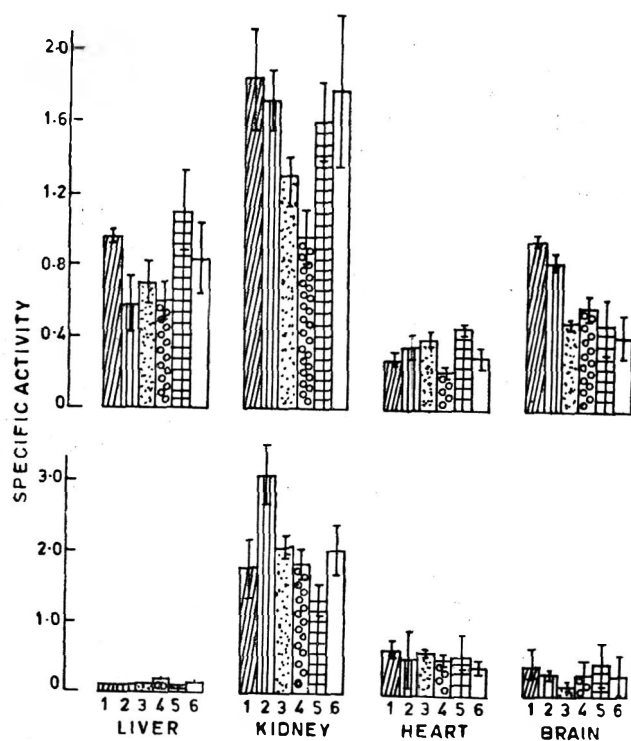
TTC=Triphenyl tetrazolium chloride.

Fig. 3. Specific activity (activity/mg protein) of acid phosphatase
(a) and alkaline phosphatase (b) in different organs of rat.
Legend as in Fig. 1

and SDH of rats fed different levels of proteins may be
due to the presence of unknown substances in the
protein preparations.

The present studies indicate that the solvent extracted
protein fractions, though not toxic, are inferior in
quality and castor bean meal protein could not be
recommended as the sole protein source for laboratory
animals.

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WATER VAPOUR ADSORPTION BY CORN AND RICE FLOURS

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Equilibrium moisture content of corn flour (cv. 'Ganga-5') and rice flour (cv. 'PBN-1') was studied at 15, 25 and 40°C and in the relative humidity ranges of 11 to 89%. At a given temperature and relative humidity, products having higher starch and protein contents had higher equilibrium moisture. Free energy change, isosteric heat and net heat of adsorption have been calculated. Out of four isotherm equations tested, Smith's equation gave the best fit to the data.

Several factors such as surrounding atmosphere, temperature and moisture content affecting the hygroscopic properties of corn and rice flours have not been so well defined as would be desirable for handling, storage and processing operations. For proper storage of food products, the humidity and temperature of the surrounding atmosphere should be such that the moisture contents are maintained within the safe limits without affecting the quality. Therefore, a study of equilibrium moisture content of foods as a function of temperature and relative humidity is of considerable practical utility. Products such as corn and rice flours are important food items, as also raw materials for many processed foods.

Sorption of water vapour by wheat flour[1-4] and flours of soybean, Bengal gram, chickpea have been reported[5]. However, there is no published information on adsorption of water vapour by rice and corn flours. This paper describes equilibrium moisture content of these flours under different temperature and humidity conditions along with associated free energy change and heats of adsorption over a water activity of 0.11 to 0.89 at 15, 25 and 40°C. Applicability of some isotherm equations available for biological materials has been examined.

Paddy (cv. PBN-1') and corn (cv. 'Ganga-5') were obtained from the Central Farm, Marathwada Agricultural University, Parbhani. Moisture, per cent protein for the whole flours (particle size 0.212 mm)

of polished rice and corn were determined by standard AOAC methods[6]. Quantitative estimation of starch of both the flours was carried out according to the method of Wankhede *et al*[7].

The samples dried in vacuum oven at 60°C for 24 hr had moisture content of less than 3 per cent. Two gram samples of the flour were weighed into dishes (5 cm diameter) with lids and placed in desiccators containing appropriate salt solutions at 15, 25 and 40°C. To determine the equilibrium moisture content, the samples were weighed with lids on after every 48 hr until there was no further change in their weights. Establishment of equilibrium varied with relative humidity and the temperature.

The chemical composition revealed that whole corn flour and whole rice flour contained 75 and 65 per cent starch and 10.5 and 6.7 per cent proteins, respectively.

The equilibrium moisture content values are given in Table 1. Caking was observed in both materials at

TABLE 1. EQUILIBRIUM MOISTURE CONTENT (EMC) OF CORN FLOUR AND RICE FLOUR AT DIFFERENT RELATIVE HUMIDITIES AND TEMPERATURES*

R.H. (%)	EMC (%d.b.) at indicated temp. (°C)		
	15	25	40
Corn flour			
11	7.0	5.9	5.1
32	9.7	8.7	8.2
44	11.3	10.5	9.8
49	12.1	11.5	10.6
62	14.9	13.9	13.1
72	18.1	16.8	14.8
80	21.1	19.8	18.2
89	26.4	25.1	23.8
Rice flour			
11	5.8	5.1	4.5
32	8.5	7.7	7.0
44	10.0	9.1	8.4
49	10.9	9.8	9.1
62	13.3	12.3	11.2
72	16.2	14.8	13.6
80	18.8	17.2	15.9
89	23.1	21.8	19.9

*Each value of EMC is an average of 3 replicates.

80 to 89 per cent R.H. At 15, 25 and 40°C and at 89 per cent R.H., mold growth was observed at both samples. Corn flour has higher sorptive capacity than rice flour which is due to the higher amount of starch and protein in the former. This may be attributed to the water holding potential of both the macromolecules.

Isotherm equations of Henderson[8], Smith[9], Bradley[10] and Harkins and Jura[11] were examined for applicability to the present data. Smith's equation gave the best fit over the entire range of R.H. and temperatures for both products.

Free energy change is the energy required to transfer water molecules from vapour state to the solid surface or *vice versa*. This quantity is considered as a measure of work done by the system to accomplish the adsorption process. It is expressed by the relation

$$- \triangle F = RT \ \text{In} \ \frac{P}{P_o}$$

Where, R is the universal gas constant, T is the absolute temperature, P is partial pressure of water vapour or equilibrium vapour pressure of adsorbate, and P_o is the saturation pressure of pure water at T. The values of $\triangle F$ using the test data for corn and rice flour ranged from 950 to 70 kcal/kg mole and 749 to 45 kcal/kg mole respectively, indicating a continuous decrease with increase in moisture content (Fig. 1). The relationship

between $\triangle F$ and moisture content, X, has been reported for cereal grains and their products[12]. It is possible to predict the $\triangle F$ of adsorption from the equation

$$- \triangle F = Ae^{-BX};$$

constants A and B can be evaluated from intercept (In A) and slope respectively.

Heat of adsorption is the quantity of heat which is released when water vapour is adsorbed on a surface. The isosteric heat of sorption is expressed by the equation

$$\triangle H_{st} = R \cdot \frac{T_1 \ T_2}{T_2 - T_1} \cdot \text{In} \ \frac{P_2}{P_1}$$

where, P_1 and P_2 are equilibrium vapour pressures at absolute temperatures T_1 and T_2 respectively. The values of $\triangle H_{st}$ may be taken as pertaining to an isotherm whose temperature is an average between T_1 and T_2. The values of the $\triangle H_{st}$ were obtained from the relationship between moisture content and relative humidity for both flours at 15 and 40°C. The calculated values of $\triangle H_{st}$ of adsorption for corn and rice flours decreased from 14.54 to 10.99 kcal/g mole and 13.65 to 11.04 kcal/g mole respectively for the moisture range of 8 to 22 per cent (Table 2). These values are comparable to those obtained for cereal grains and their products[12]. Since the heats of sorption can be considered as an indication of intermolecular attraction between sorptive sites and water vapour molecules, the relationship between $\triangle H_{st}$ and moisture content can be used to study the magnitude of binding energy or the availability of active sites to water vapour as adsorption proceeds.

In addition to heat of adsorption, the net heat of adsorption, q was calculated by the equation.
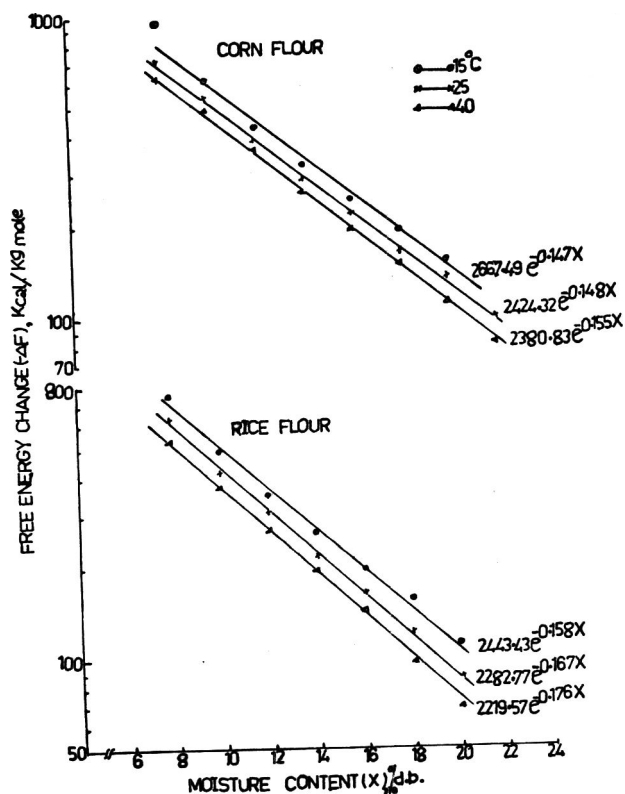
$$q = \triangle H_{st} - \lambda$$

Fig. 1. Free energy change as a function of moisture content and temperature for corn and rice flours

TABLE 2. ISOSTERIC HEAT ($\triangle H_{st}$) AND NET HEAT (q net), KCAL/G MOLE AS A FUNCTION OF AMOUNT OF WATER SORBED AT 27°C FOR CORN AND RICE FLOURS

Moisture content (% d.b.)	Corn flour		Rice flour	
	$\triangle H_{st}$	q net	$\triangle H_{st}$	q net
8	14.54	4.07	13.65	3.17
10	12.49	2.01	12.25	1.77
12	11.59	1.11	11.57	1.09
14	11.46	0.98	11.54	1.06
16	11.20	0.73	11.34	0.90
18	11.15	0.67	11.27	0.79
20	11.11	0.63	11.06	0.59
22	11.99	0.51	11.04	0.56

where, λ is the latent heat of vapourization of water at specific temperature. These values are in the range of 4.07 to 0.51 kcal/g mole for corn flour and 3.17 to 0.56 kcal/g mole for rice flour for moisture contents of 8 and 22 per cent (Table 2). Net heat of adsorption is considered as additional heat required over the heat of condensation of water and it approaches zero as the moisture content increases. This indicates that the nature of adsorbed water vapour approaches that of pure water at higher moisture content or relative humidity.

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COMPARATIVE EFFICACIES OF CHEMICAL SPROUT INHIBITORS AND VAPOUR HEAT TREATMENTS ON THE CONTROL OF SPROUTING IN STORED POTATOES

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The effects of maleic hydrazide (MH) as pre-harvest spray and methyl ester of naphthalene acetic acid (MENA), sodium salt of naphthalene acetic acid (NAA), tetrachloro-nitrobenzene (TCNB), isopropyl-N-chlorophenyl carbamate (CIPC) (each at 1000 p.p.m.) and vapour heat treatment (VHT) (at 60°C for 60 min) as post-harvest treatments on sprouting, spoilage and physiological loss in weight (PLW) during storage at 22–35, 20 and 10°C were studied. The efficacy of MH to curtail sprouting increased with decrease in the storage temperature while that of MENA, NAA and VHT decreased. At 22–35°C, the efficacy of VHT to suppress sprouting was equal to that of MH treatment (2000 p.p.m.). CIPC was effective only at 10°C. PLW was not significantly altered by any of the treatments. The spoilage in VHT tubers was not significantly different from chemically treated tubers.

Extensive work has been done on the control of sprouting of potatoes by chemical sprout suppressants[1-5]. In our earlier reports[6,7] we have shown that vapour heat treatment (VHT) at 60°C for min, controls sprouting of potatoes during storage at 20–35°C. Commercial inhibitors like isopropyl-N-chlorophenyl carbamate and isopropyl phenyl carbamate are ineffective when the storage temperature exceeds 18°C[4]. On the other hand, methyl ester of naphthalene acetic acid and sodium salt of naphthalene acetic acid are known[8,9] to suppress sprouting of potatoes even at high storage temperatures of 22–35°C.

The present study was undertaken to assess the relative efficacies of some of the chemical sprout inhibitors of potatoes during storage at ambient and low temperatures. The effects of these treatments on the spoilage and weight loss of tubers were also studied.

Pre-harvest foliar spray: Plots of size 10×10 ft, each consisting of 100 to 110 sixty day old plants were selected for the experiment. Maleic hydrazide solution containing 0.1 per cent Tween 80 and 250 p.p.m. benlate were sprayed on plants at 1000 and 2000 p.p.m. levels, at the rate of 4 lit per plot. Unsprayed plants served as control. Six replicate plots were allotted in randomised block design for each treatment. They

were cured in the field for three days and transported to the laboratory. The harvested tubers from each plot were divided into three lots to serve as replicates for storage at three temperatures.

Post-harvest chemical treatment: The tubers were treated with sodium salt of naphthalene acetic acid (NAA), methyl ester of naphthalene acetic acid (MENA), isoprophyl-N-chlorophenyl carbamate (CIPC) and tetrachloronitrobenzene (TCNB). Each chemical was applied to tubers five days after harvest, as a dip treatment at 1000 p.p.m. level for 15 min. The solutions also contained 1000 p.p.m. benlate and 0.1 per cent Tween 80. The effective concentrations of NAA (sodium salt) and MENA were established in a separate study and the concentration of the other two were kept the same for confirmation[9]. Each treatment consisted of five replicates of 5 kg potatoes and stored in gunny bags at 22-35, 20 and 10°C.

Vapour heat treatment (VHT): Potato tubers 5 weeks after harvest were subjected to vapour heat treatment at 60°C with 95±5 per cent RH, for 60 min and were stored at 22-35, 20 and 10°C. After 3 weeks, one lot from each of the above (temperature) was given a second VHT at 60°C for 60 min. Each treatment consisted of five replicates of 3 kg potatoes and they were stored in ventilated polyethylene bags at their respective temperatures. Tubers were observed periodically for sprout yield, spoilage and physiological loss in weight and the results are given as the means of five replicates.

Effect of sprout inhibitors on sprout yield: Among the five chemicals, MENA and NAA at 1000 p.p.m. and MH at 2000 p.p.m. effectively reduced the sprout yield during storage at the three storage temperatures (Table 1). MH at 1000 p.p.m. was effective only at low temperatures and the sprout yield after three months was 7.1, 0.3 and 1.7 g in 2000 p.p.m. MH, MENA and NAA (each at 1000 p.p.m.) treated tubers respectively as against 24.6 g in control, during storage at ambient temperature (22-35°C). Thus, among the three effective chemicals, MENA had reduced the sprout yield to the maximum extent. TCNB was found to be ineffective at the three temperatures, while CIPC controlled sprouting only at 10°C.

Vapour heat treatment was more effective at 22-35°C than at 20 and 10°C (Table 2). However, at 20 and 10°C, two vapour heat treatments were needed to give significantly less sprout yield than their corresponding controls.

The efficacy of MENA and NAA decreased with decrease in the storage temperature. On the contrary, MH fared better at low temperatures. The efficacy of MH is reported to be dependent on the storage temperature[10]. At ambient temperature (22-35°C), the per cent reduction in sprout yield was 83 and 71 per cent in twice VHT tubes and MH 2000 p.p.m. tubers respectively. Thus, two VHTs were as good as 2000 p.p.m. MH in reducing the sprout yield.

Effect of sprout inhibitors on PLW and spoilage: Though the tubers treated with MENA, NAA and MH 2000 p.p.m. showed slightly less weight loss, it was not significantly different from control (Fig. 1). VHT did not affect the rate of PLW when compared to control tubers (Fig. 1).

Of the post-harvest treatments, CIPC, MENA and NAA resulted in significantly more spoilage than control. A similar increased spoilage was seen in tubers treated with vapour heat once and twice (Table 3).

TABLE 1. EFFECT OF CHEMICAL SPROUT INHIBITORS ON SPROUT YIELD OF POTATOES DURING STORAGE

Sprout inhibitors		Sprout yield(g)/100 tubers at indicated periods (months)		
Name	Concn	3		6
	(p.p.m.)	22-35°C	20°C	10°C
MH	1000	27.6±15.9	15.9±1.3*	30.5±8.4*
MH	2000	7.1±5.3*	6.4±4.1*	15.2±8.7*
CIPC	1000	29.3±11.2	42.3±3.8	115.2±33.3*
MENA	1000	0.3±0.1*	4.0±1.5*	72.1±33.7*
NAA	1000	1.7±0.7*	13.5±2.6*	127.9±32.4*
TCNB	1000	30.5±6.9	40.3±6.9	262.0±26.2
Control	—	24.0±0.5	56.9±13.7	267.6±32.8

*Significantly less than control at 0.05% level. Each value is the mean of 5 replicates.

TABLE 2. EFFECT OF VAPOUR HEAT TREATMENT ON SPROUT YIELD

Frequency of	Sprout yield (g)/100 tuber[a]		
VHT	22-35°C	20°C	10°C
Once	22.5±9.2**	37.7±4.0	28.1±5.2
Twice	10.1±3.5*	29.3±3.3*	19.4±1.9*
Control	58.7±9.7	43.2±5.9	33.3±5.9

*, ** —Significantly less than control of 0.05 and 0.1% levels, respectively.
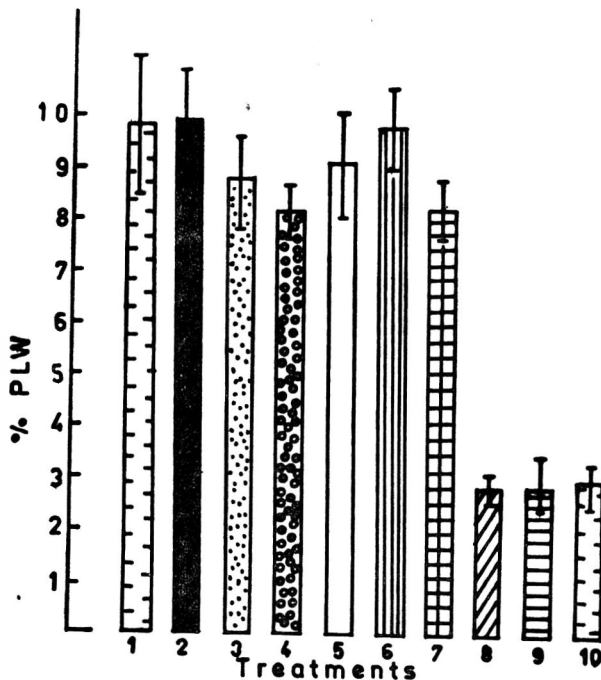
a   —Sprout yield recorded 5 wk after 2nd VHT.

Fig. 1. Effect of chemical inhibitors and vapour heat on PLW of potaoes during storage at ambient conditions. 1. Control 2. MH 1000 ppm 3. MH 2000 ppm 4. NAA 1000 ppm 5. TCNB 1000 ppm 6. CIPC 1000 ppm 7. MENA 1000 ppm 8. VHT once 9. VHT twice 10. Control. 1-7 stored in gunny bags for 90 days. 8-10 stored in polyethylene bags for 45 days.

TABLE 3. EFFECT OF SPROUT INHIBITOR TREATMENTS ON SPOILAGE‡ DURING STORAGE AT 22-35°C

Sprout inhibitors/treatment	Spoiled tubers[a] (%)
CIPC	9.2±5.4*
MENA	11.4±7.0*
TCNB	1.4±2.2
NAA	9.4±2.0*
MH (1000 ppm)	5.4±3.1[NS]
MH (2000 ppm)	5.2±0.8[NS]
Control	3.4±1.1
VHT (once)	10.5±0.8*
VHT (twice)	7.6±2.7*
Control (for VHT)	4.6±1.0

a —Cumulative spoilage at the end of 3 months.

* —Significantly more than control at 0.5% level.

NS —Not significant.

‡ —The spoilage in control was more due to *Fusarium oxysporum* (fungal) while in treated lots, it was due to *Erwinia carotovora* (bacterial).

It is evident from the results that, MENA and NAA at 1000 p.p.m. as post-harvest treatments and 2000 p.p.m. of MH at pre-harvest treatment can effectively check sprouting of potatoes during storage at 22-35°C As reported earlier, CIPC was effective only at 10°C. The effect of VHT was equal to 2000 p.p.m. of MH treatment when applied twice. Though vapour heat suppressed sprouting even at low temperatures, its efficacy was not as much as that of MENA and NAA. The rate of spoilage in VHT tubers was at par with the chemical treatments. Thus properly administered, VHT may become a very effective non-chemical method for the sprout control of potatoes during post-harvest storage, especially at the ambient conditions prevailing in tropics.

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SOLUBLE AND INSOLUBLE OXALATES IN SELECTED FOODS

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Oxalates which are known to influence the calcium absorption in foods were determined as total, soluble and insoluble oxalates along with the calcium contents in seven types of foods. The soluble oxalate contents (as per cent of total oxalates) in amaranth (Amaranthus gangeticus), kilkeerai (Amaranthus tricolor), drum stick (Moringa oleifera), leaves, Horse gram (Dolichos biflorus), sesame (Sesamum indicum), cashewnut (Anacardium occidentale), and almond (Prunus amygdalus) was 52, 37, 28, 40, 19, 27 and 18 respectively.

Oxalic acid is found widely in the vegetable kingdom mostly as calcium or potassium salt.[1,2] The significance of oxalate content of foods in human nutrition has been studied by several workers[3-9]. It is a well-established fact that oxalic acid in diets decreases the physiological availability of calcium[3,10]. Soluble oxalate is considered to have deleterious effect on calcium absorption from foods undergoing digestion simultaneously, whereas insoluble oxalate binds calcium within the food and renders it unavailable to the body. Therefore, the calcium content of foods is of little nutritional signifi-

cance unless it is considered in relation to oxalic acid and other interfering substances. The investigations by Singh et al.[5] have emphasized the need to study the soluble or free oxalate content of foods in addition to total oxalic acid. The total oxalic acid content of a large number of foods has been published by the Indian Council of Medical Research[11]. However, information regarding the free oxalic acid content of various foods is very limited. Therefore, the present investigation was undertaken to analyse a few foods for their calcium as well as total, soluble and insoluble oxalate contents.

Seven foods namely, amaranth (Amaranthus gangeticus), kilkeerai (Amaranthus tricolor), drumstick leaves (Moringa oleifera), horse gram (Dolichos biflorus) sesame (Sesamum indicum), cashewnut (Anacardium occidentale), and almond (Prunus amygdalus) were selected considering their usage and local availability. Calcium was estimated by precipitating as oxalate and titrating against potassium permanganate[12]. Total and soluble oxalates were determined by the method of Baker[2], insoluble oxalates were calculated according to Singh and Saxena[13]. The analysis was done in six replicates for each constituent.

The results are summarized in Table 1. The calcium contents of greens namely amaranth, kilkeerai and drumstick were found to be 363, 424 and 865 mg/100 g respectively. Total oxalates in amaranth and kilkeerai greens were veryhigh with a calcium/oxalate ratio of 0.6 whereas drumstick greens had less of total oxalates with a calcium/oxalate ratio of 3.6. The percentage of soluble oxalate was more in amaranth and less in kilkeerai and drumstick greens.

TABLE 1. CALCIUM AND OXALATE CONTENTS OF SOME FOODS*

Food	Calcium (mg/100g)	Oxalates			Ca: Oxalate ratio
		Total (mg/100g)	Soluble (mg/100g)	Soluble as (% of total)	
Amaranth	363±9	598±57	313±8	52	0.6
Kilkeerai	424±25	732±57	274±40	37	0.6
Drumstick leaves	865±4	235±17	66±3	28	3.6
Horse gram	249±3	217±21	86±5	40	1.2
Sesame	1300±90	1500±51	289±7	19	0.9
Cashewnuts	41±3	231±17	63±4	27	0.2
Almond	194±5	261±34	48±4	18	0.7

*Values are mean ± SD of six samples.

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Among legumes, horse gram is a very good source of calcium, (249 mg/100 g) but it also contains a high amount of oxalates, 217 mg/100 g, most of which is insoluble. Sesame seeds are very rich in calcium as well as oxalates (1300 and 1500 mg/100 g respectively). Cashewnut, although only a fair source of calcium, contains good amount of oxalates with insoluble oxalates in larger proportion. Almonds were found to contain 194 mg per cent calcium and 261 mg per cent total oxalates. Here also, the proportion of insoluble oxalates was high.

Insoluble oxalate as per cent of total oxalates is high in all food samples except amaranth and the availability of calcium from these foods may be low since calcium is bound to the oxalate. Soluble oxalates can be more harmful because they may bind with calcium present in other foods. It is of significance particularly in South Indian dietaries where greens and horse gram which contain soluble oxalates are consumed with ragi (*Eleucine coracana*), a millet which is a rich source of calcium. Nuts like cashew and almond are generally used in sweet preparations including those based on milk. Since they do not contain much of soluble oxalates they may not interfere with the absorption of calcium present in milk.

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CHEMICAL CHANGES IN APPLES DUE TO AFLATOXIN PRODUCING ASPERGILLI

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Some chemical changes have been reported in apple fruits due to aflatoxin producing Aspergilli i.e., *Aspergillus flavus* and *A. parasiticus*. Losses in the levels of total, reducing and non-reducing sugars, protein and ascorbic acid were recorded whereas phenol content of fruit tissues increased during fungal metabolism.

Aspergillus flavus Link ex Fries and *A. parasiticus* Speare are known aflatoxin producers[1]. Besides producing aflatoxins, these fungi also cause deterioration of nutritive quality of the food materials[2,3]. Changes in the chemical constituents of fruits due to toxigenic fungi have not received much attention. Present report deals with some chemical changes taking place in apple fruits due to aflatoxin producing fungi.

Apple fruits (*Pyrus malus* L.) of equal weight were surface sterilized and inoculated separately with *A. flavus* (BG-19) and *A. parasiticus* (NRRL-3240) which are known to produce aflatoxin. These were subsequently incubated for 7 days at constant R.H. of 96 per cent before chemical estimations were done.

Total and reducing sugars were estimated by standard methods[4,5] whereas non-reducing sugar was calculated by subtracting the value of reducing sugar from total sugar. Phenol and ascorbic acid contents were determined by the methods of Singh et al.[6] and Roe and Kuether[7] respectively whereas protein content of the fruit tissues was estimated according to Lowry[8].

It is evident from Table 1 that aflatoxin producing strains of *Aspergillus* caused considerable losses in the quality of total, reducing and non-reducing sugars as well as in protein and ascorbic acid contents of apple fruits during their infection. Maximum depletion of sugar content was shown by *A. parasiticus* whereas maximum loss in protein and ascorbic acid was revealed by *A. flavus*. An increase was observed in phenol content of the fruit tissues due to infection by both the fungal species. When analysed statistically, the changes in apple fruits by these fungi were found highly significant except in the case of ascorbic acid (Table 1).

A. flavus (BG-19) and *A. parasiticus* (NRRL-3240) elaborated 0.066 and 0.042 p.p.m. of aflatoxin B_1 on apple fruits under similar conditions[9]. It is thus clear from the present investigation that besides producing aflatoxins, the toxigenic strains of *Aspergillus* caused

TABLE 1. CHEMICAL CHANGES IN APPLE FRUITS DUE TO INFECTION WITH ASPERGILLUS FLAVUS AND A. PARASITICUS

Chemical constituents	Control fruits		Infected fruits		't' test value	
	Initial	7 days	A. flavus	A. parasiticus	A. flavus	A. parasiticus
Total sugars(%)	8. 90±0.062	8.66 ±0.036	6. 93±0.056	6. 16±0.014	45.28*	47.85*
Reducing sugars(%)	6. 41±0.056	6.22 ±0.082	5. 30±0.042	5. 01±0.072	17.94*	19.23*
Non-reducing sugars(%)	2. 49±0.095	2.44 ±0.046	1. 63±0.085	1. 15±0.06	14.48*	11.45*
Phenols(%)	0.242±0.004	0.262±0.007	0.380±0.009	0.303±0.002	8.36*	6.33*
Ascorbic acid(%)	0.112±0.005	0.095±0.003	0.077±0.006	0.086±0.007	4.33**	1.99
Protein(%)	0.370±0.002	0.340±0.008	0.186±0.005	0.280±0.001	13.63*	11.93*

* P < 0.01
** P < 0.05

considerable changes in the chemical constituents of apple fruits.

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REPRODUCTIVE RESPONSE OF RATS FED TURMERIC (CURCUMA LONGA L.) AND ITS ALCOHOLIC EXTRACT

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Reproductive response of rats fed turmeric (500 mg/kg body weight) and its alcoholic extract (60 mg/kg body weight) was examined for three generations. In general, no significant difference was noticed in the fertility index, gestation index, average number of pups born alive, viability index and lactation index between experimental animals and controls. Average weight of pups at birth on 12th and 21st days did not show marked deviation from that of respective control values. The data suggest that turmeric or its alcoholic extract is safe at the levels tested as far as the reproductive efficiency is concerned.

Turmeric (Curcuma longa L.) is a traditional spice in India. It is widely used in the Indian system of medicine[1]. As a food colour, turmeric, its alcoholic extract and its pigment—curcumin (diferuloyl methane) are being used in various parts of the world. Some of the beneficial effects of turmeric (and its components) such as the anti-inflammatory[2,3], choleretic and cholagoguic[4,5], hypocholesteremic[6] and antibacterial[7,8] properties have been elucidated. However, a systematic toxicological study on turmeric was lacking although some isolated

studies were conducted to show that sodium curcuminate has low toxicity[9] and turmeric causes chromosomal aberrations in mammalian tissue culture[10]. Rat growth studies conducted by feeding turmeric, curcumin and alcoholic extract of turmeric did not show any adverse effects[11,12]. With a view to obtaining toxicological data on turmeric and its alcoholic extract, studies were initiated in our laboratory. The results of acute and chronic toxicity experiments have been reported earlier[13,14] and the data obtained from multigeneration experiments on rats are reported in this communication.

Test materials and dosage: Turmeric powder containing 2.5 per cent curcumin and the alcoholic extract of turmeric were supplied by the Department of Plantation Products and Flavour Technology of the Institute. Turmeric was fed at 500 mg/kg body weight while alcoholic extract was fed at 60 mg/kg body weight which was equivalent to the dose of turmeric on the basis of curcumin content. The dosage of test materials used here is one fifth of the dose used in our earlier acute toxicity experiments[13].

Animals and diet: Weaned albino rats of the Wistar strain weighing about 32g were divided into two groups, each having 10 male and 20 female animals. All the animals were housed in individual cages and were given diet and water *ad libitum.* The basal diet had the following composition (%). corn starch, 72; casein, 15; refined groundnut oil, 10; salt[15] mixture, 2; and vitamin[16] mixture, 1. The test materials were fed throughout the experimental period and they were incorporated into the diet as described in our earlier report on the acute toxicity studies[13].

Reproduction programme: The reproduction programme was initiated shortly after 12 weeks on the two test diets, when the animals were sexually mature. Matings were set up with one male and two female rats per cage. The male and female animals were paired on littermate basis. When pregnancy was recognized either visually or by palpation, the males were separated and the females were transferred to individual cages. Lactation was permitted for three weeks. During lactation period, the mothers were given milk and cooked liver pieces. Following weaning, the females were allowed a two weeks rest period before remating. First litters were discarded after weaning. From the second litters, 10 male and 20 female rats were selected from each group after weaning. They were raised to maturity and mated like the parent generation. This mating schedule continued until the first litters from F_2 generation were weaned by which time the F_o generation rats were two years old.

Observations: Observations were made of physical appearance and behaviour of the animals throughout the experimental period. In the reproduction phase of

the experiment, number of matings resulting in pregnancy and number of pregnancies resulting in the birth of live litters in each group was recorded. The pups were weighed at birth and on days 4, 12 and 21. The number of pups which died during weaning period was also recorded. From the above records of performance, following indices of reproductive and lactating efficiency were calculated as described by Oser and Oser[17].

(*i*) Fertility index (FI), the percentage of matings resulting in pregnancy;

(*ii*) Gestation index (GI), the percentage of pregnancies resulting in the birth of live litters;

(*iii*) Viability index (VI), the percentage of pups born that survived for 4 days or longer;

(*iv*) Lactation index (LI), the percentage of pups alive at 4 days that survived the 21 days lactation period.

At the termination of each experiment with the two test diets, the animals were anaesthetised by ether. Liver, kidney, heart, brain, spleen, gonads, pituitary, adrenals and thyroid were excised from six animals of each sex in each group. The tissues were processed for histological examination.

Physical appearance and behaviour of rats belonging to various groups were normal. The reproductive response of rats of different generations fed turmeric or its alcoholic extract is given in Table 1. There was no significant difference in FI, GI, average number of pups born alive and VI among various groups of rats belonging to different generations. Even the LI of the experimental groups was not significantly different from the respective control groups except in an isolated case. In F_1 generation –2nd litters, LI in the group fed alcoholic extract of turmeric was 100 per cent as against 96.4 per cent in the control group. The difference was statistically significant (P<0.05). Since the LI in alcoholic extract fed group was higher than that of control group, and the value is in the normal range, it does not indicate any toxic effect.

Average weight of pups of various groups at different intervals of weaning is recorded in Table 2. Significant difference was not registered in the average weight of pups belonging to different groups in different generations except a few instances. Average weight of pups at birth was significantly higher in the group fed alcoholic extract than that of the control group in the F_1 generation—2nd litters and F_2 generation—1st litters. The average weight on 12th day was significantly lesser in alcoholic extract fed group in the F_o generation—2nd litters. However, the difference in weight was not consistent in all the generations and in the two litters of the same generation Moreover, the average weight at birth was more in the alcoholic extract fed group than that of the control group suggesting that the test material had no toxic effect. Histological examination

TABLE 1. REPRODUCTIVE RESPONSE OF RATS FED TURMERIC AND ITS ALCOHOLIC EXTRACT

Diet	FI	GI	No. of pups born alive Mean±S.D.	VI Mean±S.D.	LI Mean±S.D.
			F_0 1st litters		
Control	100	100	8.6±1.20	98.8± 0.80	96.3± 1.64
Turmeric	100	100	8.7±1.49	99.4± 0.63	96.7± 1.17
Control	95	100	7.1±1.84	97.3± 1.89	99.3± 0.68
Alcoholic extract	100	100	7.5±2.27	92.8± 3.28	97.8± 1.28
			F_0 2nd litters		
Control	100	100	8.9±1.43	97.2± 1.44	93.3± 2.10
Turmeric	100	100	8.6±1.78	97.5± 1.21	88.0± 2.66
Control	89	100	7.6±2.12	91.2± 5.86	81.1± 7.99
Alcoholic extract	100	100	8.7±1.75	92.6± 3.03	81.3± 5.94
			F_1 1st litters		
Control	100	94	7.3±2.54	93.2±20.65	99.1± 3.93
Turmeric	100	100	5.8±2.18	95.4±11.89	97.2± 8.59
Control	100	100	7.4±1.77	92.3±14.01	95.6± 8.70
Alcoholic extract	100	100	6.6±2.11	96.7± 7.07	94.9±15.68
			F_1 2nd litters		
Control	100	100	7.8±1.46	100.0± 0.00	98.8± 3.73
Turmeric	100	100	7.0±2.54	95.8±11.23	99.4± 2.36
Control	75	100	7.2±2.09	97.3± 7.84	96.4± 8.06
Alcoholic extract	80	100	7.8±1.78	98.2± 4.88	100.0± 0.00*
			F_2 1st litters		
Control	100	100	8.1±2.31	91.1±10.67	92.7±15.07
Turmeric	100	100	9.1±2.20	92.2±15.14	94.4± 8.90
Control	95	95	8.0±1.50	96.6± 8.09	95.7±10.28
Alcoholic extract	95	100	7.2±1.92	96.6± 8.21	97.2± 6.75

FI — Fertility Index (% of matings resulting in pregnancy)

GI — Gestation Index (% of pregnancies resulting in live litters)

VI — Viability Index (% of pups born that survived 4 days or longer)

LI — Lactation Index (% of pups alive at 4 days that survived 21 days)

* Significantly different from the respective control value (P<0.05) according to Student's 't' test.

TABLE 2. AVERAGE WEIGHT OF PUPS (IN GRAMS) AT DIFFERENT INTERVALS OF WEANING

Group	1st litters			2nd litters		
	At birth	12th day	21st day	At birth	12th day	21st day
F_0 Generation						
Control	5.0±0.17	12.8±0.43	23.5±1.04	4.8±0.16	13.4±0.56	20.4±1.47
Turmeric	5.1±0.13	13.0±0.42	22.2±1.68	4.7±0.12	13.6±0.32	18.7±1.80
Control	5.7±0.51	13.6±1.20	20.0±4.38	4.8±0.43	12.3±1.29	20.0±3.56
Alcoholic extract	5.2±0.67	13.2±3.14	20.7±7.64	4.8±0.47	11.2±2.34*	19.6±6.58
F_1 Generation						
Control	5.1±0.74	15.2±3.09	22.9±5.14	5.2±0.34	15.9±2.70	28.8±4.93
Turmeric	5.0±0.59	15.2±2.95	25.4±5.35	5.3±0.64	17.6±6.37	32.2±7.84
Control	5.0±0.57	16.1±2.76	26.2±6.48	4.8±0.41	13.7±1.53	28.9±4.73
Alcoholic extract	5.2±0.71	15.9±2.65	26.1±4.37	5.2±0.34*	14.2±2.23	29.8±6.21
F_2 Generation						
Control	4.7±0.59	12.3±2.22	22.9±3.73	—	—	—
Turmeric	4.7±0.54	12.4±1.22	22.4±3.00	—	—	—
Control	4.6±0.53	13.1±0.78	23.2±1.21	—	—	—
Alcoholic extract	5.0±0.41*	13.1±0.87	22.9±1.46	—	—	—

*Significantly different from the respective control value (P<0.05) according to Student's 't' test.
Mean ± S.D.

of various tissues did not reveal any abnormalities in any of the groups. Goodpasture and Arrhigi[10] have reported chromosomal aberrations in cultured mammalian cells treated with turmeric even at a very low concentration. However, our earlier acute[13] and chronic[14] toxicity studies and the present study have not shown any adverse effects in rats, guinea pigs, and monkeys fed turmeric and its alcoholic extract for different durations. Thus, consumption of turmeric or its alcoholic extract at the levels tested appears to be safe.

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RESIDUES OF PHOSPHAMIDON AND MONOCROTOPHOS ON CORIANDER*

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A field study was conducted to determine the residues of phosphamidon and monocrotophos on coriander. Samples of whole plant, leaf and green grain were drawn from experimental plots sprayed with insecticidal emulsion at different intervals as also dry grains at harvest (35 days after treatment). Phosphamidon and monocrotophos residues were below tolerance levels in 9 to 11 and 13 to 15 days respectively in various parts of coriander. The harvest time residue of both the insecticides (35 days after application) were below the detectable level.

Among insect pests of coriander, aphid (*Hyadaphis coriandari* Das) was found causing serious damage to coriander[1,2]. Foliar application of phosphamidon and monocrotophos has been recommended for the control of this pest[2]. As no information is available on the persistence and degradation of these insecticides on coriander crop, this study was conducted to determine the residues in whole plant, leaf, green grain at different intervals and in dry grains at harvest.

The experiment was laid out in randomized block design with four replications at the Research Farm of College of Agriculture, Jobner, during November 1981. The individual plot consisted of five rows of 3 meter length with a distance of 40 cm between two rows. Coriander variety 'UD-20' was sown on November 11, 1981. All recommended agronomical practices were followed. Phosphamidon (0.03 and 0.05 per cent emulsion) and monocrotophos (0.04 and 0.06 per cent emulsion) at 500 l/ha were sprayed on the crop on February 14, 1982. There was no rainfall during the entire period of trial.

For residues of both the insecticides, the samples of whole plant, and leaf were drawn randomly at intervals of 0, 1, 3, 7, 10, 15 and 21 days of application. The samples of green grain were taken 10 days after application as the grain formation started 7 days after application of insecticides. Zero day sample was drawn just after two hr of application and dry grain samples were taken at the time of harvest (35 days after application). Samples of whole plant, leaf and green grain were macerated in a waring blender with distilled chloroform at 4 g/ml and the slurry was filtered through a Buchner funnel containing a thin layer of anhydrous sodium sulphate placed over Whatman No. 1 filter paper. The filtrate was freed from interfering substance by adding 5 g activated charcoal to the extract and passed under pressure through a column of 4 cm layer of anhydrous sodium sulphate and 4 cm layer of adsorbant mixture containing celite. MgO. charcoal (1:1:1). The traces of insecticides were washed down the column with 3×20 ml chloroform. The extraction of insecticides from dry grain samples was done by tumbling on a motorised shaker for an hour and filtering through a Whatman No. 1 filter paper, the filtrate needed no further clean up. Ten ml aliquots were used for residue estimation.

The insecticides were determined by the colorimetric method[3]. The calculations for residues in p.p.m. T_{tol} (time taken for the residues to reach below the tolerance level), RL_{50} (half life) and T_{sen} (expected time for the residue to reach the level of detectability) were done according to Hoskins[4]. To find out the sensitivity 5, 10 and 20 μg of phosphamidon and monocrotophos were added to 50 g each of coriander whole

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TABLE 1. RESIDUES OF PHOSPHAMIDON (P.P.M.) IN CORIANDER

Days after treatment	Whole plant		Leaf		Green grain		Dry grain	
	0.03% Mean±SD	0.05% Mean±SD	0.03% Mean±SD	0.05% Mean±SD	0.03%	0.05%	0.03%	0.05%
0	9.59±0.19	12.94±0.33	6.90±0.22	9.46±0.03	—	—	—	—
1	6.87±0.51 (28.36)	7.75±0.10 (40.11)	5.02±0.02 (27.25)	6.21±0.20 (34.36)	—	—	—	—
3	2.92±0.11 (69.55)	4.03±0.07 (68.86)	2.25±0.14 (67.39)	4.20±0.15 (57.72)	—	—	—	—
7	1.19±0.06 (87.59)	2.33±0.03 (81.89)	1.12±0.08 (83.77)	1.70±0.14 (82.03)	—	—	—	—
10	0.52±0.01 (94.58)	0.65±0.08 (94.98)	0.48±0.08 (93.04)	0.54±0.06 (94.29)	BDL	BDL	—	—
15	BDL (100.00)	BDL (100.00)	BDL (100.00)	BDL (100.00)	BDL	BDL	—	—
35	—	—	—	—	—	—	BDL	BDL
RL$_{50}$	2.51	2.51	2.74	2.50				
T$_{sol}$	10.33	11.26	9.91	10.51				
T$_{sen}$	12.83	13.77	12.64	13.02				

No. of replications= 3.. Crop was harvested 35 days after treatment.

RL$_{50}$=Half life in days.

T$_{tol}$=Minimum days required to reach the tolerance limit.

T$_{sen}$=Minimum days required to reach the level of detectability.

BDL=Below detectable level.

Data in parentheses are per cent reduction.

plant, leaves, green grain and dry grain and the average recovery of phosphamidon and monocrotophos from fortified samples ranged between 85–90 and 82–86 per cent respectively.

Residues of phosphamidon: The application of phosphamidon 0.03 and 0.05 per cent spray resulted in initial deposit of 9.53 and 12.94 p.p.m. respectively in whole plant of coriander and 6.90 and 9.46 p.p.m. respectively in leaves (Table 1). The residue reached the tolerance limit (0.5 p.p.m.) in 11 and 12 days in whole plant, in 10 and 11 days in leaves. The residue in whole plant and leaves fell below detectable level in 15 days. The residue half life was 2.51 days at both the concentrations in whole plant, while on leaves it was 2.74 and 2.50 days respectively. The residue in green grain reached below detectable level in 10 days after application with lower and higher doses of treatment. At harvest (35 days after application) no detectable

residues were found. It is concluded that after 12 days of application of the insecticide at these doses, the plant or leaves can be consumed.

Residues of monocrotophos: An initial deposit of 7.82 and 11.59 p.p.m. resulted from 0.04 and 0.06 per cent application of monocrotophos respectively (Table 2). The residue reached the tolerance limit of 0.2 p.p.m. in 14 and 15 days respectively. This indicates that a period of 15 days is required for the safe use of the plant. The residue fell below detectable level in 18.10 and 18.09 days at the two doses. The half life value corrosponding to the dosage was 3.00 days and 2.73 days respectively. The leaves got an initial deposit of 5.82 and 8.22 p.p.m. at 0.04 and 0.06 per cent application respectively (Table 2). The residues reached the tolerance limit of 0.2 p.p.m. in 14 and 15 days and it was below detectable level after 16.60 and 16.82 days and half life values were

TABLE 2. RESIDUES OF MONOCROTOPHOS (P.P.M.) IN CORIANDER

Days after treatment	Whole plant		Leaf		Green grain		Dry grain	
	0.04%	0.06%	0.04%	0.06%	0.04%	0.06%	0.04%	0.06%
	Mean±SD	Mean±SD	Mean±SD	Mean±SD		Mean±SD		
0	7.82±0.14	11.59±0.54	5.82±0.25	8.22±0.41	—	—	—	—
1	4.36±0.01 (44.25)	7.07±0.22 (39.00)	3.73±0.18 (35.91)	5.11±0.42 (37.83)				
3	2.73±0.06 (65.09)	3.27±0.15 (71.79)	1.99±0.14 (65.81)	2.43±0.13 (70.44)	—	—	—	—
7	1.45±0.04 (81.46)	1.76±0.04 (84.81)	0.71±0.03 (87.80)	1.26±0.04 (84.67)	—	—	—	—
10	0.53±0.07 (93.22)	1.20±0.15 (89.65)	0.45±0.05 (92.27)	0.87±0.03 (89.42)	BDL	0.23±0.02	—	—
15	0.18±0.04 (97.70)	0.18±0.02 (98.45)	0.16±0.03 (97.25)	0.13±0.02 (98.42)	BDL	BDL	—	—
21	BDL (100.00)	BDL (100.00)	BDL (100.00)	BDL (100.00)	BDL	BDL	—	—
35	—	—	—	—	—	—	BDL	BDL
RL$_{50}$	3.00	2.73	3.01	2.73				
T$_{tol}$	13.69	14.56	13.60	14.08				
T$_{sen}$	18.10	18.09	16.60	16.82				

No. of replications=3 Crop was harvested 35 days after treatment.

RL$_{50}$=Half life in days.

T$_{tol}$=Minimum days required to reach the residue to tolerance limit;

T$_{sen}$=Minimum days required to reach the residue to the level of detectability;

BDL=Below detectable level;

Data in parentheses are per cent reduction.

3.01 and 2.73 days for 0.04 and 0.06 per cent application respectively. The residue in green grain was below detectable level with 0.04 per cent spray on 10 days of application. However, 0.23 p.p.m. residue was observed with 0.06 per cent spray. The residue reached below detectable level at 15 days after application of both the doses. At harvest (35 days after application) no detectable residues of monocrotophos at both the concentrations were found in dry grains.

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BOOK REVIEWS

Production and Preservation of Pears: Ed. by International Institute of Refrigeration, Paris, France, 1984, Price not stated.

This book gives the proceedings of the meeting held in "Salle de in Pannaterie", Avigon, France, during May 28–31, 1984, The meeting was organised jointly by the Food Science and Technology section of International Institute of Refrigeration and the working group on Pears of International Society of Horticultural Science. The meeting was held to discuss various aspects of pear cultivation, breeding, storage, diseases and marketing. Scientists from all parts of the world like Europe, North and South America, U.K., New Zealand and Africa presented papers on different aspects followed by discussions. The 'proceedings' is classified into 8 sections. The first two sections deal with prevention of browning of the fruit during storage and its relation to nutrient spray with minerals, influence of picking dates and interaction of various factors on storage, fruit quality, post harvest behaviour of pears, optimum harvesting indices for extended storage; optimum temperature and gas concentrations for maximum storage.

Section 3 deals with the diseases due to insects like bugs and aphids and also cold hardiness of young pear trees. Under marketing (Section 4), the need for finding a good and economic prepackaging practice is stressed since the marketing problems are unique in pears. Under sections 5 and 6, work done in different countries on breeding of pears and new varieties and hybrids evolved have been mentioned. Biochemical aspects regarding pear resistance to psylla, pest resistance by interspecific hybridization and back crosses and susceptibility of hybrids to firblight disease which is the main worry for the grower are also discussed.

In section 7, effect of auxins for fruit thinning and prevention of preharvest fruit drop, effect of agrostemin for increasing yield and quality of pear, effect of planting density on bearing and on available radiant energy are discussed. Section 8 covers the effect of pollinator density on behaviour of pear and its relation to maximum fruit set, performance of different rootstocks and propagation.

The book serves as an interesting reference book for those working on pear as well as those in the field of different disciplines in fruit crops—Pomology, entomology, breeding and post harvest technology. One lacuna is that no information on processing aspects of pear has been included. The 'proceedings' is covered in 245 pages in French and English. It is edited by International Institute of Refrigeration, Paris.

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Protein Tailoring for Food and Medical Uses: by Robert E. Feeney and John R. Whitaker, (Ed), 1986; pp. 408; Price: US$ 69.75 (US and Canada), $ 83.50 (All other countries).

'Protein Tailoring for Food and Medical Uses' is the third in the series of American Chemical Society symposia on the modification of proteins of food and medical interest. The first two were 'Food Proteins: Improvement through chemical and enzymatic modification' and 'Modification of Proteins: Food, Nutritional and Pharmacological Aspects', AC S, Adv. Chem. Series, Vol. 160, 1977 and Vol. 198, 1982 respectively.

Chapter 1 gives an excellent introductory survey. Chapters 2 to 6 deal with food proteins and chapters 7 to 14 with medical applications of protein modification. All are of current scientific interest, authoritative and well written. The topics covered in food proteins include covalent attachment of essential amino acids to proteins for improving their nutritional quality (Ch2) and covalent attachment of L-leucine-n-dodecyl ester to gelatin for imparting to it surface active properties (Ch3). The second half of the volume dealing with medical applications includes new and exotic topics like protein engineering (Ch 7), mechanism based enzyme inactivators (Ch8), immunotoxins (Ch 11), tailoring of enzymes as therapeutic agents (Ch 12), and tailoring of antitumor drugs (Ch 14).

Undoubtedly, it is a valuable volume of current interest for food and medical scientists interested in proteins. However, in the view of this reviewer, the prospects of developing acceptable foods may not be good. Here, in addition to the quality of the new protein, factors like economic constraints and cultural preferences are also important. Such considerations are likely to play a much smaller role in the acceptance of useful medical product and the future of protein tailoring may well be in medical applications.

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Advances in Biochemical Engineering/Biotechnology (31) *Plant Cell Culture:* by A. Fiechter, (Ed). Springer—Verlag, Berlin, Heidelberg, 1985, pp.140; Price. DM 90

Nineteen Eighties have been the decade of Biotechnological innovations and the book series 'Advances in Biochemical Engineering/biotechnology' highlights the same. Plant cell culture as an emerging field of biotechnology is receiving international attention. Presently, though micropropagation of plants in *in vitro* systems as a tissue culture technique is known, the production of secondary metabolites by exploiting chemical totipotency of plant cells has been much less studied and least understood. In this Scenerio, the book on plant cell culture in the series of advances in biochemical engineering/biotechnology (No.31) makes a very interesting reading. The book has five chapters contributed by different authors.

The range of secondary metabolites like alkaloids, phenols and terpenes and its derivatives that could be obtained by cell cultures and the associated problems are reviewed in the first chapter. The steps involved in biosynthesis of these compounds and the characterization of the connected enzymes and sensitive analytical techniques, biotransformations where it is possible are carefully detailed in the chapter on biosynthesis of secondary products by cell culture of higher plants. Immobilization of plant cells for enhancing the production of phytochemicals is elaborated in the second chapter. This technique is still at its infancy but considered to be more economical than mere culturing of cells for the production of extracellular metabolites. The benefits, unique features and products that could be obtained by immobilised cells are clearly defined. The reactor design and operation, makes an interesting reading. Plant cell immobilization has a good application potential which needs greater attention.

Production of metabolites from higher plant cells comparable to microbial fermentations is of prime importance. However, breakthroughs are yet to come. Only Shikonin obtained from *Lithospermum* is commercialised as bio-lipsticks. The conditions to increase productivity of cells, addition of precursors and biotransformation, selection of high yielding cell lines, induction of mutants and the effect of morphological differentiation and productivity are well discussed. The products of specific interests to industry like alkaloids, steroids, quinones and terpenoids are indicated, though information on this subject is quite limited in published literature. Photosynthetic potential of plant cell cultures are detailed in the fourth chapter. Selected green cell lines grown like algal cultures provide for new potentials in research on photosynthesis. Photo-

autotrophism is vital for productivity of plant cells. In this very brief chapter, the criteria for selection of specific characters of photoautotrophic cells, factors affecting their photosynthetic potential and prospective uses are outlined. This type of cell culturing totally eliminates the supply of sugar in the medium with the supply of inorganic carbon as CO_2. This is a basic study which has overbearing influence to exploit the application potential.

Selection and screening techniques for plant cell cultures are discussed in the final chapter. Isolation of variants are crucial to understand plant metabolism and development. Appropriate techniques for selection of such variants from callus and the limitations of analytical screening are effectively brought out. The techniques include plating of cells and protoplasts, microculture of single cells, mutagenic treatments etc. However, the best technique to be chosen depends on the researchers themselves and their innovative ideas considering the plant material in question.

The material presented in this book are overlapping which is inevitable with the limited information available in literature. The tables and extensive references given at the end of every chapter are fairly comprehensive. All the chapters are well written and highly readable and the problems of plant cell culture work are clearly presented. More advances in basic aspects of plant cell culture work have yet to achieve breakthroughs in programmes of applied research. This book will immensely benefit research workers in the field not only to inspire them in this difficult area but also to carefully plan their strategies to achieve success.

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

Sorption Isotherms and Water Activity of Food Materials: A Bibliography compiled by W. Wolf, W. E. L. Spiess and G. Jung. Science and Technology Publishers Ltd., 33 Woodlands Ave., Hornchurch, Essex RM 11 2 Q T, England, 1985; PP: 236; Price: £ 28.00 plus £ 2.00 post / packing.

The effects produced by the presence of water in food are of immense concern to a food processor. The book under review is like a keyboard where the keys of relevant information about water related topics on food available elsewhere can be obtained. The book contains 2201 references on the use, application, measurement and theory of equilibrium water conditions in food and food-related materials. Without a book of

this kind it was difficult to believe the existence of such a volume of reference materials regarding water related topics on food.

The bibliography is arranged alphabetically according to the names of the first author. The list is numbered consecutively and each reference is supplemented by a code number indicating the subject of the paper. The subjects and the number of reference materials under each are (i) General descriptions and reviews: 232; (ii) Thermodynamics of sorption process: 999, (iii) Measuring methods. 550, (iv) Influence of water activity on product stability. 803 and (v) Sorption data. 999. References of sorption data of about 620 materials have been separately listed. Persons working on these materials would find much use from this classification.

A classification based on bound water determination and on oxidative, textural, rheological, microbial, enzymic and non-enzymic changes in food as affected by water activity would have enhanced the usefulness of the book further. The authors may incorporate these in the subsequent edition of the book.

Reprints of most of the papers included in the book, are held at Bundesforschungsastalt fur Ernahrungen, Engesserstr. 20, 7500 karlsruhe, Federal Republic of Germany. Copies of individual papers may be had upon request in accordance with the International copyright agreement.

The effort made by the compilers is commendable. The book is worthy of its possession in personal and institutional libraries.

H. DAS
I.I.T., KHARAGPUR.

AFST(I) News

OBITUARY

Shri K. C. Ravindran Pillai passed away on 27th October 1986 at the age of 47. He had specialised in Food and Nutrition Planning and was working as the Deputy Technical Adviser in the Department of Food, Ministry of Food and Civil Supplies, Govt. of India. He was the President of the Madras Chapter of the Association of Food Scientists and Technologists (India) during 1985-86. In his untimely death, the Association has lost an active member who strived for its progress.

Born: 25—12—1939
Died: 27—10—1986

ADDITIONAL INSTRUCTIONS FOR REPORTING RESULTS OF SENSORY ANALYSIS

1. *Objectives*: The objective of the study should be stated clearly.

2. *Sensory test methods:* The methods are classified under two major categories; Analytical and Affective. Laboratory analysis with trained or semi-trained panels must use analytical methods. Affective methods can be used in consumer studies. Adequate details and references should be provided regarding methods used for pattern of collection, analyses and interpretation of data.

   Analytical (Trained Panel): The Major types of tests which can be made use of are:
   Discriminative. Difference or similarity testing and sensitivity assessments using difference tests, ranking, thresholds, dilutions, etc.,

   Descriptive/Quantitative: Flavour profiles, Texture profiles, Interval Scaling, Ratio Scaling, Descriptive Quality Scoring etc.,

   Affective (Untrained/consumer Panels): Difference/Preference, Hedonic rating, FACT ratings-Preference rankings etc.,

3. *Experimental designs:* The designs used are to be clearly stated. eg. Randomized block, Latin squares, Factorials, Fractional factorials, incomplete blocks and so on.

4. *Panel*: For analytical tests, the source of panel, whether inhouse or outside organisation to be indicated. The number of panelists should be stated, which should be normally not less than 15. Also whether the same panelists or different panelists have participated in testing the samples has to be indicated. Information on the composition (age, sex, etc) of the panel to be provided. The Panel should be trained to function as a human analytical instrument, with periodic re-orientation and at required sensitivity.

   For affective test, the panel (sample of population) should be representative of target population selected on the basis of defined sampling procedures. The number should not be less than 200. The composition (age, sex, income group, etc) of the panel should be indicated.

5. *Physical requirements*: For the analytical tests, the Laboratory set up should be reported eg. conducted in a booth with soft neutral shade walls or separators, without distraction from external sound or odour, with comfortable room tempareture (22°–25°C) and relative humidity conditions (35—40%) and suitably illuminated.

   The equipment and methods of sample preparation, testing temperature conditions, sample size and number of samples evaluted per panelist and per session should be reported.

   The time of evaluating and sequence of testing and data entry carriers, if any, and nature of palate-clearing agents used should be indicated.

   For affective test, the location of testing (stores, home, central location etc) has to be specified. The instructions given regarding sample preparation if they are not ready-to-eat, the order of testing if more than one sample is given and the questionnaire used for collection of data should be indicated.

6. *Statistical analysis:* The data handling procedure should be appropriate to the design, and should be clearly indicated including any transformations or derivations that are carried out, eg. assignment of numbers to intervals, categories and the like. The type of analysis carried out, categories, the level of significance and the decisions made are to be provided with appropriate tables and graphs. Appropriate and adequate data should be provided to justify conclusions and enable repeatability. For eg. while reporting results of tests of significance, the relevant tests like F, x^2, t, r, Rank sum, Mann–whitney, Rank correlations and so on. The probability levels, degrees of freedom, the observed value of the test criterion, the direction of the effect and the decision based on these are to be indicated.

INSTRUCTIONS TO AUTHORS

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

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

3. Names of chemical compounds and not their formulae should be used in the text. Methods of sampling, number of replications and relevant statistical analyses should be indicated. Superscripts and subscripts should be legibly and carefully placed. Foot notes especially for text should be avoided as far as possible.

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

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

6. **Illustrations:** Graphs and other line drawings should be drawn in *Indian ink* on tracing paper or white drawing paper preferably art paper not bigger than 20 cm (oy axis) ×16 cm (ox axis). The lettering should be such that they are legible after reduction to column width. Photographs must be on glossy paper and must have good contrast; *three copies* should be sent.

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

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

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

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

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

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

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

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

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

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

9. Consult the latest issue of the *Journal* for guidance and for "Additional Instructions for Reporting Results of Sensory Analysis" see **issue No. 1** of the Journal.

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